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SESSION 1 (Plenary Symposium)
Implementing One World One Health
Friday, February 13, 2009
Room: Park Congress/Ground Level
14:30–16:00

1.001 OIE Activities for the Global Improvement of Animal Health and their Benefits for Public Health
B. Vallat. OIE, Paris, France

The OIE, the World Organisation for Animal Health, which was created in 1924 to prevent animal diseases from spreading around the world, has, since then, enlarged its mandate to improve animal health worldwide. The OIE continuously adapts its strategy, which is supported by its 172 Member Countries and Territories to address their needs, as a changing world requires reactivity and scientifically and technically sound answers. One of the recent examples of an emerging disease that necessitated a quick and adapted response was avian influenza that presented not only great challenges but also opportunities. It emphasized the need to build common strategies with FAO and WHO, taking into account new concepts and parameters, such as appropriate governance, control of pathogens at the animal source, prevention of human cases among poultry owners and prevention of a pandemic.

Together we learned that diseases like avian influenza and other animal diseases, including zoonoses, that might arise in the future, cannot be managed without implementing coordinated policies. Therefore a broader concept, the “One World, One Health” strategy, has been developed by four international technical agencies (FAO/OIE/WHO/UNICEF). This strategy relates to the prevention and control of emerging infectious diseases at the animal/human interface; those with the potential to cause epidemics and pandemics, but also those animal diseases that have an impact on food security and poverty, as that is also of public health concern.

We also learned that animal diseases need to be tackled at the source and this cannot be done without functioning Veterinary Services. With the understanding that Veterinary Services are a global public good and with the help of donors, OIE was able to establish and run a programme for the evaluation of the performance of Veterinary Services (PVs). It enables interested countries to be evaluated according to the OIE international standards. When gaps are identified, the Veterinary Services can get the support needed to reach the required minimal international standards.

Improving governance, capacity building and helping countries to be ready to face new animal health threats is crucial. OIE has and will continue to take actions towards achieving these objectives and also advocates collaboration among national authorities on the animal/human interface.

1.002 Tracking Disease in Wild Animals
W.B. Karesh. Wildlife Conservation Society, New York, NY, USA

As human populations increase, global transportation speeds, changes in natural resource expand and ecosystem disruptions grow, the threat of emerging infectious diseases will continue to rise. No government or agency is responsible for, or currently capable of, the surveillance and prevention of the myriad of diseases residing around the world and the least developed monitoring subset of these diseases is for wildlife. For much of the world, there are no systems of inspection for animal markets and few people involved in trade or consumption have access to good health care, education on hygiene, common vaccinations or antibiotics. Land-use choices are still primarily based on short-term economic incentives and rarely quantify the contribution of ecosystem services to health. The inadvertent movement of infectious agents due to wildlife handling and trade and domestic animal movement, impacts not only human and livestock health, but also includes agents that can devastate native wildlife which serve as biological lynchpins for environmental integrity and provide a range of cultural and quantifiable economic values. For the near-term, much of wildlife disease surveillance will be carried out by individual organizations and government agencies, with any overview function being dependent on a spirit of cooperation. During this current decade, a tremendous interest in wildlife disease information sharing has grown around the world. A number of well-developed initiatives have built strong foundations and are now finding ways to create bridges among themselves and expand the network to include others. Opportunities still exist for determining how these efforts can serve as models for disease surveillance in domestic animals and human, and how these systems can best serve one another.

SESSION 2 (Plenary Lecture)
Avian Influenza
Friday, February 13, 2009
Room: Park Congress/Ground Level
16:30–17:15

2.001 Avian Influenza — A Unique Opportunity for Public Health
I. Capua. OIE/FAO Reference Laboratory for Newcastle Disease and Avian Influenza, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

The ongoing animal and human health crisis caused by influenza viruses of the H5N1 subtype has polarised attention of international organisations and donors on the need for improved veterinary infrastructure in developing countries and on the need for improved communication between the human and animal health sectors. Significant investments in capacity
building have resulted in the development of diagnostic laboratories and in the improvement of scientific know-how in the field of diagnostic virology. It is known that the animal reservoir (including arthropods) is the source of the majority of emerging pathogens which threaten global public health and also that most emerging pathogens originate (or cross the species barrier) in developing countries. It would therefore seem reasonable for the international community to capitalise on the investments that have been made as a result of the avian influenza emergency and expand the areas of diagnostic competence, possibly on a regional basis, to set up early warning systems and improved response capacities to manage diseases of public health relevance.

Possibly the biggest challenge we have is to find novel ways to maximise the use of the information which is generated as a result of the improved networking and diagnostic capacities. In the era of globalization, emerging and re-emerging diseases of public health relevance are a concern to developing and developed countries and are a real threat due to the interdependence of the global economy. Communication and analysis systems available should be tailored to meet the global health priorities, and used to develop and constantly improve novel systems for the exploitation of information to generate knowledge.

SESSION 3 (Parallel Session)
Vector-Borne Viruses in the 21st Century
Saturday, February 14, 2009
Room: Park Congress/Ground Level
08:30–10:30

3.001 Dengue Transmission and Control
M.B. Nathan. Consultant, Farges, France

Every decade since the 1970’s there has been an approximate doubling of the average annual number of reported dengue cases globally and the trend continues into the 21st Century. According to WHO there are an estimated 2.5 billion infections annually. All tropical regions are affected, yet dengue remains a truly neglected tropical disease. Currently, virus transmission can only be curtailed using vector control methods and possibly personal protection measures, but contemporary, large scale successes remain the exception rather than the rule. Moreover, they appear to bring their own new challenges. Given the magnitude of the problem, novel approaches to the delivery of vector control are needed, including for the mitigation of epidemics. Several of these are discussed.

3.002 Chikungunya Outbreaks: History and Lessons for the Future
R.N. Charrel. Université de la Méditerranée, Marseille, France

Chikungunya virus has been first isolated in Tanzania during a large outbreak in the 1950’s. Large outbreaks of chikungunya fever will be reviewed based on historical records. In 2006, an outbreak supposedly started in the islands of Indian Ocean (the Comoros, Mauritius, the Seychelles, Madagascar, Mayotte, and Reunion). In fact this outbreak most likely started in Kenya in 2004, and ended in India in 2007, several points make this chikungunya epidemic very interesting to investigate. For the first time, the outbreak hit a region where the medical level was as high as in developed countries, which allowed extensive investigations to be performed timely to gain knowledge on the disease (clinics, morbidity and mortality), the virus, the transmission, and other critical factors such as the environment and the perception of risk and subsequent population attitude regarding the epidemic. Whilst originally vectored by *Aedes aegypti* mosquitoes, chikungunya virus acquired the capacity to shift to *Aedes albopictus* mosquitoes in 3 different occasions almost simultaneously in Indian Ocean islands, India and Gabon. This phenomenon known as converging evolution has been rarely reported for viruses in the past. Due to adaptation to *Aedes albopictus*, chikungunya virus has expanded its geographic range of circulation, which has resulted in a significant outbreak in the Ravenna province of the Emilia Romagna region in 2007. The reasons for the occurrence of an outbreak in temperate regions will be addressed. The Italian outbreak illustrates that similar outbreaks may easily occur in other temperate countries where *Aedes albopictus* is prevalent. Other arboviral diseases could manifest themselves in epidemics. This underlines that as vectors disperse more widely, arboviruses previously believed to cause only tropical diseases might now cause outbreaks in temperate countries.

3.003 Urbanization of Yellow Fever
T. Monath. Kleiner Perkins Caufield & Byers, Menlo Park, CA, USA

Yellow fever remains a significant public health problem throughout much of tropical Africa and South America. Virus transmission between nonhuman primates and sylvatic vectors (tree-hole breeding *Aedes and Haemagogus spp.*), is amplified periodically dependent on rainfall, el Niño patterns, and other poorly understood factors, leading to epizootic waves, which may extend beyond normal geographic boundaries of the enzootic region. This probably occurs annually to some extent in Africa, where the virus emerges in the rainy season at the forest-savanna ecotone, extends into savanna habitat and is transmitted in more complex cycles involving humans, monkeys and a broad array of *Aedes spp.* In South America and Africa, *Ae. aegypti*, the domestic "urban" vector and sympatric human hosts may become secondarily involved in virus transmission. This spill-over of virus from sylvatic to urban transmission was a common occurrence in South America up until serious efforts to eradicate the urban vector in the 1940s. The situation in South America is again precarious because of the reinfection by *Ae. aegypti* endemic regions and the absence of large contiguous human populations in *Ae. aegypti* infested coastal areas. While high vaccine coverage of the human population in the endemic regions provides a barrier to urban yellow fever, that barrier is absent in adjacent coastal zones. Two instances of yellow fever urbanization have occurred in modern times (Bolivia, 1998; Paraguay, 2008); the latter will be described in some detail. In West Africa, in contrast, urbanization is the norm during repeated epidemics. The risk that yellow fever could be exported (by a viremic traveler) to a distant location infested with *Ae. aegypti* has increased due to expanding air travel and changing demographics. These risks will be discussed.

3.004 A Zika Virus Disease Outbreak on Yap Island

In the spring of 2007, physicians on Yap Island reported a community outbreak of an acute illness characterized by rash, conjunctivitis and arthralgia. Although serum from some patients had IgM antibody against dengue virus (DENV), the illness seemed clinically distinct from previously detected dengue. Subsequent testing of acute patient sera detected Zika virus (ZIKV) nucleic acid but no DENV nucleic acid. No previous outbreaks and only 18 clinical cases of ZIKV illness have been described in the literature. We obtained diagnostic serum samples and interviewed patients using a standard questionnaire to describe clinical signs and symptoms of ZIKV illness. We conducted a household survey to determine the proportion of Yap residents recently infected with ZIKV and the most likely ZIKV mosquito vectors.

We identified 49 laboratory-confirmed and 59 probable ZIKV illness cases. Laboratory-confirmed cases resided in 9 of 10 municipalities on Yap. No hospitalizations, hemorrhagic manifestations or deaths due to ZIKV illness were reported.
ZIKV were reported. Based on the results of a community serosurvey, we estimated that 73% (95% CI 69-77%) of Yap residents aged ≥3 years had recent ZIKV infection. *Aedes hensilli* was the predominant mosquito species identified at surveyed households and was the most likely transmission vector.

To our knowledge, this is the first documented outbreak caused by ZIKV and the first report of ZIKV transmission outside of Africa or Asia. ZIKV illness was generally mild, and occurred on all areas of the island. ZIKV should be considered a new human pathogen in the Pacific region with potential for further spread to areas outside Asia and Africa.

Background: Health care workers (HCW) are optimally positioned for early detection of signals of unusual health events or public health risks. Early warning aids mounting of a timely and adapted response, facilitating containment at the source and prevention of detrimental health consequences for the patients, community and HCW per se. The current legally binding International Health Regulations (IHR) entered into force in June 2003 and oblige States Parties to maintain core capacity requirements for containment at the source and prevention of detrimental health consequences for the patients, community and HCW per se. The current legally binding International Health Regulations (IHR) entered into force in June 2003 and oblige States Parties to maintain core capacity requirements for containment at the source and prevention of detrimental health consequences for the patients, community and HCW per se. The current legally binding International Health Regulations (IHR) entered into force in June 2003 and oblige States Parties to maintain core capacity requirements for containment at the source and prevention of detrimental health consequences for the patients, community and HCW per se. The current legally binding International Health Regulations (IHR) entered into force in June 2003 and oblige States Parties to maintain core capacity requirements for containment at the source and prevention of detrimental health consequences for the patients, community and HCW per se.

**Conclusion:** In a context of limited resources, institutions are facing similar difficulties: multiple information sources, of a variable quality, scarcity of reliable sources and increasing volume of information to process. To meet the challenges international collaboration must be strengthened. EpiSouth, by disseminating validated information on common health threats will eventually contribute to minimize duplication. E.I. is only one of the components of the WP and from 2009 a secure early warning platform will allow EpiSouth countries to share national alerts, hence boosting synergies on expertise available in the region.

Within two years, 26 Mediterranean countries have joined the initiative. This rate of adhesion underlines the need for enhanced sharing of public health information in the area. EpiSouth can meet this challenge and will become an essential information source to strengthen health security in the region.
ABSTRACTS

International Meeting on Emerging Diseases and Surveillance 2009

4.004 A European Syndromic Surveillance Approach Based on Routine Emergency Care Data—First Results from the SIDARTHA Project

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The European Commission co-funded project SIDARTHA (Grant Agreement No. HT 2007208) aims at improving timeliness and cost-effectiveness of European and national health threat detection by providing for the first time a basis for systematic syndromic surveillance in Europe. The project group conceptualises, develops, implements/tests and evaluates a real-time web-Geographic Information System-based syndromic surveillance system that automatically monitors routinely collected emergency department and ambulance service data. During the conceptualisation phase, international state-of-the-art and the European possibilities and needs are analysed. On this basis the surveillance system is implemented during the second phase. The paper presents the project’s approach and initial results.

International policies and good practice in health threat detection and European (national and regional) policies and practice of health surveillance were assessed during the first work package using internet search, literature review and a standardised online survey among the project group members from 12 different European countries. During the first two workshops, the interdisciplinary expert group identified a set of threats and syndromes that can be detected using emergency data. During the second work package the real possibilities of data provision by the participating emergency care providers to generate the data for these syndromes were assessed.

Suitable links for the intended SIDARTHA surveillance system to existing systems at regional and national level were identified. A set of syndromes that can be generated using emergency data was identified and justified by cross-checking with real emergency data sets. Detailed rationales consisting of definitions of terms, an explanation on the variables and the coding principles, a case definition for each syndrome, and inclusion/exclusion criteria have been defined.

The consortium analysed the possibilities of emergency data to detect health threats in Europe. In the next step, the public health authority needs at local/regional, national and European level will be investigated using a Delphi-type study.

4.005 EMPRES-i, An Integrated Tool for Early Warning and Disease Control


Early warning of potential disease outbreaks and forecasting the spread of pathogens into new areas is essential to effectively contain and control transboundary animal diseases (TADs), including emergent zoonoses. To support early warning and early reaction, FAO’s Emergency Prevention System (EMPRES) has developed a web-based Global Animal Health Information System, EMPRES-i, that provides a platform for storing, analysing and sharing timely and reliable information with FAO animal health officers, collaborating institutions and the public (through the public website).

EMPRES aims to verify disease events received from a wide range of sources (FAO in-country representations, cooperating institutions, UN parties, national ministries, the media and web-based health surveillance systems). EMPRES is in a privileged position to verify information through FAO’s worldwide network of field officers and personal contacts in other institutions. This information is fed into the EMPRES-i secure Internet-based database and presented in an up-to-date, flexible and user-friendly interface, providing the perfect mechanism for increasing awareness on TADs and zoonoses at the national, regional and global levels. The information is also used to generate and disseminate periodic disease-specific status reports and early warning messages on disease threats.

The public EMPRES-i interface was conceived in response to the growing demand of users for an interactive and flexible animal health information system. Information can be easily searched, analysed and exported according to the user’s granted level of access and privileges. EMPRES-i incorporates graphing and mapping tools that can represent outbreaks/cases in chart form (by time or location), or as maps that can be custom-designed with a wide variety of optional layers on livestock densities, biophysical layers, socioeconomic动物health status or trade. EMPRES-i also provides access to the latest publications, manuals and other resources, and to a directory of national veterinary services and FAO reference centres.

The system is under continuous improvement and new features, such as such as modules on disease surveillance, FAO response, and integration of genetic information, will be added in the future. Currently, EMPRES-i contains over 17,000 records and is in the process of integrating other existing databases (e.g. on wild life). Collaboration with partners is allowing the analysis of this vast amount of data.

4.006 Democratizing Information Technology for Biosurveillance and Outbreak Investigation

K.D. Mandl, B.Y. Reis, J.S. Brownstein, C. Gilbert, L. Hadden, C. Kirby, B. Addia. Children’s Hospital Informatics Program at the Harvard-MIT Division of Health Sciences and Technology, Children’s Hospital Boston, Boston, MA, USA

Background: Participation in electronic biosurveillance and outbreak investigation requires computational, database, and networking expertise that often exceeds the capacity of many if not most local health departments in the developed world. Despite the intent of the International Health Regulations, the developing world may continue to largely exclude from participation in modern public health practice. We sought to explore the feasibility and challenges of providing, on a large scale, resources to global public health, leveraging emerging open source technology, systems of scalable web services (cloud computing), and standardized application engines.

Methods: We defined desiderata and use cases for low cost, ubiquitous biosurveillance systems and tested three models of the AEGIS surveillance system: (1) A centralized, traditional application service; (2) An open source software release; (3) Freely available Web 2.0 applications built on the Google Application Engine, with additional computational resources offered on the Amazon ‘Cloud.’
Results: Core desiderata included: (1) Ready use of resources by health authorities with Internet access, but no other required information technology resources; (2) A platform that enables rapid evolution of a public health toolkit by supporting distributed development and rapid deployment of new applications by researchers, public health authorities, and industry; (3) Sufficient assurances for individual privacy and information security for public health authorities to be willing, and legally empowered, to participate. The results of our experience with the three models of the AEGIS surveillance system are shown in the table. The Google Application Engine version of AEGIS is shown in the figure.

Conclusion: Only the freely available Web 2.0 model system fulfilled the first two core desiderata. Whether the approach becomes feasible depends on the evolving cost models of the service providers, and whether the privacy issues can be adequately addressed to enable public health data exchange in support of global health and security.

<table>
<thead>
<tr>
<th>AEGIS User Group</th>
<th>Description</th>
<th>Target Users</th>
<th>Advantages</th>
<th>Challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEGIS Open Source</td>
<td>Client application (constant evolution since 1995)</td>
<td>High tech public health departments</td>
<td>Powerful, integrated</td>
<td>Requires ad hoc, e.g., network administrator</td>
</tr>
<tr>
<td>AEGIS service</td>
<td>Centralized hosted application service</td>
<td>Medium tech public health agencies</td>
<td>Requires ad hoc, integrated</td>
<td>Difficult to integrate, requires separate systems</td>
</tr>
<tr>
<td>AEGIS on Google App Engine (Web 2.0)</td>
<td>Web-based application, universally available</td>
<td>Low tech public health agencies</td>
<td>Requires web browser, distributed</td>
<td>Requires web browser, distributed</td>
</tr>
</tbody>
</table>

Table 1: Experience with three models of surveillance.

Figure 1: AEGIS on Google Application Engine (Flu Surveillance).

4.007 Monitoring International Outbreaks with an Outbreak Surveillance Database

N. Bryant1, J. Lawrence2, C. Wong1, A. Jordan1, H. Simons1, J. Jones2, D.R. Hill1. National Travel Health Network and Centre, London, United Kingdom, Health Protection Agency, London, United Kingdom

Background: The National Travel Health Network and Centre (NaTHNaC) maintains a database of international disease outbreaks that have the potential to affect UK travellers. The Outbreak Surveillance Database (OSD) was created in 2004, and made available on NaTHNaC’s website (www.nathnac.org) in 2007. The OSD provides NaTHNaC staff, stakeholders and the public with a comprehensive report of international outbreaks. Multiple reports of the same outbreak are linked allowing the progression of individual outbreaks to be analysed.

Method: Each working day, resources including country authorities, the World Health Organization, ProMED-mail and media reports are reviewed. Outbreaks meeting set criteria are entered into the OSD. For this analysis, data were extracted into Access and analysed using Excel, STATA and ArcGIS.

Results: From April 2004 to October 2008, 6,699 records were entered into the OSD, organised into 3,592 outbreaks. Outbreaks were reported in 197 countries. The most frequently reported outbreak diseases were H5N1 in birds (13%), cholera (12%), dengue (11%), H5N1 in humans (8%), and chikungunya (3%). 16% of outbreaks used information from both official and informal sources, while 57% used only informal sources (e.g. media reports on ProMed) and 27% only official sources. During 2007, 94 outbreaks of cholera were reported in 33 countries. 60% of cholera outbreaks and 79% of cholera cases recorded in 2007 occurred in Africa. The largest outbreak recorded was in Angola (23,477 cases).

Conclusion: The OSD allows NaTHNaC to identify important disease outbreaks, investigate changing patterns of disease, and identify emerging global threats. The content and format is globally unique. Using information from the OSD, NaTHNaC can alert health professionals and travellers via website postings and recommend appropriate risk management. As NaTHNaC’s website is open access, the OSD can also be used by individuals and health bodies throughout the world to better understand the pattern of infectious diseases.

4.008 The Role of Multinational Corporations in Surveillance and Control of Infectious Diseases

C.E. Johnson1, R.V. Lee1. ExxonMobil Corporation, Fairfax, VA, USA, 1State University of New York at Buffalo, Buffalo, NY, USA

Multinational corporations that do business in agriculture, transportation, manufacturing, and resource extraction construct and maintain facilities in virtually all of the environmental and ecologic regions of the globe. Because such operations alter the ecology and environment at the sites of construction, operations, and transportation, there is high probability of transforming pathogen traffic among arthropods, terrestrial and marine wildlife, and human beings. Before multinational development projects begin preliminary environmental impact assessments (EIAs) can identify needed surveillance for risks of emerging and established infections by identifying reservoir and vector species present in the development site and the surrounding region. For recent EIAs in Africa by our companies special emphasis has been given to the following: 1) Rodents: reservoir species for Lassa and hantaviruses; 2) Bats: reservoir species for lyssa and Nipah viruses, and potential reservoirs for filoviruses, coronaviruses, and influenza viruses; 3) Primates: potential sources of Ebola virus; 4) Mosquitoes: vectors for dengue, Chikungunya, yellow fever viruses, filaria, and malaria parasites; 5) Aquatic species: snail hosts for schistosomiasis.

Preparation for a bauxite mining project in West Africa included field identification of critical habitat locations for chimpanzees and other primates. The evaluation was directed by concern for protection of endangered species and for the human population that would congregate about the mine and processing operations. The assessment included potential for bat, porcine, and human pathogens to enter and circulate through bushmeat hunting and arthropod vectors. For a South American pipeline and road project we recommended the retention of canopy bridges to allow canopy creatures to cross without having to come down to the ground. The design avoided contact between canopy arthropods and yellow fever hosts and mosquitoes and humans on the ground in yellow fever endemic regions.

We believe that developing collaborative affiliations among multinational industries and the research, epidemiologic, and clinical branches of academic and government veterinary and human health services is an important and urgent need.
Short Message Service Gateway as Tracking and Reporting Communication Tool During Active Highly Pathogenic Avian Influenza Surveillance in Bangladesh

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Background: Highly Pathogenic Avian Influenza (HPAI) was first reported in poultry in Bangladesh in March 2007. Since then the Department of Livestock Services responded to 287 outbreaks. To strengthen the national HPAI disease response, the Government of Bangladesh, in close collaboration with the Food and Agriculture Organisation (FAO) of the United Nations, established active surveillance in 150 Upazillas (sub-districts). This paper reports on using Short Message Service (SMS) gateway (=method of sending and receiving SMS messages between computer and mobile phones) as a reporting tool when monitoring disease and death in poultry in Bangladesh.

Methods: The name, location (Upazilla) and mobile phone numbers of the employees conducting the surveys were added to the purposefully developed gateway system. All 450 Community Animal Health Workers (CAHWs) and 150 Upazilla Livestock Veterinarians (ULOs) were trained in using the SMS gateway system in groups of 50 in five hours. The CAHWs recorded animal health data on purposefully designed forms. At the end of the working day the total number of all investigated chickens, ducks and other birds and their health status were coded and sent by the CAHWs by SMS to the gateway system. The ULOs sent a message every time they investigate poultry disease or death. All SMS messages received by the gateway did get an automated reply; one reply if the code was correct, or two messages with the second message requesting the correct code. Individuals were monitored for compliance with the system and if necessary were individually contacted and re-trained.

Results: Daily 1200 SMS coded text messages were received at the Department of Livestock Services. The total number of chickens, ducks and other birds investigated that day in 150 Upazillas and the number of sick and dead birds was recorded. The data was analysed by individual surveyor, per sub-district, district and nationally.

Conclusion: SMS gateway proved to be a cost effective, efficient, real-time reporting method during active HPAI surveillance in Bangladesh. Data generated by the system was of great epidemiological value and allowed accurate disease tracking. With sufficient funding, the system could be enhanced further by reporting after every farm visit instead of daily reports and adding spatial farm location using Global Positioning Systems. Due to its success, the system will also be used in other countries where FAO has established active HPAI surveillance.

Predictive Vector-Borne Disease Surveillance: A Multidisciplinary Approach for the Early Detection and Response to Outbreaks

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The morbidity and mortality associated with vector-borne human and zoonotic diseases is a rapidly increasing threat to global public health. Many factors are associated with this growing risk: vector-habitat expansion due to environmental degradation and climate variability and change; shifting animal and human population dynamics that promote exposure to zoonotic and vector-borne diseases; and, resource-limited public health infrastructures insufficient to establish and sustain routine disease surveillance activities for early detection and potential mitigation of human and animal disease outbreaks.

A multidisciplinary coordination program has been initiated by the U.S. Department of Defense Global Emerging Infections Surveillance and Response System (DOD-GEIS) to link data sets and information from: eco-climatic remote sensing activities; mosquito vector geo-spatial mapping; disease vector and reservoir behavior and habitat characterizations; and, human health electronic disease surveillance data for febrile illnesses. With the specific intent of generating timely outbreak alerts to public health authorities so that they can prepare for and implement necessary outbreak prevention and control measures, the multidisciplinary program is building a model-based public health informatics tool for accurately predicting increased risk for vector-borne disease outbreaks.

This approach has proven viability exemplified by a DOD-GEIS sponsored model implemented for Africa and the Arabian Peninsula. This model successfully predicted outbreaks of Rift Valley Fever in Kenya, Tanzania, Somalia, Sudan, Madagascar and South Africa from 2006 through 2008. These predictions were used by host country ministries of health and agriculture, WHO and FAO to prepare for and mitigate the impact of Rift Valley fever outbreaks. The model is being expanded and modified to include other geographic areas including Asia and North America, and will be applied to other vector-borne diseases such as malaria, dengue and JE.

This existing model is being enhanced by the incorporation of the above-referenced new data sources. Public health authorities are being consulted to determine the best ways to visualize and deliver model results in a web-based, easy, accessible manner. Model usage to date has, and with enhancements will continue to facilitate early awareness of impending outbreaks and allow for timely preparedness and control implementation activities.
Figure 1. Positive NDVI anomalies over central Sudan for August 2007 associated with above normal rainfall. These positive anomalies created ecological conditions that promoted the emergence and propagation of RVF vectors.

However, a new dichotomy may form. This time with 4–5 billion people moving through the health transition towards longer lives with cell phones, washing machines, less infections, more diabetes and more human rights. In contrast to this majority 1–2 billion people are stuck in vicious circles of poverty and infections. This world view will be animated using software from www.gapminder.org.

SESSION 6 (Parallel Session)
Emerging Zoonoses
Saturday, February 14, 2009
Room: Park Congress/Ground Level
14:30–16:00

6.001 Rift Valley Fever
A. Duse, South Africa
NO ABSTRACT RECEIVED

6.002 Recent Developments and Controversies in Rabies
H. Wilde. Chulalongkorn University, Bangkok, Thailand
Rabies remains a significant public health menace in most of south and southeast Asia. The large uncontrolled canine population is virtually the only sustaining cause. Finding ways to reduce this large vector population and vaccinate sustainably the rest must be the target of our efforts. Pre- and post-exposure prophylactic methods have been well established but cost and availability of quality biologicals remain a problem worldwide. The intriguing question of why we have two forms of rabies (furious and paralytic), using modern molecular biology and imaging technology, is now nearing some explanation. Recent transplant transmission cases in Europe and the United States demonstrated that rabies awareness among physicians, even in tertiary care centers, is still unsatisfactory. Better dissemination of clinical and laboratory knowledge for diagnosis are needed. Survival of two, and possibly three, young rabies patients infected by bats, has resulted in considerable media attention and discussions of how to deal with rabies patients, still considered virtually 100% fatal. There is need for humane, low cost and culturally acceptable methods for population control for dogs in developing countries with a canine rabies problem. Governments must be motivated to provide more resources to combat this disease.

6.003 Crimean-Congo Haemorrhagic Fever in Turkey
O. Ergonul. Marmara University School of Medicine, Istanbul, Turkey
CCHF is a fatal viral infection found in parts of Africa, Asia, Southeastern Europe, and the Middle East. The CCHF virus (CCHFV) belongs to the genus Nairovirus in the family Bunyaviridae and causes a severe disease in humans, with a reported mortality rate of 5–40%. The geographic range of CCHFV is the most extensive of the medically significant tick-borne viruses. Humans become infected through the bites of ticks, by contact with a patient with CCHF during the acute phase of infection, or by contact with blood or tissues from viremic livestock. The widespread geographical distribution of CCHFV, its ability to produce severe human disease with high fatality rates, and fears about its intentional use as a bioterrorism agent makes CCHFV a significant human pathogen. The geographic range of CCHFV is known to be the most extensive of the tick-borne viruses important to human health, and the second most widespread of all medically important arboviruses, after dengue viruses. Since 2000, new outbreaks have been reported from Southeastern Europe, from Albania, Yugoslavia, Bulgaria, Turkey, and Greece. In Portugal, Hungary,
and France only serologic evidence of CCHFV infection exist. Since 2002, about 3000 confirmed cases of CCHF were reported in Turkey. The case fatality rate was 5%. The disease was milder among the children. Since 2002, 15 health care workers got the the disease, and 3 of them died. The global warming is one of the leading factors among the biotic changes for such a dramatic increase in the number of the cases in Turkey. Especially, early use of ribavirin could be useful in treatment of CCHF infection. There is no well accepted effective vaccination against CCHFV. The personal protection from the ticks and body fluids of the patients are the methods for minimizing the effect of the infection.

SESSION 7 (Parallel Session)
New Food-Borne Threats
Saturday, February 14, 2009
Room: Klimt Ballroom 2 & 3 / First Level
14:30—16:00

7.001 Orally-acquired Chagas’ Disease. An Emerging Urban Threat in the Americas
J. Torres, Instituto de Medicina Tropical, Universidad Central de Venezuela, Caracas, Venezuela

Chagas’ disease (CD) is an important public health problem in 17 countries in Latin America, where about 9.8 million of people are currently infected and more than 13,000 of them die every year as a consequence of it. The burden of disease caused by CD remains the second highest among the endemic Tropical Diseases in the Americas, representing more than two million DALYs, annually. However, the complexity of this zoonotic disease that affects a large number of mammalian reservoirs and vectors, makes its eradication an impossible task. In addition to vectorial transmission, congenital, transfusion-related, organ transplantation-related and oral transmission, remain as secondary mechanisms of infection. Oral transmission of Chagas’ disease is possible through the fecal vector contamination of food or beverages.

In humans, an increasing number of microepidemics of Chagas’ Disease probably transmitted by the oral route, have been reported from Brazil, Mexico, Argentina and Colombia. Most of them involved a low number of infected persons from rural or semirural communities, who consumed potentially contaminated fresh fruit juices (açai, bacaba and sugar cane), and resulted in a high lethality. However, little is known about the epidemiology, pathogenesis, and clinical course of this unique route of infection.

A large urban outbreak of orally-acquired infection affecting a public school community in Caracas, Venezuela, has recently been described by us. It was unique in affecting a predominantly young, healthy population occurring in an affluent urban area where vectorial transmission does not occur. One hundred three out of 1,000 exposed individuals were infected for an attack rate of 10.3%. Morbidity was high (75% were symptomatic, and 28.34% had clinical and/or EKG findings of acute cardiac involvement); however, lethality was low (0.97%), probably due to the rapid diagnosis and treatment. Circumstantial epidemiological evidence, multivariate logistic regression analysis and genetic characterization of the isolates, indicated a common source of infection and incriminated a contaminated fresh guava juice prepared at a distant location under precarious sanitary conditions.

Food-borne transmission of T. cruzi may occur more often than currently recognized. In endemic countries, CD must be considered in the differential diagnosis of urban outbreaks of prolonged febrile illness associated or not with cardiac involvement.

7.002 Food-Borne Transmission of Nipah Virus in Bangladesh
S.P. Luby1, M.J. Hossain1, E.S. Gurley1, N. Nahar2, B.N. Ahmed2, S.U. Khan1, M.A. Rahman3, N. Homaira1, R. Rota4, P.E. Rollin4, J.A. Comer4, T.G. Ksiazek2, M. Rahman1, 1ICDDR,B, Dhaka, Bangladesh, 2Institute for Epidemiology Disease Control and Research, Dhaka, Bangladesh, 3ICDDR, Institute for Epidemiology Disease Control and Research, Dhaka, Bangladesh, 4Centers for Disease Control and Prevention, Atlanta, GA, USA

Fruit bats are the reservoir host for Nipah virus. We reviewed human infections with Nipah virus in Bangladesh from 2001–2007, studied fruit bat access to date palm sap harvesting using infrared wildlife photography, and conducted in depth anthropological investigations with date palm sap collectors. We identified 23 introductions of Nipah virus into human populations in central and north-western Bangladesh between 2001 and 2007. Ten introductions affected multiple persons (median 10). Illness onset occurred from December through May. We identified 122 cases. Their mean age was 27 years; 87 (71%) died. In an outbreak investigation in Tangail District in 2005 persons with Nipah infection were seven times more likely than controls to report drinking raw date palm sap (OR 7.0, 58 vs. 17% 95% CI 1.6, 3.05). Similarly in a 2008 outbreak investigation Nipah cases were eight times more likely to report drinking raw date palm sap than controls (OR 18, 100 vs. 25% 95% CI 2.2, inf). The highest concentration of date palm sap trees are in the regions where human Nipah investigations have repeatedly been identified. Fresh date palm sap is a common seasonal treat in rural Bangladesh. Date palm sap collectors are low income agricultural workers who supplement their income a median US$ 5.00 per week by collecting date palm sap. Infrared wildlife photography recorded a mean of 4.5 bat visits to the shaved part of a tree during date palm sap collection. The bats stayed a mean of 111 seconds, and 92% licked the sap. Highly pathogenic Nipah virus is occasionally transmitted from bats to people via food-borne transmission. Once people are infected they can pass the virus from person to person. Current prevention efforts are focused on reducing bat access to date palm sap that is destined for direct human consumption.

7.003 Food- and Non-Food-Borne Zoonotic Hepatitis E
C.G. Teo. Division of Viral Hepatitis, Centers for Disease Control and Prevention, Atlanta, GA, USA

Hepatitis E is classically encountered in developing countries as large, water-borne outbreaks of jaundice. These arise from anthropogenic transmission of hepatitis E virus (HEV) along the fecal-oral route. At the turn of the century, small outbreaks and sporadic cases of hepatitis E linked to ingestion of undercooked boar meat, venison and pig liver began to be reported from Japan. They heightened awareness that hepatitis E can be zoonotic, and led to studies revealing the disease to be endemic not only in East Asia but also in Western countries. The food-borne nature of the disease in the West is not as firmly established as in Japan, however. Nonetheless, ingestion of undercooked meat of boars and pigs, and ingestion of their offal have been identified as risk factors. Moreover, a wide variety of animals other than artiodactyls are being implicated as hosts, and therefore reservoirs of HEV. While such broad enzooticity of HEV requires further confirmation, it does raise the possibility that zoonotic HEV transmission can occur along non-food-borne routes, e.g., following occupational, recreational and environmental exposure to animals and their waste products. Definitive data have not emerged to determine the extent by which such exposures contribute to transmission. Furthermore, factors that lead to the manifestation of illness following HEV infection have yet to be identified. Resolution of the uncertainties surrounding transmission and pathogenesis should permit more precise targeting of measures to control and prevent both infection and disease.
SESSION 8 (Parallel Session)
Antibiotic Resistance: The Future is Now
Saturday, February 14, 2009
Room: Park Congress/Ground Level
16:30–18:00

8.001 Antibiotics as a Limited Natural Resources
R. Laxminarayan. Resources for the Future and Princeton University, Washington, DC, USA

Modern medicine depends on effective antibiotics to control bacterial infections. Antibiotic effectiveness can be thought of as a natural resource, much like fish, oil or forests; it is a resource that is accessible to anyone who can purchase it. All antibiotic use, appropriate or not, "uses up" some of the effectiveness of that antibiotic, diminishing our ability to use it in the future. The lecture will discuss incentive-altering strategies to use antibiotics sustainably, both today and in the future.

8.002 Balancing Human and Animal Health
Fedorka-Cray P. USDA-ARS Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Athens, GA, USA

Bacteria and antibiotics have likely co-existed since the beginning of time; one seeks only to survive (bacteria) while the other (antibiotics) serves multiple functions. The discovery of antimicrobials began a "golden age" in medicine as previously untreatable diseases were cured. Animals benefited shortly thereafter as veterinarians began routine use of these compounds. Unfortunately, bacteria develop mechanisms of resistance almost as fast as new drugs come to market. Today, the luster of the "golden age" has dimmed as both medical and veterinary communities scramble to arrest the ever increasing problem of antimicrobial resistance.

Initially, the numbers and types of antimicrobials increased exponentially. However, for the past two decades the introduction of new, and the efficacy of existing, antimicrobials has dramatically declined. Globally, health officials are continuing to develop policy to arrest the spread of antimicrobial resistance while increasing the call for production of newer and better treatments.

Because resistance is now emerging faster than the time it takes to bring new compounds to market, human and veterinary officials are at odds as both sides balance their needs to use antimicrobials with the global need to slow/prevent emerging resistance. Compounding the issue is the need to regulate use in both developed and underdeveloped countries. Can we achieve balance and restore the "lost luster" that once looked so promising? The answer lies in understanding the consequences associated with limiting use of antimicrobials in human and veterinary medicine.

8.003 A Worst Case Scenario: XDR TB
E. Gotuzzo. Lima, Peru

NO ABSTRACT RECEIVED

SESSION 9 (Parallel Session)
Oral Presentations:
Hot Topics in Emerging Diseases
Saturday, February 14, 2009
Room: Klimt Ballroom 2 & 3 / First Level
16:30–18:00

9.001 Rapid, Simultaneous Detection of Multiple Animal Viruses by Bead-Based Multiplexed RT-PCR
P. Naraghi-Arani, R.J. Lenhoff, J.B. Thissen, A.C. Carillo, J.A. Olivas, S.M. Michele. Lawrence Livermore National Laboratory, Livermore, CA, USA

Background: The rapid and reliable detection of emerging zoonoses is critical to effective management of these diseases. We have developed four multiplexed RT-PCR assay panels that can rapidly detect high priority agricultural pathogens, including Foot and Mouth Disease Virus and Avian Influenza in environmental and clinical samples. The use of bead-based liquid arrays has proven to be a well adapted and versatile technology that can be custom tailored to rapidly screen for both DNA and RNA in a single tube. Previously we had, in collaboration with the CDC, developed another panel for human biological threats. Our assays have been tested by researchers in multiple public health and USDA laboratories and proven robust.

Methods: Multiplexed PCR assays are developed at LLNL through a proven process that includes sequencing and bioinformatics leading to TaqMan and multiplexed PCR assays. We have utilized the Luminex X-Map system for development of all panels. The process involves an end-point RT-PCR followed by hybridization to a mixture of probe-conjugated polystyrene beads that can be readily classified by flow-cytometry. Up to a maximum of 100 unique bead classes, and thus RT-PCR products can be identified using this system.

Figure 1. Multiplex Assay Process

Results: With the USDA and multiple university partners, multiplexed RT-PCR assays both sensitive and specific for multiple biothreat agents have been developed. We have demonstrated that these assays are as selective as TaqMan assays with sensitivities ranging from 10–10000 genomes. These assays have been tested for proficiency in the field and proven of value.

Conclusions: Multiplexed assays provide many advantages over other methods. In the event of a public health emergency, these assays can provide rapid, sensitive, specific and cost effective means of screening many samples. Additionally, the rapid detection of agricultural pathogens is of great importance as most emerging infectious diseases are zoonoses.

9.002 Universal Virus Detection in Clinical Samples and Cell Culture—New Diagnostic Potential
C. Uhlenhaut, S. McLennahan, A.M. Sierra-Honigmann, P.R. Krause. FDA/CBER, Bethesda, MD, USA

Background: Rapid, reliable, and unambiguous identification of viruses is critical not only for solving clinical problems (including those related to bioterrorism and emerging infections), but also to assure safety of regu-
lated products. Our improved degenerate oligonucleotide primer PCR (DOP-PCR) does not require prior sequence information of the virus present in a given sample and allows the generic detection and identification of viruses using a single assay. It combines the open view of other non-specific virus detection tools with a detection limit close to that of specific NATs.

Methods: This universal method is based on enzymatic purification and physical separation of host nucleic acids away from virus capsids. Nucleic acids are extracted from virus capsids, and RNA is transcribed into cDNA. Both DNA and cDNA are used as template in a highly degenerate PCR optimized for the detection of virus sized genomes. The PCR products are analyzed either by conventional cloning and sequencing or by high throughput sequencing.

Results: DNA and RNA viruses from a wide range of virus families with diverse genomes and structural properties were detected by our DOP-PCR. In cell culture, SV40, VZV, AA2V, EBV, HSV-1, HSV-2, PPV, bovine parvovirus, polio virus, feline calicivirus, HTLV-I, HTLV-II, squirrel monkey retrovirus, BVDV, and other viruses were detected. In clinical samples and tissues we identified HAV, human calicivirus, human metapneumovirus, and human coronavirus HKU1.

Conclusion: The DOP-PCR assay can be used to identify viruses directly from biological specimens and cell culture. This novel technique requires no prior sequence information to amplify viral genomes, but is nonetheless able to amplify at least a portion of each viral genome. It can detect viruses in a variety of samples using only one aliquot of each sample per assay. These features render it ideal for virus discovery and detection in samples where other methods have failed. The ability of the DOP assay to detect all viruses, human and zoonotic, allows the detection of emerging viruses and suggests that this (or a similar) approach may find utility in identifying contaminants (or providing viral safety assurance) in non-clinical samples, potentially including cell substrates and regulated biological products. Also, DOP-PCR can facilitate the timely identification of viral agents in immuno-compromised patients, who often suffer from unusual infections.

9.003 Donors of the First Highly Pathogenic Avian Influenza H5N1 Virus in Asia

M.M. Mukhtar1, S.T. Rasool2, D. Song3, C. Zhu2, Q. Hao2, Y. Zhu2, J. Wu2. 1College of Life Sciences, Wuhan University, Wuhan, China, Present Address: Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan, 2College of Life Sciences, Wuhan University, Wuhan, China, 3College of Life Sciences, Guangxi Normal University, Guilin, China

Background: Highly pathogenic avian influenza (HPAI) viruses of H5N1 subtype were first isolated in United Kingdom (A/Chicken/Scotland/59) and have since been isolated from poultry for more than four decades. In Asia, HPAI H5N1 viruses have been circulating since the discovery of A/Goose/Guangdong/1/1996 (Gs/Gd/1/96) virus in 1996. Gs/Gd/1/96 virus is related to high morbidity and mortality in geese in Guangdong province of China. Genetic characterization of the gene segments of viruses circulating in southern China indicated that the H5N1 viruses were generated by reassortment. We conducted this study to determine the origin of first HPAI H51N1 in China and Asia.

Methods: To find the progenitor of HPAI Gs/Gd/1/96 virus in China, we analyzed the influenza virus sequences available in influenza genome databases. Published nucleotide and amino acid sequences of influenza viruses, used for sequence and phylogenetic comparison in this study, were obtained from GenBank (NCBI; http://www.ncbi.nlm.nih.gov/genomes/FLU/), the Influenza Sequence Database (ISD; http://www.flu.lanl.gov) and the Influenza Virus DataBase (IVDB; http://influenza.genomics.org.cn). We also took into account the genetic characteristics of donor and recipient viruses.

Results: Phylogenetic analyses of all eight genes of two Nanchang avian influenza viruses, A/Duck/Nanchang/1681/92 (H3N8-1681) and A/Duck/Nanchang/1904/92 (H7N1-1904), isolated from Jiangxi province, China, in 1992, showed that six internal genes of H3N8-1981 virus and five internal (except NS gene) genes of H7N1-1904 virus were similar to Gs/Gd/1/96 virus. The neuraminidase (NA) gene of Gs/Gd/1/96 had the highest genetic similarity with A/Duck/Hokkaido/55/96 (H1N1-55) virus. The haemagglutinin (HA) gene of Gs/Gd/1/96 virus might have originated as a result of mutation of H5 HA gene from A/Swan/Hokkaido/51/96 (H5N3-51)-like viruses. The PA gene of H5N3-51 virus had the highest similarity with Gs/Gd/1/96 virus. Analysis of amino acid residues that correlate with viral pathogenicity also showed that Gs/Gd/1/96 and Nanchang viruses have high similarity for internal genes.

Conclusion: This study explains the origin of first Asian HPAI H5N1 virus in Guangdong by the reassortment of Nanchang (close to Guangdong) and Hokkaido (Japan) (H1N1-55 and H5N3-51) viruses.

Figure 1. Phylogenetic tree is based on the nucleotide sequences of influenza A virus PB2 genes. Analysis was based on nucleotides 34-2275 (2,242bp) of the PB2. Numbers at the nodes indicate confidence levels of bootstrap analysis as a value of percent. Donor and recipient viruses of this study are indicated by (a filled triangle).

Figure 2. Phylogenetic trees are based on the nucleotide sequences of influenza A virus NA(a) and HA(b) genes.

IMED 2009
9.004 PB2 and Temperature Sensitivity of Avian, Swine, and Human Influenza A Viruses in a Novel Porcine in vitro Model

S. Kasloff1, H. Weingart2. 1University of Manitoba, Winnipeg, Canada, 2National Centre for Foreign Animal Disease, Canadian Food Inspection Agency, Winnipeg, Canada

Background: The ability of influenza A viruses to successfully cross the species barrier depends on numerous factors, including the ability to replicate at the temperature of the new host. Function of the viral polymerase complex at varied temperatures is attributed to the PB2 gene and residue 627 is considered a major determinant, though 16 other residues are associated with avian or mammalian adaptation. This project served to evaluate replication of viruses from varied host backgrounds in a novel porcine in vitro model to further investigate the notion of temperature sensitivity through phenotypic and genotypic comparisons.


Results: All avian viruses, including one with a human-characteristic residue 627, reached high titers at 37˚C compared to 41˚C, but low titers at 33˚C. Three human viruses reached similar titers at 37˚C and 33˚C; two of which contained only human-associated PB2 residues and one that contained six avian-associated residues. The 1918 human virus also contained six avian-associated residues but displayed an avian-like temperature preference, preferring 41˚C over 33˚C.

Conclusions: The ability of influenza viruses to replicate at different temperatures in the swine in vitro system is not entirely predictable based on virus natural host species, and comparisons of residues associated with human or avian host tropism did not always correlate with virus phenotypes. Differences between these results and published findings indicate that temperature-dependent replication is system specific, and a human system is required in order to speculate the ability of viruses to adapt to the human host.

9.005 Rapid Identification and Laboratory-Guided Response to an Outbreak of Ebola Hemorrhagic Fever Caused by a New Species, Uganda—August-December, 2007

A. MacNeil1, J. Wamala2, E. Farnon3, T. Ksiazek4, S. Okware5, R. Downing6, J. Towne7, P. Rollin8, the Ebola Outbreak Response Team9, 1The Centers for Disease Control and Prevention, Atlanta, GA, USA, 2Ministry of Health, Kampala Uganda, 3CDC-Uganda, Entebbe, Uganda, 4International collaboration, Bundibugyo, Uganda

This late breaker abstract will be included in the program supplement.

9.006 Participatory Impact Assessment of Competing Intervention Strategies Against the Highly Pathogenic Avian Influenza in Indonesia

B. Bett1, M. McLaws1, F. Unger1, E. Sawitri2, J. Yani3, C. Jost4, J. Mariner5, 1ILRI, Nairobi, Kenya, 2ILRI, Jakarta, Indonesia, 3ILRI, Bangkok, Thailand, 4CMU, MOA., Jakarta, Indonesia, 5Ontario Public Health Laboratories Branch, Toronto, Canada

The highly pathogenic avian influenza (HPAI) is currently endemic in Indonesia. A participatory disease surveillance and response (PDSR) program has been implemented to improve detection and control of the disease. The program, however, is being compromised by lack of reliable intervention strategies and favourable institutional environment for implementation of the existing interventions. We describe an on-going operational research with backyard and small intensive poultry farms to assess the effectiveness of preventive vaccination against AI alone and both AI and Newcastle disease (ND) using Legok 2003 H5N1 and HB1 vaccines. The study will run between April 08 and July 09.

A total of 16 districts in West Java, Central Java, and Yogyakarta provinces were purposefully selected. Within each district, three treatment blocks were randomly selected and assigned treatments (control, AI vaccination and AI and ND vaccination). Participatory epidemiological techniques are being used to collect information on the incidence of clinical HPAI outbreaks and coverage of the vaccination programmes. Clinical HPAI outbreaks are defined as cases with high mortality rates of at least 80%; peracute death or death within 4 hours from the recognition of symptoms, or birds being found dead with no premonitory signs; and infectious pattern involving more than one household over time.

On average, 74.5% of the households in the study areas keep poultry. The poultry species commonly raised include kampung chickens, Muscovy ducks, ducks, pigeons and swans/geese. A total of 151 sudden death events comprising 106 clinical HPAI outbreaks were observed between April and June. Only five of these cases were recorded in the PDSR database. The clinical signs associated with clinical HPAI include sudden death with no symptoms, blue head/comb, nasal discharge, full blue or black body and flock mortality of greater than 80%. The mortality rate caused by the clinical HPAI in kampung chickens was 49.5%. Of the kampung chickens that remained healthy, 34.2% were sold off in the course of these outbreaks.

Most farmers sell chickens in the face of an outbreak. This practice contributes immensely to the spread of the disease. There is a need therefore to develop behavior change interventions that aim to break incentives that promote this trade e.g. by using culling and compensation approaches. This study would provide data for the assessment of preventive mass vaccination programs in Indonesia.

9.007 Seasonal Patterns and Environmental Predictors of Invasive Meningococcal Disease in London, England; Philadelphia, United States; Sydney, Australia; and Toronto, Canada

L.M. Kinlin1, V. Ng1, N.S. Crowcroft2, J. Grannerod3, G. Fraser3, C.V. Spain4, C.C. Johnson5, F. Jamieson6, E.M. Brown7, D.N. Fisman8. 1Research Institute of the Hospital for Sick Children, Toronto, Canada, 2Ontario Agency for Health Protection and Promotion, Toronto, Canada, 3Health Protection Agency, London, United Kingdom, 4Philadelphia Department of Public Health, Philadelphia, PA, USA, 5Ontario Public Health Laboratories Branch, Toronto, Canada

Background: Seasonal variation in the incidence of invasive meningococcal disease (IMD) is well-described; however, mechanisms underlying this seasonality have been poorly understood. With recent concerns related to climate change, identifying environmental predictors of disease occurrence has taken on new urgency. We sought to evaluate the effect of environmental conditions on IMD incidence in four urban areas: London, England; Philadelphia, United States; Sydney, Australia; and Toronto, Canada.


Results: The seasonal distribution of IMD cases was similar in all cities ($\chi^2=1.1.0.3(P=0.27$), with peak incidence in the winter and spring. Periodograms suggested annual periodicity of disease occurrence. Poisson regression models confirmed the oscillatory nature of meningococcal infections (P for seasonality $<0.001$ for London, Philadelphia and Sydney; $P=0.10$ for Toronto). Analysis of monthly IMD incidence in London revealed an association with both carbon monoxide (CM) and sulphur dioxide (SD) levels (IRR per mgm$^{-3}$ increase in CM 1.41 [95% CI 1.05–1.90]; IRR per mgm$^{-3}$ increase in SD 1.03 [1.01–1.06]). In Philadelphia, incidence humidity was associated with elevated risk (IRR per 1% increase 1.04 [1.004–1.08]); case-crossover methods identified an inverse
relationship between ultraviolet index 1–4 days prior to onset and risk (OR 0.55 [0.38–0.84]). Monthly incidence in Sydney was associated with humidity and wind speed (IRR per 1% increase in humidity 0.99 [0.98–0.99]; IRR per 1 km/h increase in wind speed 1.37 [1.21–1.55]). No environmental predictors were identified using Toronto data.

Conclusion: Despite synchronous patterns of case occurrence, environmental predictors of IMD are not consistent across regions. This finding—while underscoring the role of environment in communicable disease dynamics—supports the theory that seasonal fluctuations in infection may be caused by small exogenous changes interacting with population dynamics. Effects of climate change are likely to be region-specific and difficult to predict.

**9.008 Possible New Hepatitis B Virus Genotype, Southeast Asia**

C.M. Olinger1, P. Jutavijitum1, J.M. Hübschen1, A. Yousukh2, B. Samountry3, T. Thammavong4, K. Toriyama5, I.E. Andernach6, C.P. Muller1. 1Institute of Immunology, Laboratoire National de Santé/CRP-Santé, Luxembourg, 2Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, 3Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Luxembourg, 4Department of Medicine, Faculty of Medical Sciences, National University of Lao, Vientiane, Lao People’s Democratic Republic, 5National Blood Transfusion Centre, Lao Red Sciences, National University of Lao, Vientiane, Lao People’s City and Central provinces of Lao PDR were carried out.

Methods: Phylogenetic analysis of sequences obtained from 453 HBsAg-positive first-time blood donors from donation centres in Vientiane City and Central provinces of Lao PDR had never been done.

Results: Excluding the potentially mixed infected and recombinant sequences, 163 strains (42.2%) belonged to genotype B and 204 (52.8%) to genotype C. 19 strains (4.9%), including 15 complete genome sequences, did not group with any of the known genotypes A to H. These sequences formed 2 clusters emerging from a common node. One of the clusters grouped with a recently reported single strain from Vietnam for which the authors defined a new genotype I and aberrant strains from Hanoi, Vietnam, reported eight years ago and is referred to as subgenotype II. The second cluster (I2) contains exclusively strains from Lao PDR. Full length genotype I strains were most closely related to the genotype C group of strains (average Kimura distance of 7.89%), while on the S gene level, genotype I was most closely related to genotype G with a distance of 4.23%. A complex recombination pattern involving genotypes C, A and G suggests that genotype I was formed by recombinations outside of Southeast Asia before spreading within Vietnam and Laos, where it later recombined with regional strains.

Conclusion: The new clusters of sequences formally comply with the definition of a new genotype ("I") and 2 subgenotypes of it. This emerging new genotype developed probably outside of Southeast Asia and is now found also in mixed infections and in recombinations with local strains in a geographically confined region.

**SESSION 10 (Poster Presentations I) Antibiotic Resistance: The Future is Now**

Saturday, February 14, 2009

10.001 – 10.026 Room: Klimt Ballroom I – First Level

10.001 Prevalence of *Yersinia enterocolitica* in Irish Slaughter Pigs and Their Environment

B.P. Murphy1, N. Drummond1, T. Ringwood1, P. Whyte1, J.F. Buckley2, M.B. Prentice1, S. Fanning1. 1Cork County Council, Cork, Ireland, 2University College, Dublin, Ireland, 3National University of Ireland, Cork, Ireland

*Yersinia enterocolitica* produce enterocolitis accompanied by diarrhea, low grade fever and abdominal pain, following consumption of contaminated food or by direct inoculation through blood transfusion. *Yersinia* infections mimic appendicitis when pain manifests in the lower right abdomen resulting in potentially unnecessary appendectomies. The first comprehensive surveillance study on the prevalence of *Y. enterocolitica* in Irish slaughter pigs is complete. Four pig slaughterhouses were visited five times over a two-year period. Duplicate porcine tonsil tissue, neck muscle, nasal, head and carcass swabs from 10 pig carcasses and ten environmental samples from each slaughterhouse were examined microbiologically using a rapid direct plating method and the traditional cold enrichment method. Molecular screen for virulence determinants was performed on recovered isolates using a triplex PCR, targeting genus-specific 16SrRNA and species-specific chromosomal *all* genes and virulence plasmid encoded *pYad*. *Y. enterocolitica* was recovered from 279/1902 samples (15%). The majority are *bio*/*serotype* 3:09; with 4:03 and 1A also identified. Antibiotic resistance profiles show 100% of the collection are resistant to Cephalotin (Kf) an antibiotic used in both human and veterinary medicine. In addition multi-drug resistance was observed in some of the recovered isolates. Comparative studies of pork products and human clinical isolates recovered from diarrhoeal patients in Ireland were simultaneously undertaken by the group. Multi-locus variable number tandem repeat analysis (MLVA) will be applied to the recovered collection. Whole genome comparisons will be carried out on 50 selected *Y. enterocolitica* isolates based on their profiles, using a *Yersinia enterocolitica* 8081 genomic array containing 4208 chromosomal and 83 plasmid predicted protein-coding sequences. The role of disease surveillance is to provide current information on emerging diseases and to heighten awareness of threats and trends in the “farm-to-fork” continuum, so that prevention and control measures may be put in place to protect public health.

10.002 *Yersinia enterocolitica* in Retail Markets in Ireland: How Prevalent is it in Raw Pork?

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*Yersinia enterocolitica* is the causative agent of yersiniosis in warm blooded animals, the predominant symptoms include; diarrhoea, fever, abdominal pain and vomiting. The disease can range in severity from self-limiting gastroenteritis to potentially fatal septicaemia. Pseudoappendicular syndrome has been reported resulting in unnecessary appendectomies. *Y. enterocolitica* is considered to be a food-borne pathogen as it has the ability to multiply in foods at low temperatures, below 4˚C. Two hundred retail outlets in Ireland were surveyed over a two-year period. Pork chops, pork mince and sausage meat were analysed using a traditional cold enrichment method (phosphate buffered saline at 4˚C) and an overnight enrichment method (Oxsmen broth at 30˚C in year 1; direct plating at 28˚C in year 2). Phenotypic and genotypic characterisation was undertaken i.e. bio- and serotyping, antimicrobial resistance profiling using a panel of 12 antibiotics used in both human and veterinary medicine. Molecular typing applying a multiplex PCR to simultaneously amplify the *Yersinia*-specific 16S rRNA gene along with the virulence factors *all* and *pYad* genes. A relatively new method known as multi-locus variable number tandem repeat analysis (MLVA) is being developed to sub-type the collection. To date 35/185 (19%) *Y. enterocolitica* were recovered from the retail samples.
All were biotype 1A, and 16S rRNA positive. Forty four percent showed resistance to ampicillin and cephalotin (AmpKl), 29% to Kf and 3% showed additional resistance to amoxicillin (Amc). Biotype 1A is described as avirulent (non-pathogenic) however; there is a significant body of scientific evidence to indicate that, at least, some strains of biotype 1A can cause gastrointestinal disease in humans. This has been corroborated during a collaborative study, by University College Cork, of isolates recovered from humans in Irish hospitals. The presence of this pathogen is a significant concern from a food safety and public health perspective.

10.003 Occurrence Escherichia coli in Wild Guinea Pigs Fecal Pellets in Iran Karkas Mountain

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Background: Guinea pigs are rodents belonging to the family Caviidae and the genus Cavia. Guinea pigs are herbivores (strict vegetarians) and have a similar digestive tract to horses, rabbits and chinchillas. Intestinal flora in their digestive tract is specialized in order to help breakdown and digest plant materials. Knowledge of the intestinal flora of this animal is important both from a public health standpoint and for use guinea pigs as a model of infectious disease. In this survey Enterobacteriaceae bacteria role in digestive tract of guinea pigs has been identified in wild guinea pigs fecal pellets that they live around Karkas Mountain camping.

Methods: Two small groups of wild guinea pigs selected around Karks climbers camping. One groups live above and another group live below of Karks climbers Camp. 80 Guinea pigs fecal pellets were collected twice in a week. Then each one of samples emulsified with 10 ml of T.S.B medium and for recovery of enteric bacteria 0.1 ml spreading in differential and selective media and identified by IMViC tests.

Results: 1) The group lives above of Karks climbers Camp E.coli were not isolated from their fecal pellets. 2) There were not found Salmonella SPP from two groups that were investigated. 3) The groups lives below of Karks climbers Camp E.coli were isolated from their fecal pellets and there were E.coli in their fecal temporarily.

Conclusion: To our knowledge, no complete study of the bacterial fecal flora of guinea pigs has been made. Guinea pigs digestive tract bacterial flora consists largely of lactobacillus with various number of Bifidobacterium, Bacteroides, Clostridium, Enterococcus and some anaerobic gram positive cocci. There are not found Escherichia coli in the digestive tract of guinea pigs. But wild guinea pigs that they live below of Karks climbers Camp were have been contaminated with Escherichia coli that were in climbers digestive tract bacterial flora.

Keywords: Wild guinea pigs, Escherichia coli, Fecal pellets, Iran Karkas Mountain

10.004 Survey of the Prevalence of Malassezia Spp. on the Domestic Animals Skin in the Northern Iran and Identify of Them

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Background: The genus Malassezia consist of lipophilic yeasts are known to be as component of the normal micro flora of human skin and many mammals and birds. The purpose of is study was to determine the prevalence of Malassezia spp. on the domestic animals skin in the Tonekabon city and identify them according to animals sex, age, breed and species most frequent.

Methods: During the 9 month-period, from October 2007 to June 2008 sampling was carried out by stilt moquet (kind of carpet) and scraping from 230 (36 horses, 51 goats, 41 sheep and 102 cows) animals perineum, ear, axilla, groin and dorsum. The identification of Malassezia spp. after uphold by microscopic and macromorphology of culture was based on microscopic, biochemistry and physiological characteristics for examples the ability to use Tween5, catalase reaction, splitting of Esulin. Growth on SGA supplement with olive oil 37, 40 and accomplishment with morphological characteristic on SGA supplement olive oil, as described by Guil- lot Mayser and Gueho (1996).

Results: Of 113 animal (49.13%) have positive culture of malassezia spp. Melassezia spp. were isolated from 19 of horses (16.81%), 12 of sheep (10.69%), 23 of goats (20.35%) and 59 of cows (52.21%). Among the results obtained, the most frequently isolated species was M. furfur with 31 cases (27.43%) followed by M. globosa 12 case (23.9%), M. pachydermatis 15 cases (13.27%), M. obtuse 12 cases (10.61%), M. restricta 11 cases (9.73%), M. slooffiae 11 cases(9.73%) and M. sympo- dialis 6 cases (5.3%).

Conclusion: To the authors' knowledge, in this survey significance correlation was observed between the animals age and breed with prevalence malassezia, whereas significance correlation was not found between animals sex and prevalence of malassezia.

10.005 Cutaneous Leishmaniasis in Rodent Reservoirs in Fars Province, Southern Iran

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Background: Leishmania spp. is an intracellular protozoa which is transmitted by the bite of hematophagous sand flies. This study was performed to determine the rodent reservoir hosts for human CL caused by L. major in Fars Province, southern Iran.

Methods: Rodents were caught alive in suitable wire traps and were identified using the accepted taxonomic criteria. Animals were euthanized and impression smears were taken from the ears, tails, and feet and stained with Giemsa and also were cultured. DNA was only extracted from cultured promastigotes. Six enzymatic systems were used together with specific and RAPD PCR to demonstrate the presence of parasites in the tissues. Foot pad, tail, ear, lymph nodes, spleen, liver and femoral bones were histopathologically studied by H&E and toluidene blue. Serimithin and ultrathin sections were used for electron microscopy.

Results: Three hundred and twenty four rodents were caught and comprised five different species while infection to L. major was confirmed in 12 rodents. In Larestan; T. indica; and Gerbillus spp., in Kharameh; T. indica, in Marvdasht; R. norvegicus, in Fasa; T. indica, and in Estahban; T. indica. Phlebotomus papatasii has the ability to survive in all these habitats that accounts for the wide geographical range of the parasite. Three different zymodemes of L. major were identified using their isoenzyme profiles indicating to the role of new zymodemes in natural reservoir hosts in the area. In histological and ultrastructural studies of the animals, the bone marrow of femoral bone was just the tissue demonstrating the macrophages infected to amastigote form of L. major.

Conclusion: It was shown that T. indica is an even more widely distributed animal reservoir host in Iran and added species of the genus Gerbil- lus and Rattus norvegicus to the list of different rodent species involved in the epidemiology of CL caused by L. major in Iran and the bone marrow of femoral bone was just the tissue demonstrating the macrophages infected to amastigote form of L. major.

10.006 The Parasitic Infestation in Laboratory Animals and the Potential Risks for Researchers

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Background: A reliable research is dependent on using infection-free laboratory animals. Laboratory animals’ facilities and structure should achieve hygiene standards. If these animals were infected by zoonotic agents such as Hymenolepis nana, Hymenolepis diminuta, Syphacia, a risk of transmission to humans would be possible. So this study was done in the Animal House of Shiraz University of Medical Sciences to determine the prevalence of helminth infestation in these animals.

Methods: 38 laboratory animals including 28 mice of different strains and 12 rats, randomly selected from both sexes, were sacrificed and necropsied. The gastro-intestinal tracts were fixed in 10% formol-saline solution. The gastro-intestinal tracts were opened and all helminths were collected and identified using standard methods.

Results: Syphacia muris, Syphacia obvelata, and Hymenolepis nana were the recovered zoonotic parasites. The prevalence of infection was 83.3% in rats, 90% in inbred Balb/C mice, 100% in outbred Balb/C mice, and 100% in C57BL/6J mice.

Conclusion: Our results denote to the high risk of infection of laboratory animals for researchers and the personnel taking care of the animals. So there is a need to educate the personnel and researchers working with these animals. Periodical tests, prevention and treatment of infestations of laboratory animals would decrease this risk.

10.007 Genomic Sequence Determination, Analysis and Comparison of Avian Hepatitis E Viruses from Australia and Europe Indicate the Existence of Different Genotypes

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Background: Avian Hepatitis E infections were detected in chickens suffering from big liver and spleen disease or hepatitis-splenomegaly syndrome in Australia, USA and Europe. So far the complete genomic sequences of two avian HEV strains from USA have been determined. The genome of both avian HEV strains is 6.6kb long which is 600bp shorter than mammalian HEV genomes. Avian HEVs share the same genome organisation and antigenic epitopes with mammalian HEVs. Since there are just very limited data about genetic characteristics of a single isolate from Australia and none from Europe the genomes of two isolates from Australia and Europe were sequenced to allow substantial comparison.

Methods: Fragments of viral genome were amplified by RT-PCR and cloned into sequencing vector. Positive clones were sequenced in both directions. The consensus sequence for each clone was derived from at least 3 independent cDNA clones that originated from independent RT-PCRs. Subsequent analysis included GenBank database searches (BlastN, BlastP and specialized BLASTs for conserved domains and conserved domains architecture) and phylogenetic analysis (PHYLIP package v3.68).

Results: In the present study we report the genomic sequences of an Australian isolate of chicken big liver and spleen disease virus (AaHEV) (8651 bp), and an avian HEV isolate from Hungary, Europe (EaHEV) (8655 bp). The genomic and phylogenetic analysis of these two strains and their relationship to other avian and mammalian HEV strains are determined. Sequence analyses of these isolates identified major genetic differences among avian HEVs. Most of them are located within ORF1 region, although only a few within conserved motifs of predicted domains. The non-silent mutations in ORF2 region suggest the presence of potentially different epitopes among avian HEVs. Finally, the phylogenetic analysis confirmed the distant relationship to mammalian HEVs and additionally suggested that the avian HEVs can be separated in three different genotypes.

Conclusion: This study reports genomic sequences of two genetically distinct avian HEV isolates from Australia and Europe. Phylogenetic analysis based on the full-length genomic sequences of mammalian and avian HEV isolates confirmed that avian HEVs belong to a distinct branch and suggests further separation of avian HEVs into at least 3 different genotypes: genotype 1 (Australia), genotype 2 (USA) and genotype 3 (Europe), indicating a geographic origin similar to mammalian HEVs.

10.008 Swine MRSA ST398 in Austria


In 2003, a novel lineage of methicillin-resistant Staphylococcus aureus (MRSA) the MRSA sequence type (ST) 398 presumably associated with pigs, has been isolated from humans and animals in The Netherlands. Does this swine MRSA ST398 pose an emerging pathogen to Austrian human or animal population?

An EU-wide baseline study has obliged all member states to investigate the prevalence of MRSA in breeding pigs’ holdings. In Austria, dust samples were taken from different pens of 262 holdings selected by a randomized sampling plan. Nasal swabs of 162 people in contact with swine were investigated on a voluntary basis. Also 87 military horses were screened for MRSA carriage. Additionally, 82 raw meat products (pork, beef and mixed) were tested for MRSA. The bacteriological methods were given by European Commission Decision 2008/55/EC. 9% of dust samples were positive and 8% of people in contact with swine were identified as MRSA carriers. No MRSA isolates were detected in the specimens taken from the 87 military horses. One sample of the mixed meat products was found being contaminated with MRSA. All isolated MRSA strains revealed tetracycline resistance and belonged to spa type 1011, which is characteristic for the ST398 lineage.

The results indicate that the novel MRSA lineage has reached Austria. Although the colonisation rate in Austrian pigs seems lower compared to previous results from The Netherlands or Belgium, a main concern is that the ST398 lineage has a zoonotic potential with transmission to farm workers, and perhaps further spread from those living at pig farms to other individuals in the community. This spread could proceed either by human-to-human contact, or via food. MRSA has to be regarded as a new emerging zoonotic agent and livestock may constitute a growing reservoir of the MRSA ST398 lineage, thereby becoming an important source of community-acquired MRSA.

10.009 Screening and Quantification of Leishmania infantum using Real Time PCR in 140 Dogs in South-East of France

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Background: Leishmania infantum leishmaniasis is endemic in southeast of France. As dogs are the main reservoir, epidemiological survey of canine leishmaniasis is relevant. The main goal of our study was to evaluate the real prevalence of asymptomatic carriage in military dogs by means of real time qPCR and serology.

Methods: We included prospectively 140 military dogs working in eight different military camps located in three areas of southern France: Var, Bouches-du-Rhône and Corsica. All animals were wearing deltamethrine-impregnated collars. Samples of peripheral blood were taken between December 2007 and April 2008. Parasitaemia levels were then measured by means of quantitative real time PCR targeting kinetoplast DNA with TaqMan® chemistry. ELISA and Western Blot (WB) were used for serological screening.

Results: We tested 140 dogs, all male. Mean age was 5.3 years. Mean time of stay in an enzootic area was 3.7 years. ELISA and WB were positive in one (0.71%) and 19 (14%) dogs respectively. Fifty eight dogs (41.4%) had a positive parasitaemia. Global prevalence (positive WB and/or positive qPCR) was 50% (n=70). Mean parasitaemia was 0.043 parasites/mL in positive dogs [min:0.0002–max: 2]. The concordance percent for WB and qPCR results was 55% (n=77). Regarding the prevalence of positive parasitaemia, a significant difference was noticed between dogs living in the Var region and the others coming from the two other areas. Parasitaemia was rapidly positive within the first semester of stay in an enzootic area.
Conclusion: To our knowledge, it is the first screening of *Leishmania infantum* in a canine healthy population using real time PCR quantification on peripheral blood. Despite the use of deltamethrine-impregnated collars, the proportion of dogs with low parasitaemia is important. Thus, it may be relevant to evaluate the effect of screening and treating asymptomatic canine reservoirs on human infection by performing further studies comparing both populations.

**10.010 Oral Cavity as Natural Reservoir for *Streptococcus sinesis***

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**Background:** *Streptococcus sinesis (S. sinesis)*, a novel species of viridans streptococcus, was firstly isolated from several blood cultures of a 42-year old Chinese woman with infective endocarditis in Hong Kong. Subsequently, more cases of *S. sinesis* infective endocarditis have been identified in Hong Kong, Switzerland and France. The organism is now recognized as a cause of the disease in worldwide.

According to the clinical history of one of three patients we identified with *S. sinesis* infective endocarditis in Hong Kong, dental procedures had been performed prior to the development of endocarditis. This suggests that the oral cavity might be a reservoir of this bacterium. Therefore, we carried out a molecular surveillance study in saliva samples from healthy volunteers in Hong Kong using two independent gene targets.

**Methods:** Saliva samples from 100 healthy volunteers with median age 35 (21–58) were collected. All subjects were not on antibiotics in the past four weeks before sample collection. Each saliva specimen was plated out on horse blood agar and incubated for 24 hours. All bacterial colonies observed were harvested for DNA extraction.

The molecular detection was done by polymerase chain reaction using *S. sinesis* 16S rRNA and groEL gene specific primers, a 128-bp fragment of the 16S rRNA gene and a 435-bp fragment of the groEL gene can be amplified from bacterial DNA contains *S. sinesis*.

**Results:** *S. sinesis* was identified in 22 out of 100 (22%) healthy volunteers’ saliva specimens. By comparing the sequences of corresponding regions of *S. sinesis* strain HKU4™ to those of positive purified PCR products, no nucleotide difference between 88-bp sequences of the 16S rRNA genes was showed, while there were 0-8 nucleotide differences between the 511-bp sequences of the groEL gene amplified from the 22 saliva samples.

**Conclusion:** As *S. sinesis* was detected in the saliva of more than 20% of healthy volunteers using the two housekeeping gene targets, oral cavity should be the natural reservoir of the bacterium and probably the source of the infection in patients with infective endocarditis.

**10.011 Discovery of Two Novel Parvoviruses Closely Related to Human Parvovirus 4 in Hong Kong***

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**Background:** Human parvovirus 4 (PARV4), a recently discovered parvovirus found exclusively in human plasma and liver tissue, was considered phylogenetically distinct from other parvoviruses. Unlike the other human parvoviruses with related viruses in animals, PARV4 was phylogenetically distinct without closely related animal counterparts. To identify possible animal origins of PARV4 or related viruses, a surveillance study for PARV4-like viruses in human and animal samples was conducted.

**Methods:** A total of 303 samples from pigs (including lymph nodes, liver, serum, nasopharyngeal and faecal samples) were obtained from slaughter houses and pig farms in Hong Kong over a 2 year period, 30 porcine liver and 32 bovine spleen samples were collected from food markets. Viral DNA extraction and PCR were then performed to identify PARV4-like viruses. Seven porcine and three bovine positive samples were selected to perform the nearly full-length genome sequencing.

**Results:** PARV4-like viruses were detected in 44.4% of porcine samples 13% of bovine samples. Analysis of genome sequences showed that the two animal parvoviruses were most similar to PARV4 with 61.5 to 63% nucleotide identities and, together with PARV4, formed a distinct cluster within the family Paroviridae. The three parvoviruses also differed from other paroviruses by their relatively large predicted VP1 protein and the presence of a small unique conserved putative protein.

**Conclusion:** Two novel paroviruses closely related to PARV4, namely Porcine Hokovirus (PHoV) and Bovine Hokovirus (BH0V) were identified from porcine and bovine samples in Hong Kong. From the results of genome organization and phylogenetic analysis, we propose a separate genus, *Hokovirus*, to describe these three paroviruses.

**10.012 Identification of Novel Avian Coronaviruses***

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**Background:** Birds are known to be the major reservoir of emerging viruses, for example, avian influenza viruses. The number of known coronaviruses (CoVs) in birds is relatively small as compared to bats. Therefore, we hypothesized that previously unrecognized coronaviruses may be present in birds.

**Methods:** 1541 dead wild birds were involved in a territory-wide molecular epidemiology study for CoVs. Viral RNA were extracted and RT-PCR was performed. Complete genomes of the novel CoVs were sequenced. Chymotrypsin-like protease (SCLP™), RNA-dependent RNA polymerase (Pol), helicase, spike and nucleocapsid genes were used to construct the phylogenetic trees.

**Results:** Three novel CoVs were identified in three different bird families. The sequenced genomes (26425-26581 bases) represent the smallest genome among known CoVs. In all the phylogenetic trees constructed, the three novel CoVs formed a cluster distantly related to group 3a CoVs. For helicase, spike and nucleocapsid, they were clustered with Asian leop...
guidelines. MRSA that were defined as CA-MRSA, following the CDC criteria were subsequently spa-typed. Determination of the genetic relatedness of strains belonging to MRSA CC398 spa types was performed using rep-PCR on the new DiversiLab system (BioMérieux), because MRSA CC398 are not typeable with PFGE.

**Results:** In total 13 MRSA CC398 isolates could be detected. The first MRSA CC398 appeared in 2004. MRSA CC398 was then present in every following year, with one MRSA CC398 strain in 2004 up to four MRSA CC398 strains in 2008.

Eleven of the 13 MRSA CC398 belonged to the spa-type 1011, one to spa-type 1034 and one to spa-type 11451.

Resistance patterns of all MRSA CC398 isolates included, beside the resistance to all β-lactam antibiotics, resistance to tetracycline. Only the spa-type 1034 isolate showed additional resistances to clindamycin, erythromycin and trimethoprim/sulfamethoxazole.

Determination of the genetic relatedness using rep-PCR on the DiversiLab System resulted in surprisingly one single cluster for all MRSA CC398 isolates from 2002–2007.

All affected patients had had contact to animals, in 12 cases with pigs; one person has contact to horses only.

**Conclusion:** MRSA CC398 could be for the first time described in patients in Austria, with a first appearance of MRSA CC398 in 2004. MRSA CC398 could only be detected in patients with close contact to animals, particularly pigs, whereas Styria holds one of the biggest concentrations of pig farms in Austria. Even if there was only a small number of MRSACC398 from 2002 to 2007 active surveillance is needed to monitor the spread of this new MRSA subtype.

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**10.015** *Bartonella bovis* Infection in Cattle Farms in North-eastern Italy

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*Bartonella* spp. are haemotrophic small gram-negative bacteria. These microorganisms have been a wide range of mammal and are considered emerging pathogens in humans and animals. Even if data reported are very few, *B. bovis* infection has been demonstrated in dairy and/or beef cattle of three continents: America (United States), Africa (Ivory Coast), and Europe. In the European farms, the microorganism have been detected from dairy cattle in France and recently, also in a mixed production cattle farm from Italy. After the first Italian evidence, we conducted a survey aimed to verify and quantify the presence of this microorganism in other farms around the first infected premise. In this study we examined all the six farms located in an area of 2 km of radius around the first infected herd in the municipality of Mira (Venice). During 2007 we collected blood samples from 217 out of 318 cows. All the samples collected have been screened by PCR for *Bartonella* 16S-23S intergenic region (ITS). 78 out of 217 samples resulted positive and all the farms showed at least one positive animal. Herd prevalence ranged from 8% to 55%. No clinical symptoms have been observed in positive animals. Positive samples have been found in cows of every ages, with an higher frequency in young animals (less than 2 years). The finding of a positive sample in a one-day calf speaks in favour of the vertical transmission of this infection, as supposed but never demonstrated by other Authors. *B. bovis* infection is present and widespread in the area we investigated even if with different level of frequency. The high levels of prevalence of infection suggest efficient routes of transmission which should be accurately investigated.

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**10.014** Network “Rodent-Borne Pathogens”: First Germany-Wide Epidemiology of Hantavirus Infections in Rodent Reservoir Hosts

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In conclusion, our investigations confirmed a broad geographical distribution of three different hantavirus species and the presence of different genetic lineages in Germany. Further investigations shall identify reasons for the non-synchronized oscillation of the numbers of human cases in different regions of Germany. Collaboration of scientists from several research areas in the network will be highly beneficial for conducting these further studies.

**10.016** Investigation on Wild Rodents as Reservoirs of Tick-Borne Pathogens in a National Park of Central Italy

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**Background:** To date information on tick-borne diseases (TBD) in central Italy is still limited and scattered. For these reasons a study to investigate the potential role of rodents as reservoir for Lyme disease, human granulocytic anaplasmosis, tularemia and Q-fever, was performed in an area of the National Park of “Gran Sasso and Monti della Laga” Abruzzi Region, Italy. The area was selected for its environmental features.

**Methods:** From February to October 2008 rodents were trapped monthly, for two consecutive nights, in four sites using 47 LOT live traps. Each animal was etherised, identified, marked by ear-tag and biometric measures were recorded. Blood sample was collected from the rodent’s tail vein and spoted on FTA cards. Ticks found on the animals were collected with tweezers, stored in 70% ethanol and identified by morphological examination. All samples were tested by Real Time PCR.

**Results:** The main species of wild rodents trapped were *Myodes glareolus* and mice belonging to the genus *Apodemus*. Out of 94 blood samples tested by Real time PCR 8 were positive for *Borrelia burgdorferi* s.l. and one was positive for *Coxiella burnetii*. The main tick species collected were *Ixodes ricinus* and *Rhipicephalus turanicus*. The last two tick species were mainly larvae and nymphs. 122 ticks were found on rodents. Sixty nine pools of *Ixodes* ticks were tested; 6 were positive for *Borrelia burgdorferi* s.l. and 4 for *Coxiella burnetii*. All samples were negative for *Francisella tularensis* and *Anaplasma phagocytophilum*. The main species of wild rodents trapped were *Myodes glareolus* and mice belonging to the genus *Apodemus*. Out of 94 blood samples tested by Real time PCR 8 were positive for *Borrelia burgdorferi* s.l. and one was positive for *Coxiella burnetii*. The main tick species collected were *Ixodes ricinus* and *Rhipicephalus turanicus*. The last two tick species were mainly larvae and nymphs. 122 ticks were found on rodents. Sixty nine pools of *Ixodes* ticks were tested; 6 were positive for *Borrelia burgdorferi* s.l. and 4 for *Coxiella burnetii*. All samples were negative for *Francisella tularensis* and *Anaplasma phagocytophilum*.
Conclusion: Results show that at least two of the selected TBD agents are circulating in the study area. Furthermore, as toxoids acuminatus was positive for *B. burgdorferi* s.l., it suggests that this species might be involved in maintaining the ecological cycle of Lyme disease. In the study area at least two of the selected TBD agents are circulating as shown by sporadic positive serological results in animals. In order to assess the risk for TBD for humans it is necessary to improve the knowledge on the epidemiological features.

**10.017 Typhoid Fever as A Public Health Problem Through Consuming the Meat and Eggs of the Carrier’s Birds**

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**Background:** Salmonellosis is one of the widest spread food-borne zoonoses in all the continents of the world, hence study was planned to characterize the fecal shedding and peripheral blood prevalence of Salmo nella enteritidis and Salmonella typhimurium in Pakistani population having clinical sings of typhoid fever.

**Methods:** In this experiment each 100 samples of human stool and blood were collected from 100 suspected patients of typhoid fever from four public hospitals, analyzed using Polymerase Chain Reaction to identify Salmonella enteritidis and Salmonella typhimurium.

**Results:** On average 14% and 10% stool samples were found positive for *Salmonella enteritidis* and *Salmonella typhimurium* of suspected typhoid fever patients, respectively. Similarly on average 6% and 2% blood samples were found positive for *Salmonella enteritidis* and *Salmonella typhimurium* respectively.

**Conclusion:** It was concluded that humans consume poultry meat and eggs and thus Salmonella species particularly *Salmonella enteritidis* and *Salmonella typhimurium* may be transmitted by poultry products to humans and might causes typhoid fever, in addition to *Salmonella typhi*.

**10.018 Survey of Worms’ Contamination Prevalence in Stray Dogs in Shahrekord in Summer and Fall of 2006**

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The digestive system of 50 collars of stray dogs, had been euthanized by Shahrekord Health Center, has been studied for worms contamination. From these numbers, 20 collars were female and 30 collars were male. Forty collars that include 17 females and 23 males and all of them older than 1.5 years, were contaminated to Cestodes. The cestodes were 1–3 scolexes. The 80% of positive samples was Taenia hydatigena and residual 10% was *Taenia multiceps*. Note that these dogs were not contaminated with *Echinococcus granulosus* and induced that Hydatid cyst was out of reach of dogs. In this study, no determined relationship between sex and contamination. In addition, survey of relationship between age of reach of dogs. In this study, no determined relationship between sex and contamination.

**Background:** Echinococcus granulosis is the most recognized reptilian zoonosis. Because, cold-blooded vertebrates including snakes, turtles and lizards, harbor *Salmonella* spp. frequently as part of their intestinal flora and excrete the pathogen intermittently. In a retrospective study the isolation rate of *Salmonella* from reptile specimens (n=764) of 74 different animals was evaluated.

**Methods:** *Salmonella* enrichment was performed in OXOID Rappaport-Vassiliadis enrichment broth (9 ml) and OXOID selenite cysteine bouillon (9 ml), incubated at 42°C. For isolation one loopful of enrichment cultures was streaked after 24th onto MacConkey and xylose lysine deoxycholate agar plates (OXOID) and incubated for 24h at 37°C. All isolates were typed with DADE Behring Salmonella test sera to O and H antigens by slide agglutination. Biochemical identification was done by using API20E test strips (bioMérieux). Detailed serotyping was conducted at the Austrian Agency for Health and Food Safety—AGES (Graz, Austria).

**Results:** Out of 173 salmonellae isolated between 1997 and 2006, 48.04% (n=86) belonged to *Salmonella* enterica subspecies enterica I, 22.35% (n=40) to subspecies diarizorziae IIb, 10.61% (n=19) isolates to subspecies houtenae IV. In total 110 different *Salmonella* serovars were isolated including some rare serotypes such as S. IV 43 z4,z23,-, S. Gatuni and S. II 4,12:a:-. The most frequently isolated serotypes were S. Newport (4.47%), S. Minnesota (3.91%) and S. Ibb 181:v2 (3.91%), S. Oranienburg (3.35%) and S. IV 1,44:z4,232:- (3.35%). The percentage of *Salmonella* positive samples was higher for lizards (42.26%) and snakes (37.63%) as compared to turtles (7.80%). 10.06% of the isolates could be assigned to serotypes which had been related to human cases of reptile associated salmonellosis. These serotypes included S. Chameleonn, S. IV 49 g251- (former S. Marina), S. Infantis, S. Java, S. Kisarawe, S. Minnesota, S. Montevideo, S. Pomona, S. Poona, S. Telekebri and S. Ila 41:z4,223:-.

**Conclusion:** Out of 764 reptile specimens examined, 22.64% (n=173) were tested positive for *Salmonella* and 10.06% of these isolates belonged again to serotypes previously related with reptile associated salmonellosis. The problem of reptile associated salmonellosis is not a new one in public health. Reptiles excrete the bacteria in most cases in their faeces without clinical symptoms. Risk groups are in particular children under 5 years, pregnant women, elderly or those with weakened immune systems. Proper hygiene measures, i.e. washing hands immediately after handling reptiles or their utensils, are imperative. Furthermore, reptiles should not be permitted free access throughout the house and in particular they should be kept out of bathrooms and food preparation areas to avoid contamination. Without doubt it is important that pet-store owners, health-care providers, and veterinarians provide information and prevention messages to owners and purchasers of reptiles.

**10.020 Isolation of Laribacter hongkongensis, a Novel Bacterium Associated with Gastroenteritis, from Commonly Consumed Frog in China and Southeast Asia**

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**Background:** *Laribacter hongkongensis* is a recently discovered novel bacterium associated with community-acquired gastroenteritis and traveler’s diarrhea. Previous studies demonstrated its isolation from freshwater fish and natural freshwater environments. Its presence in different freshwater environments suggested that it is well adapted to these conditions. It is not known if other freshwater animals could also be a source of *L. hongkongensis*.

**Methods:** 30 Malaysian prawn, 20 sand shrimp, 20 Chinese mystery snail, 10 Chinese soft-shelled turtle and 10 Chinese tiger frog were collected from local retail markets in Hong Kong. Samples were obtained from the stomach and intestines of the animals. All the samples were inoculated onto cefoperazone MacConkey agar. Colonies were identified by conventional biochemical method and suspected *L. hongkongensis* clones were further confirmed by PCR using primers specific to *L. hongkongen-
sis 16s rRNA gene. Confirmed isolates were subjected to genotype analysis by pulse-field gel electrophoresis (PFGE).

**Results:** L. *hongkongensis* was recovered from eight of 10 Chinese tiger frogs, with highest recovery rate in the large intestine, followed by the small intestine and stomach. None of the other sampled animals was found to harbor the bacterium. A total of 26 isolates of *L. hongkongensis* were obtained among the eight positive frogs. A heterogeneous population of the bacterium in frogs was found by PFGE, with 6 different patterns among the 26 isolates and a single frog carrying different strains, which correlates with the patterns found in human, freshwater fish and natural water isolates previously.

**Conclusion:** The study represents the first to describe the isolation of *L. hongkongensis* from amphibians. The high isolation rate and genetic heterogeneity of *L. hongkongensis* among the Chinese tiger frogs suggested that these animals are also natural reservoir for the bacterium. As Chinese tiger frog is a common food consumed in China and Southeast Asia, caution should be exercised in handling and cooking them.

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**Abstracts**

**10.021 High HEV Prevalence in Four Wild Boar Populations Across Germany**

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**Background:** Europe is considered to be non-endemic for Hepatitis E virus (HEV), but human autochthonous cases of hepatitis E have been reported. In the recent past an increasing number of reports described hepatitis E as a viral zoonosis. Swine HEV can be zoonotic transmitted from pig to human causing a hepatitis infection and wild boar meat was evaluated as one risk factor for transmission in Germany. A high prevalence of HEV in the wild boar population is reported for several European countries, but actual data for Germany are missing.

**Methods:** Wild boar samples were collected in four different eastern and western German regions from October to December 2007. Liver, bile and blood samples were tested for HEV RNA by highly sensitive quantitative real-time PCR. Serum samples were analysed for antiHEV IgG by two different commercially available ELISA kits and a western blot. Long-range PCR assays generated sequences within ORF1 and ORF2 for genotyping and overlapping fragments of two different full-length genomes.

**Results:** 28.7% of the samples were seropositive and 68.2% of the animals showed HEV RNA in the qPCR analysis. Animals of all age groups were HEV positive in qPCR even adult sow and male wild boars. The highest rate of positive qPCR results was found in bile compared to liver and serum samples. Phylogenetic analysis clustered all isolates within genotype 3 but the estimated subtype was dependent from the region. Genomes from Rhineland Palatine and Brandenburg belonged to subtype 3i, one isolate from the boarder Brandenburg/Saxony clustered within subtype 3f, all others of this spot within subtype 3e. A 969 nt fragment within ORF 2 indicated that the circulating HEV isolate within one population appeared to be stable, but animals in close proximity can bear different subtypes. Two full-length genomes of subtypes 3i and 3e were generated.

**Conclusion:** A high infection rate of HEV can be assumed for the general German wild boar population. The potential zoonotic transmission of HEV from highly contaminated wild boar meat as source of human autochthonous cases in Germany is discussed.

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**10.022 Listeria monocytogenes from Farmed Fish and Processing Environments- Prevalence and Typing by Multiple-Locus Variable Number of Tandem Repeat Analysis**

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*Listeria monocytogenes* is a bacterium with widespread distribution, and occasionally it may cause listeriosis among animals and humans. Due to the potentially severe outcome of listeriosis, considerable concern has been paid to its presence in foods. This study focuses on *L. monocytogenes* in Norwegian farmed fish and seafood processing facilities. A total of 303 samples were collected from three factories during May to October in 2007. All samples were examined for *L. monocytogenes* by immunofluorescence (miViidas), conventional plating and biochemical tests (API-Listeria). The Multiple-Locus Variable Number of Tandem Repeat Analysis (MLVA) was applied to type *L. monocytogenes* isolates. Of the 303 examined samples, 65 showed to harbour *L. monocytogenes*. The overall sample prevalence of *L. monocytogenes* varied from plant to plant, ranging from 5.9% to 33.5%. *L. monocytogenes* was isolated from 33.3% of the raw fish samples, 23.1% of the processing line samples, 7.4% of the finished products and 20.4% of the environmental samples. On average, *L. monocytogenes* was found in 41.7% of the samples taken from floors, 36.8% from drains, 28.6% from personnel clothing, 28% from equipment (knives, forceps, boxes), 12.5% from samples of water or ice, 10.8% from transporters (conveyor belts, wagon, car jack), and 9.7% from processing machines. Drains and floors were the sites with the highest prevalence. The MLVA typing method divided the 65 isolates into 15 distinct profiles, nine of which were found to match human profiles. Among the 15 MLVA types, 07-09-10-06, 05-08-14-10-06 and 06-14-10-06 were found to be the most common. The MLVA type 07-09-10-06 was also the most common strain isolated from human clinical samples from Norwegian patients. Even though the result from this study demonstrates a possible epidemiological link, it cannot be concluded that humans have been infected by *L. monocytogenes* through the consumption of farmed fish.

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**10.023 Sealpoxvirus at North American Marine Mammal Rehabilitation Centers**

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Sealpox is a zoonotic disease that occurs in seals and sea lions (pinipeds). Sealpoxviruses can be transmitted to humans; infections are characterized by localized nodular lesions on the skin. In humans, sealpoxvirus infections are rare and generally self-limiting, although relapses are documented. Sealpoxviruses are most genetically similar to viruses in the parapoxvirus genus, several-orf virus, pseudocowpox virus, and bovine papular stomatitis virus, all associated with domestic ruminants regularly affect humans. Little is known about the influence of animal stress on the emergence of these viruses in ruminant herbs. Because sealpox is typically observed in animals transitioning from wild to captive environments, sealpoxviruses present an attractive model for trying to understand the relationship between animal stress, viral infection and virus transmission to humans. We conducted a study of veterinary health workers at nine marine mammal rehabilitation centers in North America to ascertain their knowledge of and experience with sealpoxvirus. Facilities were included based on their having housed 1 or more pinnipeds with sealpox during the previous year. In total, 1302 pinnipeds were under observation during this time; 16 (1.2%) were clinically diagnosed as having had sealpox. At 3 facilities illness onset was reported as "prior to arrival"; at 6, onset occurred “more than 5 weeks after” arrival. Juveniles were more likely to be infected (14 compared to 2). Nine of the infected animals (56%) were moulting, 4 (25%) had concurrent illness, 3 (19%) had one or the other plus injury. Those with concurrent illness died (including all the adults diagnosed with sealpox), while most animals with malnutrition recovered. Four facilities reported confirmation of the sealpox diagnosis; 5 reported observing sealpoxvirus transmission in their facility; 4 reported transmission to unhealthy pinnipeds, 3 reported transmission to healthy pinnipeds. All reported the involvement of juveniles in transmission events. Two facilities reported instances of past human sealpox. All facilities reported use of personal protective equipment (PPE) when handling animals; and 5 respondents reported use can prevent virus transmission. This study indicates that sealpox is a consequential illness of captive pinnipeds and additional confirmatory diagnostic testing is warranted. The disease association with stress should be studied, and proper PPE may prevent transmission to humans.

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**IMED 2009**

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Aim: To characterize the observed prevalence of tick-borne Zoonosis pathogens in domestic animals in Italy during 2003-2008. Serological (indirect immunofluorescence antibody, n = 10284) and DNA tests (polymerase chain reaction, n = 5626) were conducted on horse, donkey, cattle, sheep, goat, cat and dog samples supplied to the National Centre of reference for Anaplasma, Babesia, Rickettsia and Thelleria (IZS Sicilia) for diagnosis or originated from random sampling conducted for epidemiological research. Pathogens analysed included Anaplasma phagocytophilum, Rickettsia conorii, Coxiella burnetti and Babesia microti.

Methods: Blood was collected in sterile tubes with and without anticoagulant (EDTA). Indirect immunofluorescence antibody (IFA) test kits for A. phagocytophilum, R. conorii, C. burnetii and B. microti (Fuller Laboratories) were used. Fluorescein isothiocyanate-conjugated anti-host species immunoglobulin (Ig)G (Sigma, or Fuller Laboratories) were used as secondary antibodies.

DNA was extracted using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma). PCR analysis for A. phagocytophilum (De la Fuente et al., 2005, Bown et al., 2003), R. conorii (Tejanzabos et al., 1989), Babesia microti (Persing et al., 1992) and C. burnetii (To et al., 1996) were conducted.

Results: Serological prevalence were: for A. phagocytophilum 7.7% (horses), 16.3% (donkeys), 20.1% (cattle), 11.9% (sheep), 18.6% (goats), 8.1% (cats) and 32.0% (dogs); for R. conorii 36.9% (horses), 57.5% (donkeys), 62.1% (cattle), 60.1% (sheep), 2.0% (cats) and 64.2% (dogs); for C. burnetii 0.0% (donkeys), 31.5% (cattle), 34.1% (sheep), 11.2% (dogs) and for B. microti 0.3% (dogs).

Biomolecular prevalence were: for A. phagocytophilum 1.2% (horses), 0.0% (donkeys), 8.7% (cattle), 6.9% (sheep), 3.8% (goats), 1.3% (cats) and 2.4% (dogs); for R. conorii 2.6% (horses), 4.1% (donkeys), 0.5% (cattle), 0.3% (sheep), 0.3% (cats) and 2.4% (dogs); for C. burnetii 0.0% (donkeys), 6.1% (cattle), 2.5% (sheep), 0.0% (goats) and 0.0% (dogs) and for B. microtii 0.0% (cattle), 10.6% (cats) and 0.0% (dogs).

Conclusion: The results reported herein suggested that domestic animals have an important role on the maintenance of pathogens in endemic reaons. A. phagocytophilum can infect a wide range of vertebrate species including domesticated and wild animals and humans with an impact on animal and human health. The high serological prevalence for R. conorii suggest that horses, donkeys, cattle and sheep are asinom hologenic reservoir for Rickettsia species. C. burnetii show high prevalence in cattle and sheep. Cats could serve as the major domestic reservoir for B. microtii.

Resistance to Antibiotics in E. coli Isolated from Farmed Rabbits in Spain. Could They Play Any Role as Vectors of Resistance to Humans?

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Aim: To study the distribution of selected bacterial isolates from clinical specimens submitted by veterinarians to diagnostic laboratories from rabbit farms affected by digestive outbreaks. Antibiotic susceptibility of isolates was performed by Kirby Bauer Disks Diffusion tests using CLSI and OIE criteria. Amoxicillin, ampicillin, penicillin, oxacillin, cephalothin, enrofloxacin, ciprofloxacin, flumequine and nalidixic acid were included in the susceptibility panel because their importance to human health and apramycin and neomycin were included because they are the treatment applied in digestive outbreaks in rabbits in Spain.

Of the 87 isolated E. coli strains, all were resistant to oxacillin and penicillin, 64% were resistant to ampicillin, 8% to amoxicillin and 27.9% to cephalothin (an additional 60.5% were classified intermediate resistance). The highest prevalence of strains resistant to the quinolone/fluoroquinolones class was observed for nalidixic acid (66.7%) and it decreased for the younger quinolones.

The study of resistance for antibiotics used in animal health demonstrated 24.1% of apramycin resistant E. coli strains and 40.3% with intermediate resistance. The alternative treatment is neomycin to which presented 26.4% of strains were resistant and 24.2% presented intermediate resistance.

If we take account that E. coli can be a commensal microorganism in animal populations, probably a part of these resistant strains could remain as commensal microorganisms after disease outbreaks. These E. coli strains could play the most important role as reservoir of resistance genes.

The Bicolored White-Toothed Shrew (Crocidura leucodon) is an Indigenous Host and Possible Reservoir of Borna Disease (BD) Virus in the BD-Endemic Region of Eastern Germany

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Background: Borna disease (BD) is a fatal meningencephalitis of horses and sheep, which occurs sporadically in certain endemic regions of central Europe. Due to its unique features neither the natural reservoir of Borna disease virus (BDV) nor the possible infection cycle could be explained over decades, until two years ago three BDV-infected Crocidura leucodon shrews were identified in Switzerland. For confirmation, we collected and investigated 52 shrews of 5 different species originating from the eastern part of Germany, where BD is endemic, too. Since shrews are protected species by German law we focused only on shrews which were caught by cats or trapped accidentally in rodent traps.

Methods: All shrews were weighed and measured, and the exact species were determined. Photos were taken of each shrew shortly before dissection. Various organ samples were investigated by BDV RT-PCR, and amplification products were sequenced. From PCR-positive individuals, a number of organ samples were examined by immunohistochermistry, in order to assess the distribution pattern of BDV nucleoprotein. To further clarify the existence of BDV in shrews, the determined sequences were subjected to BLAST search, comparative alignment and phylogenetic analyses.

Results: Two out of the 52 shrews investigated were positive for BDV nucleic acid in RT-PCR. Both positive shrews belonged to the species C. leucodon. The BDV infection was demonstrated in all organs of the infected animals by RT-PCR, and was confirmed by immunohistological analysis. The obtained nucleotide sequences showed 99% identity to other related BDV sequences, and they exactly fitted to the epidemiological sequence cluster of the corresponding endemic territory.

Conclusion: To our knowledge, this is the first report of BDV infection in bicolored white-toothed shrews in Germany. Our investigations confirmed the pattern of persistent infection with a widespread distribution of BDV within the central, peripheral and vegetative nervous system of these animals. Thus C. leucodon represents a natural host and possibly reservoir species of BDV. In the poster, we present our hypothesis how BDV may be transmitted from shrews to the final hosts such as horses and sheep.
**10.027**

**Optimal Duration of Gentamicin Containing Regimen for the Treatment of Uncomplicated Brucellosis**

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**Background:** Optimal duration of gentamicin containing regimen for therapy of human brucellosis is not clearly determined.

**Methods:** This randomized clinical study was conducted to compare the efficacy of gentamicin 5 mg/day for 5 days plus doxycline 100mg twice daily for eight weeks (GD group) versus streptomycin 1gr IM for 2 weeks plus the same dose of doxycline for 45 days (SD group). All cases were followed for one year after cessation of therapy. Efficacy of both regimens (failure of therapy or relapse) were compared.

**Results:** Seventy-nine patients with the mean age of 35±14.5 years and 75 cases with the mean age of 36.7±13.9 years were treated with regimen of GD or SD, respectively. The clinical manifestations in these two treated groups were similar. Failure of therapy was seen in one patient in GD group and in the case in SD group (95% CI, 0.042 to 0.271, OR=5.468, p=0.613). Relapse was seen in 2 (2.5%) cases in GD group and in 5 (6.7%) cases in SD group (95% CI, 0.067 to 1.905, OR=0.358, p=0.264). Efficacy of therapy was seen in 76 (96.2%) cases treated in GD group and in 68 (90.7%) cases in SD group (95% CI, 0.649-10.486, OR=2.61, p=0.201).

**Conclusion:** The efficacy of gentamicin for 5 days plus doxycline for 8 weeks is a little better than that of streptomycin for 2 weeks plus doxycline for 45 days although the difference is not statistically significant.

**10.028**

**Giardiasis in Pakistan: A Review**

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Giardia duodenalis is a flagellated protozoan parasite that inhibits the small intestine in trophozoite and cystic forms, causing gastrointestinal illness (giardiasis) in both human and animal. The water-borne transmission of this protozoan has been well documented worldwide. Fecal contamination of potable water by sewage is a common cause of waterborne outbreaks. Like other developing countries, Pakistan is also suffering with poor sewer system. 20 to 40% beds are occupied in the hospitals of Pakistan by patients suffering from water related diseases. Disease such as cholera, dysentery, hepatitis, giardiasis and cryptosporidiosis infections are about 80% of all diseases and are responsible for 33% of deaths. In children, prevalence of Giardia has been reported 51.2% in South Punjab, 50% in peri-urban areas of Karachi, 18.3 to 23.7% in Islamabad, 0.1 to 3.3% in Northern area and 67 % in Faisalabad, Pakistan. In Pakistan, 70% human population is related with livestock related business. Livestock specific assemblage E of G. duodenalis appears to be the most frequent genotype in calves, but the potentially zoonotic assemblage A has also been found in calves and other mammals which suggests its zoonotic importance. In one study, 33% of buffaloes near district Lahore (Pakistan) were found positive for G. duodenalis infection. High shedding intensity rate (45384 cysts per gram of feces) of G. duodenalis cysts in feces in infected calves are the major threat to environment, human and other animals in Pakistan. Untreated waste water irrigation in Pakistan is associated with an increase risk of Giardia infection in occupationally and environmentally exposed households. So far no research has been conducted on the taxonomy and detection of Giardia in water in Pakistan. Keeping in view the high prevalence of Giardia in human and animal in Pakistan, its diagnosis, treatment and control is warranted.

**10.030**

**Prevalence of Stx1, Stx2, EaeA and O157 Genes in STEC O157 and Non-O157 Strains Isolated from Cattle, in Southern Iran**

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**Background:** Shiga toxin-producing *Escherichia coli* (STEC) strains are human pathogens linked to hemorrhagic colitis and hemolytic uremic syndrome. There is limited information about these bacteria present in the cattle gastrointestinal tract that are susceptible to human infection in Iran.

**Methods:** In the periods from September to November 2007 and 2008, carcass surfaces and rectal anal mucosal swab samples from Shiraz cattle were collected at the main slaughterhouses of the Shiraz southern of Iran. The samples were analyzed by conventional plating and then compared with PCR. 440 samples from 220 cattle were collected.

**Results:** STEC were detected from 86 of 220 cattle. Most of the isolates belonged to *E. coli* serotypes other than O157, suggesting a low prevalence of strains of this serotype. *E. coli* O157:H7 was present in 4.54% of all samples from 10 cattle (10/220) from different herds. Six (2.72%) of them were from carcass surfaces and four (1.81%) from rectal anal samples. With the exception of four isolates from adult cattle which appeared to be negative for stx genes, all *E. coli* O157 isolates were positive for both stx1 and stx2 and *E. coli* attaching-and-effacing gene sequences, and therefore, they were regarded as potential human pathogens. The presence of stx1, stx2, eaeA and O157 genes in 15.47, 31.19, 19.04 and 2.61% strains of *E. coli* isolates was investigated. Fifty four carcasses were contaminated with *E. coli* those have at least one gene (Stx1, Stx2) versus 47 recto anal samples, indicated that most carcasses might be cross contamination. Ten strains were isolated by conventional plating while 14 *E. coli* O157 was diagnosed by PCR. So there was appeared PCR to be significantly more sensitive than conventional plating for detection of the organism in feces and from carcass surfaces.

**Conclusion:** Variation in isolates according to Stx1, Stx2, Eae and O157 genes will be appear the causative agents of different STEC. Keeping animals together in pens, which enhances faecal-oral contact and close contact in slaughter lines, is suggested as a possible explanation for the differences seen in stx occurrence in rectum and carcass of the same cattle. We were further interested in the role of cattle as a reservoir for STEC in southern of Iran. Our report demonstrates that domestic cattle were identified as an important natural reservoir for these organisms in this country which can be life-threatening.

During the Korean War, an outbreak of an unknown disease, identified as Korean Hemorrhagic Fever (KHF), occurred in US forces in 1951 with relatively high morbidity and mortality. The etiologic agent was identified in 1976, and since that time other rodent-borne hantaviruses, Seoul, Soochong, Mju viruses, and one shrew-borne hantavirus, Imjin virus, have been identified. All of the rodent-borne hantaviruses in Korea cause disease in humans, while it has not been determined if Imjin virus causes disease in humans. Following the Korean War where there were >2,000 US cases (1951–1953), the number of cases among US service- men decreased, with annual cases/outbreaks of 1–14 cases occurring sporadically.

In response to Hantavirus cases in 2000, a rodent-borne disease surveil lance program was established by the Department of Preventive Medicine, USAMEDDAC-Korea, and the 65th Medical Brigade, Korea, to identify small mammal population densities, infection rates among Apodemos agrarius, the primary reservoir of Hantaan virus, and later expanded to include other sylvatic rodents and insectivores to identify risk factors associated with transmission. Three training sites were surveyed seasonally from 2001–2005, while two installations and another 10 training sites were surveyed periodically and the relative rodent densities and Hantavirus rates determined. Later, viral sequencing of Hantaan virus showed an association with genomic sequences and geographic distribution among rodents captured at selected US and ROK operated training sites, leading to the identification in 2005 of the location of transmission of virus among four US Soldiers and provided important information on the long incubation period (10–35 days). Furthermore, these data described environmental factors, rodent reproductive cycle, and Soldier activities that increase risk of Hantavirus infection.
Rift Valley fever virus (RVFV), a mosquito-borne zoonotic agent, is of growing importance to veterinary and human health. Historically, RVF was restricted to sub-Saharan Africa but recent large outbreaks in non-endemic regions caused international concern for further spread. Classical diagnostic methods require high biocontainment facilities and are time-consuming. Therefore there is an increasing demand for rapid and safe RVF diagnostic tools. Molecular techniques might fulfill these requirements but are highly specialized which could be problematic in desolate outbreak situations and in poor-resourced developing countries. Sheep and rabbits were immunized with recombinant RVFV nucleocapsid protein to produce hyperimmune polyclonal antisera which were then used to develop a sandwich ELISA for the detection of RVFV nucleocapsid protein in thermo-chemically inactivated specimens. The ELISA identified RVFV strains of distinct geographic and historic origins and showed no cross-reaction with closely related viruses of the Bunyaviridae family. In RVFV-infected human, bovine, porcine, equine, hog, chicken, and spleen tissues and Anopheles mosquito pool homogenates the average detection limit was log10 10^7.5 TCID50/reaction volume. Compared to virus isolation the ELISA was 67.7% sensitive and 97.97% specific when testing sera from RVF patients; similar diagnostic accuracy was achieved when testing sera from experimentally infected sheep. Accuracy of 100% was achieved when testing tissues from various organs of experimentally infected mice and naturally infected buffalo fetuses. Antibodies were detectable in infective culture supernatants as early as 8 hours after infection and before cytopathic effects became microscopically visible. The ELISA is highly specific, safe and simple rendering it ideal for use in less equipped laboratories.

**10.031 Safe Detection of Rift Valley Fever Virus in Human and Animal Specimens by A Sandwich ELISA**

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Infection with Rift Valley fever virus (RVFV) in livestock is characterized by acute hepatitis, abortion and high mortality rates in young animals. Humans develop Symptoms ranging from a mild flu-like illness to hemorrhagic fever, encephalitis and death. The role of the anti-nucleocapsid response in protection against infection has not been elucidated, although it is generally accepted that anti-N antibodies do not neutralize the virus. Recombinant RVFV nucleocapsid protein (recNP) was expressed using the pET32a(+) bacterial system and purified using the histidine fusion tag. Groups of BALB/c mice were immunized and boosted with 70µg recNP, in combination with ISA50, Alhydrogel, TiterMax Gold or SaponinQ adjuvants. Adjuvant and PBS placebo control groups were included. All vaccinated mice groups generated strong Th2mediated IgG1 responses, whereas recNP/SaponinQ vaccinated mice generated a much stronger Th1-mediated IgG2A response than other adjuvants. RVFV-challenged mice were clinically monitored and tissue and blood specimens taken at regular intervals. Animal sera, homogenates of necrotic liver and spleen tissues and Anopheles mosquitoes were tested in the ELISA. 96% of the mice showed severe clinical signs or died between day 2 and 6 after challenge. Vaccinated groups showed between 40 to 100% protection against sickness or death depending on the adjuvant used. Anti-N humoral immunity did not prevent viral replication in vaccinated animals as demonstrated by RVFV recovery from the liver, brain and kidney tissues of clinically normal mice but viral loads were lower compared to non-vaccinated groups. The type of adjuvant used with recNP plays a major role in protection achieved but the possible cellular mechanism responsible needs further investigation.

**10.032 Evaluation of A Recombinant Rift Valley Fever Virus Nucleocapsid Protein As A Vaccine Immunogen in Combination with Four Adjuvants**


African livestock and wild animals are at risk of Rift Valley fever virus (RVFV) infection, a mosquito-borne disease of both domestic and wild animals. The virulent RVFV is transmitted to domestic ruminants via infected mosquitoes and, in turn, causes significant economic losses. Therefore, the development of a safe and effective vaccine is imperative for livestock producers. The present study aimed to evaluate the efficacy of a purified recombinant nucleocapsid protein of RVFV (recNP) as a vaccine immunogen in BALB/c mice, in the presence of four different commercial adjuvants: Alhydrogel, TiterMax Gold, SaponinQ and ISA50. Groups of BALB/c mice were immunized with 70µg recNP at 0, 2, 4 and 8 weeks. Adjuvant and placebo control groups were included. The immune response and protective efficacy of the recNP were determined by ELISA and challenge experiments. The highest humoral response was observed in the group vaccinated with recNP, with and without Alhydrogel. Protection against RVFV was achieved when testing tissues from various organs of experimentally infected mice and naturally infected buffalo fetuses. Accuracy of 100% was achieved when testing sera from various organs of experimentally infected mice and naturally infected buffalo fetuses. Antibodies were detectable in infective culture supernatants as early as 8 hours after infection and before cytopathic effects became microscopically visible. The ELISA is highly specific, safe and simple rendering it ideal for use in less equipped laboratories.

**10.033 Occurrence of Taenia multiceps Infection in Israel**

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Taenia multiceps (Coenurus cerebralis) infection is a common worldwide problem of small ruminants. Dogs harboring the adult worm (final host) play an important role in spreading the disease. Coenurusosis may develop in the brain, spinal cord and in other tissues of a wide range of animals, including sheep, goats and some wild animals. In Israel, the disease was first described by Landau (1957) in herd in central Israel. Since then no new information regarding the prevalence of the disease in Israel was reported. During the period 2000–2008, a prevalence of 1.3 to 9.8% was demonstrated by us in some herds in central and southern Israel, leading to mortality rate of 1.14–24.61% and culling of animals to the extent of 37.4%. High variability was observed regarding the size and cyst locations. Most infections were demonstrated in 0.5–3-year-old sheep. Clinical syndromes include vivid types of nervous symptoms with little or no change in hematological and biochemical profile. Treatment of coenuroses in sheep and goats using albendazole, niclosamide and praziquantel is only partially effective. Coenurosis is a rare disease in humans and less than 100 cases were reported from Africa, the United Kingdom, Italy, France, USA and North America. Recently, in the Negev desert area in southern Israel, an unusual case of a huge intraparenchymal cyst in a 4-year-old girl caused by T. multiceps was demonstrated.

**10.034 Anthrax in Italy: Neglected or Undervalued Disease?**

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Nowadays, common people, scientists and politicians are aware of the bioterrorism phenomenon but it is necessary to underline that anthrax is a mainly a disease which is widespread among livestock and wild animals in many countries. The impact and consequences of the disease are usually more severe when it appears in areas considered anthrax-free, where such pathogen is unknown or underestimated. A typical example was an anthrax outbreak occurred in Italy on December 2006 in a farm located at 1500 m. above sea level in Alto-Adige (south-Tyrol), north-western Italy, where, going back over folks’ memory, anthrax cases had never been suspected. The delay of the diagnosis in two sheep favoured the development of a severe form of cutaneous anthrax in a 57-year-old farmer. Another recent outbreak occurred in Tuscany, where the regional veterinary services consider anthrax a very rare condition. Indeed, disease outbreaks had not been reported for decades and in the recent past no vaccination programme had been conducted in that territory. During the end of August and September 2008, in a mountainous area of the region (Mugello), nine cattle died in ten days. Since clostridiosis is considered the main cause of death of cattle reared in that area and the preagonic symptoms were suggestive of enterotoxemia, no laboratory tests were done. When at last, a blood sample of the seventh sudden death occurred among cattle at pasture was examined, a fully virulent Bacillus anthracis was isolated. One month later, environmental samples (soil and water puddles) were collected in pastures associated with the confirmed case and the other suspected cases. Not surprisingly, anthrax spores from soil samples where cultured also from sites where in the last five years additional deaths were reported, with a presumptive diagnosis of enterotoxemia. Molecular laboratory investigations were performed to compare the isolates associated with the 2008 outbreak with those cultured from pastures where deaths had been reported in the previous years. The Multiple Loci VNTR Analysis (MLVA) with 8 VNTRs and the analysis of four Single Nucleotide Repeat (SNRs) in a multiplex assay were employed for the purpose.
The lesson learnt from these experiences induces us to consider that anthrax aetiology is often disregarded and anthrax cases are probably significantly underreported in many areas of the Italian territory.

Technical Support: Angela Aceti and Nicola Nigro.
Found: Ricerca Finalizzata 2006 “DIGNOVA”


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Background: In Japan, the numbers and cases of food poisonings must be reported to the Ministry of Health, Labour and Welfare (MHLW) as required by the Food Sanitation Law. In this study, characteristics of food-borne diseases associated with Vero toxin producing Escherichia coli were analyzed to consider potential interventions.

Methods: Annual food poisoning data published by the MHLW were reviewed from 1998 to 2005.

Results: Total 131 VTEC food-borne diseases incidents with 1,352 cases, and 9 deaths were reported to the MHLW during this study period. Each year 8–24 incidents were reported, with average 16.4 incidents per year. 100 incidents (76%) were reported from May to October. 52% of 131 incidents were involved in 2-5 cases, but 3 outbreaks with more than 100 cases were reported. Serotyping data was available in 123 incidents, and 113 incidents (92%) were associated with O157:H7. In 48 incidents (37%), implicated food were identified. Beef was identified in 22 incidents (45.8%), followed by species unspecified meat (12 incidents). Fresh produce was not identified as the source of outbreaks in any incidents. It was reported that beef were consumed raw in some incidents. In 79 incidents, food safety problems which were determined to be a cause were identified in restaurants.

Conclusion: It was indicated that food-borne outbreaks associated with VTEC were the significant public health problem in Japan. Among the implicated foods, beef was identified about the half of incidences. None of outbreaks were traced back to fresh vegetables which is a common implicated foods, beef was identified about the half of incidences. None of outbreaks were traced back to fresh vegetables which is a common

10.036 Study on Natural Infection with Chlamydia Species in a Dairy Herd in North-Eastern Italy

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In March 2008, 4 cows from a dairy herd suffering from mainly late-term abortions tested positive for Chlamydiae antibodies in complement fixation test (CFT), with titres ranging from 1:64 to 1:128. With the aim to investigate the antibody and shedding dynamics of Chlamydia sp. (C.) inside this herd, from July to November 12 animals were sampled monthly by collecting blood and vaginal swabs. Sera were examined by CFT and an indirect ELISA specific for C. abortus (Pourquier); swabs were analysed by PCR, targeting the 16S rRNA gene, followed by enzymatic restriction (RE) analysis; whenever possible, PCR amplicons were sequenced. Isolation on LLCMK2 cells and egg cultures were performed on PCR positive specimens. Results are shown in table 1. At each sampling, from 5 to 8 bovines out of 12 were CFT positive with titres ranging from 1:32 to 1:512. All over the study all animals tested negative in ELISA. At least once during our observation period 9/12 bovines showed CFT Chlamydia-positive titres but only 3/12 animals maintained positive variable titres from the beginning to the end of the study. PCR analysis revealed chlamydial DNA in 6/12 bovines and two species were detected: C. abortus and C. pecorum. Only 1/12 bovine resulted positive for both species. To date, one C. abortus strain has been isolated on egg cultures, confirmed by PCR and RE analysis. Further investigation on egg and cell cultures is ongoing.

Table 1. Summary of results obtained on samples collected.

10.037 Human Monkeypox Disease and Investigation in A Border Region of the Republic of Congo and the Democratic Republic of Congo

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Background: Monkeypox is a zoonotic virus, endemic in forested regions of Africa, which has the potential for human-to-human transmission. Human infection results in a disseminated pustular rash manifestation, with case fatalities up to 10%. Because of the possibility of geographic expansion of monkeypox, we have begun surveillance and investigation activities in the northern bordering regions of the Republic of Congo (RoC) and the Democratic Republic of Congo (DRC).

Methods: Following a monkeypox outbreak in Likouala district, RoC, in 2003, we began outreach and investigation activities, and developed a rash illness surveillance system. In response to reports of a monkeypox outbreak in Equateur Province, DRC, a region that borders Likouala, RoC, we began collaborative investigation activities in DRC in 2008.

Results: Despite enhanced surveillance activities in Likouala, RoC, we have not detected the occurrence of human monkeypox since 2003. However, following reports of a rash illness outbreak, during 2007, we identified 37 probable cases of variella through laboratory testing, in 4 rural villages, with temporal and geographic clustering suggestive of a sizable outbreak. Additionally, we documented serial transmission of variella having palm and sole lesion manifestations, a manifestation typical of monkeypox. In contrast, through investigations begun in 2008, we identified a large number of suspect human monkeypox cases in Equateur, DRC, including 21 laboratory diagnosed cases. The wide-spread geographic and temporal distribution of cases across the province suggests viral endemnicity, and ongoing animal-to-human introductions of monkeypox virus.

Conclusions: Even though Likouala, RoC and Equateur, DRC, border geographically, these regions differ in terms of the occurrence of human monkeypox, with the latter supporting endemic disease. While reasons for this difference have not yet been identified, the potential for monkeypox reemergence in RoC still exists, necessitating ongoing rash illness surveillance, as well as continued investigation and outreach in areas where monkeypox cases occur.
Recent studies designed to examine wildlife-livestock-human disease transmission at the community level have demonstrated that early detection of factors that impact on ecosystem health, including changes in the distribution and health of people and wildlife, unusual activity in disease vectors, or disruption to normal behaviour and social structures in humans and wildlife species is important if we are to enhance our ability to develop and apply early interventions to prevent the spread of emerging and re-emerging diseases such as West Nile Virus, Tuberculosis and Avian Influenza. From these studies it is apparent that, to be effective in the early detection of emerging diseases in wildlife, surveillance systems need to be designed to capture data from a range of sources such as climate studies, wildlife health monitoring projects, social and ecological impact assessments and community health programmes. Data from long term studies designed to assess the health of isolated communities, and their environment, has been used effectively to improve health interventions for wildlife species and has the potential to be used equally effectively to identify and mitigate emerging risks for livestock and public health. The value of data from these studies is that researchers and policy makers work in close collaboration with the communities that depend on wildlife and the immediate environment for their food and water supply. This poster outlines a number of collaborative studies underway at the University of Calgary designed to ensure early detection of emerging diseases in human and wildlife populations. The work depends on strengthening links between the veterinary faculty, community health sciences, faculty of medicine, social sciences and centre for policy and health studies in order to better predict risk factors for human and animal health and to better understand disease ecology.

**Abstract:**

**Title:** Ecosystem Health and Disease Surveillance

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**Abstract:**

Recent studies designed to examine wildlife-livestock-human disease transmission at the community level have demonstrated that early detection of factors that impact on ecosystem health, including changes in the distribution and health of people and wildlife, unusual activity in disease vectors, or disruption to normal behaviour and social structures in humans and wildlife species is important if we are to enhance our ability to develop and apply early interventions to prevent the spread of emerging and re-emerging diseases such as West Nile Virus, Tuberculosis and Avian Influenza. From these studies it is apparent that, to be effective in the early detection of emerging diseases in wildlife, surveillance systems need to be designed to capture data from a range of sources such as climate studies, wildlife health monitoring projects, social and ecological impact assessments and community health programmes. Data from long term studies designed to assess the health of isolated communities, and their environment, has been used effectively to improve health interventions for wildlife species and has the potential to be used equally effectively to identify and mitigate emerging risks for livestock and public health. The value of data from these studies is that researchers and policy makers work in close collaboration with the communities that depend on wildlife and the immediate environment for their food and water supply. This poster outlines a number of collaborative studies underway at the University of Calgary designed to ensure early detection of emerging diseases in human and wildlife populations. The work depends on strengthening links between the veterinary faculty, community health sciences, faculty of medicine, social sciences and centre for policy and health studies in order to better predict risk factors for human and animal health and to better understand disease ecology.
on the list. Newly identified zoonoses can be added or information on already included zoonoses can be modified without the necessity to completely redo the analyses. For this purpose, a web-based tool is being developed to allow a user-friendly, dynamic and actual priority listing.

**Conclusion:** This quantitative dynamic method is especially valuable in the priority setting of emerging zoonoses, where information is uncertain or even absent, and changes constantly. This method is more transparent and objective and can form the basis of future knowledge management systems. MCA methods are, however, more costly and time-consuming than the other priority setting methods, and are still under development.

**10.042 Development of a Blueprint for an Early Warning System for Emerging Zoonoses in the Netherlands**

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**Background:** Late 2006, the Ministry of Agriculture, Nature and Food Quality asked the Netherlands Centre for Infectious Disease control (Cib/ RIVM) to coordinate a two years research program with the aim to develop a blue print of a proactive early warning system for emerging zoonoses in the Netherlands in collaboration with the main veterinary key players. Besides this early warning system, the program needs to develop an effective and efficient infrastructure of collaborating key partners in human and veterinary infectious diseases to control (emerging) zoonoses.

**Methods:** In 2007, the consortium consisting of partners from the Faculty of Veterinary Medicine, Utrecht University, Central Veterinary Institute, the Animal health services and Cib/ RIVM was launched. The two-year program has been divided into two successive phases; an inventory and prioritization project (finalized in March 2008) and in September 2008 a research and developmental second phase has started, based on the results and gaps identified in the first phase of the program.

**Results:** Early warning and surveillance systems were evaluated in the first phase. Uneven standards of surveillance, human and animal-based, for zoonotic diseases or pathogens in the Netherlands became readily apparent during the inventory process. In summary, existing systems mainly target husbandry, and are suboptimal or lacking for wildlife, vectors, pets and exotic animals.

A dynamic database consisting of 92 emerging zoonoses supposed to be relevant for the Netherlands (Europe) has been developed. For the priority setting, an innovative method was developed, as existing methods often based on current disease burden in a country fail for ‘emerging zoonosis’ only present as a ‘threat’. In our method, newly identified zoonoses or additional information for a zoonosis already in the system can be added or modified without the necessity to completely redo the analyses. The prioritized list does however not indicate which agents are most likely to emerge.

**Conclusions:** The ultimate goal of an early warning system for emerging zoonoses is to limit the impact of a zoonotic disease for public health, trade in animal and animal products and the animal health and well-being. To effectively detect, aggregate and translate ‘emerging signals’ to actions, bridging the gaps between the veterinary, public and wild life health is the most important challenge for the future and will be the focus of the second phase of the program.

**10.043 Hepatitis E Virus Infection in Germany: A Food-Borne Zoonosis?**

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**Background:** Hepatitis E is a classic water-borne disease in developing countries. In Germany, hepatitis E virus (HEV) infections are notifiable. The number of non-travel-associated infections has increased in recent years. The route of transmission remained unknown in most of these cases. Our objective was to determine risk factors for autochthonous HEV-cases and to analyse differences in HEV-strains from autochthonous and travel-associated infections.

**Methods:** Cases were patients with defined clinical manifestations and laboratory confirmation. PCR-products from blood or stool samples were genotyped for phylogenetic analysis. A case-control study included case subjects with autochthonous HEV infection and matched controls from a population-based telephone list. We collected information about relevant exposures (eg, travel abroad, consumption of specific food items, contact to animals, surface or waste water) in the 2 months before symptom onset.

**Results:** From May 2006 trough August 2007, 76 of 96 persons for whom HEV infection had been reported to the routine surveillance system were interviewed. Sixty-six cases had disease that fulfilled the inclusion criteria: 45 (68%) had autochthonous infection, and 21 (32%) had travel-associated infection. Of the travel-associated infections, 52% had been acquired in India. Of the autochthonous cases, 76% were males and 51% lived in communities <20,000 inhabitants. In conditional logistic regression involving 45 case subjects and 135 control subjects, consumption of offal (41% vs. 19%; Odds Ratio, OR, 2.7, 95% confidence interval, CI, 1.2-6.2) and wild-boar meat (20% vs. 7%; OR 4.3, 95% CI 1.2-15.9) were independently associated with autochthonous HEV infection. Genotypes 3 (swine-like sequences) or 4 were present in 15 of 15 persons with autochthonous infection, and genotype 1 was present in 8 of 9 persons with travel-associated infection.

**Discussion:** Hepatitis E is endemic in Germany and likely exists as a food-borne zoonosis. Implicated meat products should be investigated to provide recommendations for specific preventive measures. Consumers should cook wild-boar meat and offal properly and avoid cross-contamination of food products.

**10.044 Increasing Prevalence and Spreading of Echinococcus multilocularis in the Most Western European Border Line**

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**Background:** Alveolar echinococcosis is one of the most pathogenic parasitic zoonoses in Europe, caused after oral uptake of eggs of Echinococcus multilocularis, shed by infected foxes. At present, there seems to be a trend towards increased parasite density in central Europe and spread in western Europe.

**Methods:** Red fox data from an area close to the westernmost margin of E. multilocularis habitat in Europe, Belgium and a neighbouring province Limburg in The Netherlands (NL) were analysed with the aim of studying the emergence of the parasite in this area. Foxes were analysed using mucosal scrapings. Spatial coordinates of the locations of infected and uninfected foxes have been determined by GPS. A mathematical model describing the parasite population dynamics both in time and in space was fitted to the worm burdens of foxes sampled in the NL.

**Results:** The spatial distribution of the prevalence of infection among sampled foxes has been modelled as an ellipsoidal gradient, demonstrating increasing prevalence in south-eastern direction. Moreover, E. multilocularis infection in the western border area could be shown to have a continuous distribution across national borders. We found a strong indication that the parasite’s reproduction number R0 is greater than 1 and that the parasite is spreading with a speed of 2.7 km per year to a wider region in the southern area in the Netherlands. Based on the R0 derived from the mathematical model of the parasite’s transmission, we analysed the effect of control measures aimed to eradicate the infection.

**Conclusion:** E. multilocularis is spreading in northern direction. In Belgium, human alveolar echinococcosis had been absent in the past, but several human cases have been reported since 2004. Also in the Netherlands, human cases might be expected coming years. A major task for the future is to control spreading of the parasite in the fox population.
Development of Typing ELISA for New World Hantavirus Infection

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Background: Hantaviruses cause two important rodent-borne viral zoonoses: hemorrhagic fever with renal syndrome (HFRS), found in Eurasia and caused by Old World hantaviruses, and hantavirus pulmonary syndrome (HPS), found in North and South America and caused by New World hantaviruses. We have developed serotyping ELISA technology for Old World hantaviruses that uses a truncated recombinant nucleocapsid protein (rNP) from which N-terminal located cross-reactive epitopes were deleted. The current study examined the applicability of rNP-based ELISA technology for serotyping New World hantaviruses.

Methods: The S genome RNAs encoding the NPs of two New World hantaviruses, Sin Nombre virus (SNV) and El Moro Canyon virus (ELMCV), were cloned and expressed using E. coli and baculovirus systems. Truncated NPs missing the 99 N-terminal amino acids (rNP100) of SNV and ELMCV were produced using recombinant baculoviruses, and their applicability for serological differentiation between SNV and ELMCV infections was examined using ELISA technology.

Results: Based on a comparison of the amino acid sequences of the internal variable region of the NP (aa 230-302) of 39 New World hantaviruses, they were divided into five groups with about 70% identity among the groups. The representative viruses of groups 1 to 5 are SNV, Andes virus, Bayou virus, ELMCV, and Cano Delgadito virus, respectively. The rNP100 of SNV and ELMCV, the representatives of two of these groups, could serologically differentiate homologous and heterologous patient and rodent sera using ELISA. Using the entire NP of SNV and ELMCV expressed in E. coli, we could detect both homologous and heterologous antibodies equally.

Conclusion: The entire NP and rNP100 of SNV and ELMCV are useful ELISA antigens for surveillance in regions where both viruses co-circulate. The New World hantaviruses appear to have at least five antigenic groups.

Analysis of the Hantavirus-Specific CD8+ T Cell Response in Mice

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Background: Hantaviruses cause two serious and often fatal human diseases: hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). In contrast to humans, the reservoir animals are persistently infected without signs of disease. To elucidate the immune response of reservoir animals against virus, we established a persistent infection model and transient infection model by using laboratory mouse.

Methods: Mice were infected with hantaviruses and analyzed their CD8+ T cell response.

Results: Viral antigen was detected in the lungs of the persistent infection model and transient infection model by using laboratory mouse, and analyzed their CD8+ T cell response.

Conclusion: The major epitope of the HTNV in BALB/c mouse (H-2d) was identified, and its HMC tetramer was prepared. Using these tetramers, possibility of dysfunction and deletion of HTNV-specific CD8+ T cell in the persistent infection model mice were shown.

Pathomorphological and Ultrastructural Diagnosis of West Nile-Virus Infection in Goshawks (Accipiter gentilis) in Austria

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West Nile Virus (WNV) is a member of the Japanese encephalitis anti-generic group within the genus Flavivirus. WNV was identified first in Africa and has spread over large areas of the globe due to its wide vector and host range (CDC 2008).

Here we report the pathomorphological and ultrastructural findings in three goshawks, which were molecular biologically and virologically tested positive for WNV lineage 2.

Materials and Methods: Organ samples of the affected birds (two females, one male) were investigated by means of routine histology (H&E), immunohistochemistry (ABC-technique) and electron microscopy (negative staining and ultrathin sectioning).

Results: One of the most striking histological findings was a moderate multifocal to diffuse non-purulent meningoencephalitis with formation of glial nodules. Additionally, a moderate extraparenchymal inflammation with peri-portal accentuated lymphocytic infiltrates and multifocal splenic necroses were found. Furthermore one bird displayed a slight hydropericardium and a second one showed a severe granulomatous-necrotizing pneumonia demonstrating fungal hyphae and haemorrhages caused by arrosion of blood vessels as well as a slight tubulonephrosis with dilata- tion of tubuli filled by debris and bacteria.

Viral antigen could be demonstrated by means of immunohistochem- istry in the brain, spleen, liver, heart, kidney and in the small intestine. Negatively stained virions measured 45–55nm in diameter, were icosa- hedral in shape, and showed typical flaviviral morphology, that is, a dense core surrounded by a thin, diffuse outer layer. In ultrathin sections, flavivirus-like particles were frequently seen in the cerebellum, in liver and pancreatic acinar cells. In liver and acinar pancreatic cells, the particles are found in the vacuolar system and near membranes of the endoplas- matic reticulum; in cells of the cerebellum, virus particles were usually present in cytoplasmic vacuoles.
Positive samples (12 from 8 pigs) were sequenced and determined as HEV. Based on HEV sequences from all genotypes published at the GenBank, we designed two pairs of overlapping primers which allowed us to sequence more than 1 kb of the ORF2 and ORF3 regions of the characterised Austrian pig isolates. Using these sequences and 39 GenBank sequences from human and animal isolates belonging to all HEV genotypes, a phylogenetic tree was performed (neighbour-joining method). All strains found in Austrian domestic pigs clustered to the genotype 3.

For the first time, HEV-3 was detected in Austrian domestic pigs. Moreover, the development of a very high sensitive and reliable one-step real-time RT-PCR using a TaqMan MGB-probe for detecting Austrian HEV strains in different pig samples (including liver, bile, faeces and serum) is in progress. This new method will be a key factor in determining the prevalence of HEV in Austrian domestic pigs, to define risk groups within this population and to detect hepatitis E viral RNA in commercial pork products. Finally, these preliminary results will help to establish a national surveillance program.

Prevalence of Chlamydia psittaci Determined by Direct PCR in Pigeons in Madrid

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Avian Chlamydiosis, a systematic and occasionally fatal disease in birds, is caused by Chlamydia psittaci. Chlamydial infections have been also reported in mammals (including humans), reptiles and amphibians. Efforts to detect and identify chlamydial species are important because they do not only cause disease but also interact synergistically with viruses or with other bacteria, increasing the virulence of these. Among the possible sources of chlamydial infections for humans, peridomestic birds such as pigeons represent a potential risk due to their increasing population in urban areas. However, there are only few studies describing the prevalence of this zoonotic pathogen in these hosts species.

The objective of the present study was to determine if Chlamydia psittaci was present in the population of pigeons from the city of Madrid, and to estimate its prevalence. A total of 115 pigeons were captured from different areas of Madrid and in different months, euthanized and sampled in the period 2007–2008. Pharyngeal and cloacal swabs were collected from all animals, and subjected to DNA extraction protocols. DNA samples were later analyzed using a specific PCR.

Only one sample was positive at the PCR analysis, yielding an apparent prevalence of 0.87% (C.I. 95%: 0.02-4.75%). Our data are in disagreement with previous studies performed by PCR detection of Chlamydia psittaci DNA on feral pigeon’s samples in other cities, which reported higher prevalences (ranging from 5 to 23%). These differences could be explained in part due to differences in the sampling seasons, as shedding occurs intermittently in infected animals and can be activated by stress factors present mostly in certain times of the year (such as breeding).

In summary, our data suggest that Chlamydia psittaci is present in the pigeon population of Madrid but with a low incidence. However, due to the zoonotic nature of this pathogen, surveillance programs for the detection of its presence on peridomestic animals that can act as reservoirs for humans are highly recommended.

Nipah Virus: New Entry Model, New Therapeutic Approaches

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Background: Nipah virus (NiV) is a zoonotic biosafety level 4 (BSL-4) paramyxovirus that emerged recently in Asia and causes acute encephalitis in man. At present no antiviral treatments or prophylactics are available against this dangerous pathogen whose mortality can reach 92%. NiV entry, like that of other paramyxoviruses, is believed to occur via pH-independent fusion of the virion envelope with the host cell’s plasma membrane, mediated by the concerted action of the viral glycoproteins, following receptor attachment. The NiV receptor ephrinB2 is a ligand for members of the Eph class of receptor tyrosine kinases such as EphB4. Ephrins and Ephs are plasma membrane-bound proteins which play an important role in both vasculogenesis and neurogenesis: signaling mediated by contact between opposing EphB4- and ephrinB2-expressing cells results in bi-directional trans-endocytosis of receptor-ligand complexes and subsequent cellular repulsion.

Methods: we have used a combination of mutagenesis, fluorescent tagging and confocal microscopy to determine the pathway by which NiV enters the host cell. Experiments involving live virus were carried out in the P4 laboratory in Lyon. As many steps of this pathway are known to be blockable, we have tested various drugs for their capacity to block NiV entry in vitro and have quantified their inhibitory effect by the use of a plaque reduction assay.

Results: Our results show that NiV entry into the host cell occurs not by fusion at the plasma membrane but by subversion of receptor-mediated macropinocytosis: this is achieved by the NiV attachment glycoprotein NiV-G mimicking EphB4. Although the cytoplasmic domain of ephrinB2 appears to be dispensable for post-entry virus spread via synctia formation, intracellular signaling from this domain is required for NiV entry—mere attachment to ephrinB2 is insufficient. Importantly, our results show that drugs such as amiloride (used for treating hypertension) and chloroquine (used to treat malaria) abrogate NiV entry by having an effect at different steps of the endocytic entry pathway.

Conclusions: Our finding that NiV enters by an endocytic mechanism is exciting because it should now be possible to develop a cheap, safe novel antiviral treatment effective not only for NiV but also Hendra virus.

Nipah Virus Infection in Dutch Tourist

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Background: Nipah virus (NiV) is a zoonotic biosafety level 4 (BSL-4) paramyxovirus that emerged recently in Asia and causes acute encephalitis in man. At present no antiviral treatments or prophylactics are available against this dangerous pathogen whose mortality can reach 92%. NiV entry, like that of other paramyxoviruses, is believed to occur via pH-independent fusion of the virion envelope with the host cell’s plasma membrane, mediated by the concerted action of the viral glycoproteins, following receptor attachment. The NiV receptor ephrinB2 is a ligand for members of the Eph class of receptor tyrosine kinases such as EphB4. Ephrins and Ephs are plasma membrane-bound proteins which play an important role in both vasculogenesis and neurogenesis: signaling mediated by contact between opposing EphB4- and ephrinB2-expressing cells results in bi-directional trans-endocytosis of receptor-ligand complexes and subsequent cellular repulsion.

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Conclusions: Our finding that NiV enters by an endocytic mechanism is exciting because it should now be possible to develop a cheap, safe novel antiviral treatment effective not only for NiV but also Hendra virus.
Background: Marburg hemorrhagic fever was diagnosed in a Dutch tourist after returning from a trip to Uganda. Both clinical and public health experiences are presented.

Methods: Case report

Results: A 41-year-old female had been traveling to several national parks of Uganda between 5 and 28 June 2008. On July 5th she was admitted to a hospital because of 2-day history of fever and nausea. On July 7th she was transferred to the Leiden University Medical Centre because of the rapid development of severe acute liver injury. Before transfer, strict isolation measures were taken because of the suspicion of viral hemorrhagic fever. The patient was exposed to bats during the visit to the "python cave" in the Maramagambo Forest on 19 Jun 2008.

Rapid deterioration with severe hemorrhage, shock, renal failure, and fulminant liver failure occurred. The patient died on 11 Jul 2008 because of cerebral edema.

Plasma Real Time PCR for Marburg virus was positive and sequence analysis of viral RNA showed 98% homology with the Marburg Victoria Lake Popp strain. Furthermore, Marburg virus could be cultured from the patient’s blood and demonstrated by electron microscopy (Bernard Nocht Institute for Tropical Medicine, Hamburg, Germany). No antibodies to Marburg virus could be demonstrated. Contact tracing of unprotected and protected contacts with possible exposure starting on 2 Jul 2008 was initiated. Regional, national and international follow-up and communications were intense, prolonged and successful.

Conclusion: This case report adds circumstantial evidence to the involvement of cave-dwelling Rousettus aegyptiacus bats in the transmission of Marburg virus. Visiting caves inhabited by bats in sub-Saharan Africa should be regarded as a risk factor for acquiring Marburg viral hemorrhagic fever.


Background: Rabies is a zoonotic viral disease which infects domestic and wild animals. It is transmitted to other animals and humans through close contact with saliva from infected animals. Rabies is usually fatal if adequate treatment is not started on time. WHO estimated that the number of deaths due to rabies was 55,000 in 2004. Deaths occur mostly in rural areas and 99% of all deaths occur in developing countries. Ten million people receive post-exposure anti-rabies treatment due to suspected animal bite. Accurate data are lacking in developing countries. The objective of our study is to summarize the epidemiologic trends of rabies in animals and animal bites in Jordan from 2000 to 2007.

Methods: A retrospective epidemiological analysis was performed on data collected on animal rabies and bites from surveillance. Vaccines serological sections are held in the directorate of diseases control in the Ministry of health as it is the reference diagnostic laboratory for rabies in Jordan. Specimens of rabies viral antigen in brain material from animals with suspected rabies were tested. All specimens were tested by direct fluorescent antibody technique using standard protocol. Animal bite data from annual epidemiologic reports of the communicable diseases control directorate were used and analyzed on epidemiological features comparing 2007 data with the six years preceding period (2000–2006).

Results: Between 2000 and 2007, the total number of animal bites was 15,756 with an average of 1,947 cases per year (min 1,332 (2002)–max 2,807 (2007)). The incidence rate for animal bites in 2007 was 48.9/100,000 population. More than 50% of animal bites were related to dogs. Cases distributed by geographic areas were 53% for central, 45% for North and 2% for South of the country. Data did not show any seasonality.

Human males were more exposed than females (ratio is 3:1), and the distribution of animal bites cases by age showed that cases aged more than 20 years represented 43%.

From 2000–2007, 108 animals were confirmed positive for rabies in laboratory tests. Rabies cases in animals increased from 1 case in 2003 to 50 cases in 2007. Dogs (56; 52%) represented the largest proportion of animals, cattle (20; 21%) sheep (7; 6%), and goats (6; 6%) also became infected. No human cases occurred since 1997. One human rabies case occurred in 2007.

Conclusion: Since 2003, a clear increase of animals infected with rabies was observed in Jordan. However reported cases represent only a part of the total infected animal numbers. In fact, this total number is underestimated as many infections are probably unobserved and never tested or reported. Similar patterns were observed in developing countries. In Jordan, most rabies infections occurred among dogs, and rabies in animals in spread mostly in the northern region of the country. No change in surveillance procedures for rabies has been made in Jordan during this period.

Close surveillance of rabies in animals and animal bites in human should be continued as well as provision of post-exposure treatment of humans following animal bites or exposures likely to transmit rabies. Health education should be reinforced for high endemic area especially the north.

10.053 Hydatidosis Epidemiology at Humans and Animals in the Area of the Center and North-West Romania

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Background: Studies effectuated in the latest years reveal that the prevalence of echinococcosis is increased in many countries, including Romania with the area taken in study. Cystic echinococcosis in humans represent a major problem of public health, responsible of hospitalizations and surgical interventions each year.

Methods: It was take in study 683 sera samples from 7 counties from Ardeal area, into the period of 2006–2007. The infection was detected by demonstrating the presence of specific antibodies by ELISA in humans and animals.

Results: From the total of 683 samples, 35 were positive for hydatidosis, resulting a general prevalence of 5,12 %. A significant higher level is observed in the rural (7, 24%) medium than urban (2, 77%), in females (6, 72%) than males (3, 55%). The incidence is higher in adults (5,15%) than child (5,05%). The presence of hiatidic antibody in the child reveal the recent transmission of the disease, a prove of the active maintenance of the parasite in the territory. In the area of the center and north-west of the country, it was made an epidemiological study in abattoirs. In sheep, the prevalence of hydatidosis reveal by necropsy was 69,1%, and the sera prevalence reveal by ELISA of 81,8%. The swine and the horses are less infected than the ruminants, the sera prevalence at this species being 10, 72% for the swine and 3,26% for the horses. The incidence of the disease in bovines and sheep vary between 80% and 100%. In pigs, the incidence of the disease is around 2 and 20%.

Conclusion: Our studies reveal the necessity of the active surveillance of hydatidosis, disease with important social and economic aspects and emphasize the need of national program for the control of these infections in humans and animals.

10.055 First Crimean-Congo Hemorrhagic Fever Case in Greece

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Background: Crimean-Congo Hemorrhagic Fever (CCHF) is a severe viral disease with fatality rate up to 30%. It is endemic in Asia, Southern Europe and Africa. In Greece, a CCHFV strain, AP92, was isolated in 1975 from ticks; however, no clinical cases have been reported in the country up to 2008.
Case Report: On June 21, 2008, a 46-year-old woman was admitted to University Hospital of Alexandroupolis, because of a febrile syndrome accompanied by malaise and nausea; she presented slight thrombocytopenia (158,000/mm3). She reported a tick bite three days before admission. Liver enzymes (AST, ALT, LDH) concentration elevated next day, while aPTT was undefinable. Severe ascites under pressure in all peritoneal spaces was seen in CT. Bone marrow aspiration revealed haemaphagocytosis. Antibiotics and supportive therapy with fresh frozen plasma and erythrocytes were applied. On June 23, she presented mictorrhagia and disseminated intravascular coagulation. She was transferred to ICU, and she was intubated. Next day she died with multiple organ failure.

Results: An RT-nested PCR performed on serum and whole blood samples taken the 3rd day of illness were found positive for CCHFV, while quantitative Real-Time RT-PCR showed that the woman was carrying a CCHF viral load of 2.97 x 10^8 copies/ml; this amount of virus is usually associated with fatal outcome. As expected, no IgM antibodies were detectable. Sequencing of the PCR product revealed that the causative strain was a CCHF viral strain, closely related to other European/Turkish CCHFV strains, highly different with the already known Greek strain AP92, which is suggested to be low- or not pathogenic. The highest identity was observed with CCHFV strains from Bulgaria, Kosovo and Albania rather, than from Turkey.

Conclusion: The first CCHF case was observed in Greece. It is known that Hyalomma marginatum marginatum, the main vector of the virus, is present in Greece; in addition, endemic foci are present in neighboring countries with similar ecosystem, suggesting that there is high risk for occurrence of additional cases in the near future.


Background: Former Yugoslavia was endemic for cystic echinococcosis: incidence of 3.7 (1969) and 4.7 (1985) per 100,000 population, with hyper-endemic regions with incidence of 12 per 100,000 population in Dalmatia, Hercegovina, Montenegro, Eastern Serbia, Sandzak, Kosovo, Macedonia. In Serbia, just in Institute for Digestive Diseases in Belgrade from January 1990 to December 2005, 651 patients were operated from liver echinococcosis. Cystic echinococcosis in Serbia still poses certain economic and ecologic problems due to few imported facts: (i) a large number of stray and free-roaming owned dogs in the cities as well as in the villages; (ii) numerous of small private slaughtering houses lacking adequate facilities, close to human settlements; (iii) home slaughter and consumption of non inspected meat from cattle, sheep and pigs. The aim of our investigation was to determine the presence of G1 sheep, G5 cattle and G7 pig genotypes of Echinococcus granulosus in liver cysts of operated patients in Serbia.

Methods: Liver cysts were obtained from four patients after surgical intervention. For each cyst viability test with 0.1% eosin was performed. From all examined cysts laminar layers and protoscolicides were removed and stored at -20°C until further processing. For each isolate, the genomic DNA was extract with the High Pure PCR Preparation Kit (Roche Diagnostics, Mannheim, Germany), based on Proteinase K digestion. Two mitochondrial genes were amplified by PCR: cox1, a portion of the gene coding for cytochrome c oxidase I and nad1, a portion of the gene coding for NADH dehydrogenase I.

Results: After viability test cysts were found to be viable in all four cases. The mitochondrial cox1 and nad1 fragment sequences of our samples showed that two of the samples correspond to sheep G1 strain and two of them are pig G7 strains.

Conclusion: Till the recent time, it was questionable if the pig G7 strain is infective for humans at all. Our results confirm, for the first time, that pig G7 strain is present in human liver cysts in Serbia. Further investigations should be performed in genotyping of human cysts from other sites, as well as from sheep, cattle and pigs from Serbia.


Vibrios are important members of the autochthonous flora of marine and estuarine environments and include non pathogenic bacteria and pathogenic species capable of causing seafood-borne gastroenteritis, wound infections and septicaemia. During the summer of 2006, wound infections in bathers caused by Vibrio species were reported from several European countries: three people with Vibrio vulnificus infections in Germany, three people from Sweden with non-agglutinating and non-toxin-producing V. cholerae infections and a couple of cases in Poland. In Denmark, it has been reported 15 human infections, mainly skin and ear infections with V. alginolyticus and V. parahaemolyticus. The atypically high temperatures in...
the summer of 2006 probably provided conditions favourable for the growth of Vibrio bacteria in the environment.

To investigate on the virulence potential of non pathogenic Vibrio strains, a screening has been conducted by PCR on a collection of 110 environmental strains isolated from water, plankton and sediment samples in the area of the Venetian Lagoon, Italy considering a number of virulence, fitness and antibiotic resistance genes. Virulence genes included V. parahaemolyticus yopP and thr and the nanH gene from V. cholerae encoding a neuraminidase. flaA is a gene involved in the regulation of V. cholerae flagella synthesis and response to environmental changes and vpsR is a gene involved in biofilm formation and environmental persistence of V. cholerae while V. cholerae luxA gene is involved in bioluminescent expression. int15’cs is a gene included in a cassette of antibiotic resistance determinants in V. cholerae.

About one third of the strains (36/110) resulted positive to PCR indicating that they carried at least one of the considered genes: 9 strains carried the gene nanH and 3 the yopP gene while no strains carried the thr gene. 12, 5 and 5 strains presented respectively the expected amplicons for flaA, vpsR and luxA while 6 strains carried int15’cs, a gene involved in antibiotic resistance.

Biochemical and molecular methods were applied to the PCR-positive strains in order to identify them at the species level: 20 strains resulted to be V. alginolyticus, 5 V. metschnikovii, 1 V. mimicus, and 2 Photobacterium damselae. One strain was eliminated from the study in that resulted to be V. cholerae while other 8 strains have not been yet definitively identified. Data obtained in this study indicate that the marine environment might constitute a reservoir of virulence and antibiotic resistance genes carried by autochthonous bacterial species which could represent a risk for human health.

**10.059** Documented freedom of Echinococcus multilocularis in Sweden


**Background:** Echinococcus multilocularis (EM) has never been recorded in Sweden, neither in man nor in animals. Before Sweden became a member of the EU in 1995, it was compulsory to de-worm dogs and cats against EM before entering Sweden. In 2003, when the rules for transporting pets to and within the EC were harmonized, this requirement was accepted as a Swedish additional guarantee. When EM was discovered in its natural host, the red fox (Vulpes vulpes), in Denmark, previously considered free from EM, it was decided to initiate an active surveillance of EM in wild red foxes in Sweden.

**Methods:** Between 2001 and 2007, between 200-300 wild red foxes received from the ordinary hunting bag have annually been investigated for EM; in total 1,987 animals. Fecal samples from all foxes were analyzed with a Copro-ELISA at University of Zurich (Dr. F. Grimm). From all individuals where fecal samples gave a positive OD value in the test, eggs of Taeniidae tapeworms, when found, were analyzed by PCR. In addition to the intestines from positive foxes a further 50-100 intestines per year were investigated for EM with the sedimentation and counting technique (SCT) (WHO/OIE Manual on Echinococcosis, J. Eckert et al. 2001), in total 498 samples.

**Results:** In no case was EM confirmed.

**Conclusion:** Sweden is considered to be free of Echinococcus multilocularis. This is further supported by the fact that alveolar cysts of EM never have been recorded in humans or at regular meat inspections at slaughterhouses in Sweden.

**10.060** Toxoplasma gondii in Wildlife of Svalbard

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The zoonotic protozan Toxoplasma gondii is known as an agent infecting virtually all mammals and birds worldwide. Since cats (Felidae) are the only animals known as final hosts for the parasite (sexual reproduction), the parasite is traditionally linked to presence of cats. Recent findings demonstrate widespread of the parasite also in wildlife of the high-Arctic archipelago of Svalbard, where cats are virtually absent due to harsh climate conditions. By use of a direct agglutination test, high seroprevalences of anti-T. gondii antibodies were demonstrated in the polar bear, Ursus maritimus (24.3%), arctic fox. Vulpes lagopus (43%) and barnacle goose, Branta leucopsis (7%). Also one out of 17 walrus (Odobenus rosmarus) was seropositive, whereas svalbard reindeer (Rangifer tarandus platyrhynchus) and sibling voles (Microtus levis) were seronegative, the latter indicating oocysts on the ground as not important for the spreading of the parasite in the Svalbard ecosystem. By PCR typing of the parasite from arctic fox brains, type II (strain most commonly found in European mainland) was most commonly found, suggesting an important role for migratory birds in the spread of the parasite to Svalbard. More recent findings of high seroneg+ivalences also among bearded seals (Ergathus barbatus) from Svalbard, a species feeding mostly on marine benthos and fish, suggest importance also of other spreading mechanisms linked to the marine environment.

**10.061** Potential Consequences of Introduction of Echinococcus multilocularis Into Sweden

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**Background:** Five EU member states in, including Sweden, are free from EM. Sweden requires that dogs and cats entering Sweden are treated against EM by a veterinarian with an anthelmintic before entry. Pets from Finland, Norway, Ireland Malta and United Kingdom are exempted. However, the EU Commission has indicated that due to the cost and inconvenience of the present requirements are considered disproportionate. The costs of keeping the present regulation compared to costs if EM were introduced into the country were therefore estimated. As data for cats was not available only dogs were included in the assessment.

**Methods:** Potential consequences were estimated quantitatively when possible. If EM were introduced it was assumed that all dogs were dewormed monthly. Costs for de-worming of introduced dogs were based on the estimated number of introduced dogs in 2003 and an average weight of 15 kg. Costs for de-worming were 2.2 Euro/5 kg.

**Results:** Potential consequences if EM became endemic: i) common outdoor activities such as picking berries and mushrooms would be affected. ii) Increased need for continuous awareness campaigns for the public and risk-group owners such as dog owners, farmers and hunters on how to handle potential risks. iii) Increased need for surveillance in foxes estimated to more than 5000 Euro annually. iv) It has been estimated that between 9 to 42 human cases may be diagnosed annually and the costs of living treatment per patient has been estimated to be 300 000 Euro. v) Annual costs for de-worming all dogs in Sweden, estimated to be 56 million Euro. In summary: more than 60 million Euro. Costs with the present system: costs for de-worming of all introduced dogs is approximately 0.2 million Euro per year (excl. veterinary costs)

**Conclusion:** The costs of keeping the present regulation is not considered to be disproportionate.

**10.062** Assessment of the Risk of Introducing Echinococcus multilocularis Into Sweden by Dog Movement During a 10-year-period

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**Background:** Echinococcus multilocularis (EM) causes a serious disease in humans and is considered to be an emerging pathogen in parts of Europe and Japan. Once introduced and established in a region it is, with present knowledge, impossible to eradicate.

Five member states in EU, including Sweden, consider themselves as free from EM. During a transitional period ending in 30 June 2010 require-
ments for de-worming are in place for pet movements into these countries. For all dogs and cats entering Sweden veterinary treatment with an anthelmintic against EM is required before entry. Dogs from Finland, Norway, Ireland Malta and United Kingdom are exempted. However, Commission officials have indicated that the present requirements are considered to be disproportionate. A risk assessment was done to estimate the risk of introduction of EM by pet movements from other European countries into Sweden.

Methods: A quantitative risk assessment was done to estimate the risk of introduction of EM by dog movements into Sweden. The number of potentially exposed dogs introduced into Sweden RiskUniform (700;1500) and the prevalence of EM in these dogs Beta (0.13%, ml 0.5%, max 7%) were estimated. Different scenarios of compliance between 0 to 99.9% was used. It may be assumed that a voluntary recommendation of de-worming might have between 30–50% compliance and that a compulsory requirement might have between 90 and 99.9% compliance. A model was constructed in Excel, and simulated in @RISK.

Results: The model showed that with 30–50% compliance between 116–83 (upper 95% CI 287-205) infected dogs would be introduced during a 10-year period. With no de-worming corresponding figures were 168 (upper 95% CI 410) and with 90–99.9% compliance 16–0 (upper 95% CI 41–0).

Conclusion: De-worming with a high compliance is needed to minimise the risk of introducing EM into Sweden by dog movements.

10.063 Cats as Reservoir of Tick-Borne Zoonosis

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Domestic cat blood samples from Ischia island, Naples, Italy were collected with the purpose to analyse the presence of zoonotic tick born pathogens. Several reports have demonstrated that cats can act as reservoirs for different agent of zoonosis. For this reason we analyze the degree of spreading of pathogens in these pets, due also to the possibility that handling of cats may expose human personnel to an array of important or emerging feline-associated human illnesses that occur all over the world.

Materials and Methods: We collected 57 samples of whole blood with EDTA and 52 glands Fine Needle Aspiration (FNA) belonging to 57 cats. Samples were kindly provided by a private veterinarian office of Ischia during 2008. Samples were investigated by specific PCR to: Babesia spp. (Nagore D. et al., 2004), B. microti (Persing D. et al., 1992), Rickettsia spp (Tzianabos et al. 1989; Williams et al. 1992), Coxiella burnetii (To et al. 1996), Anaplasma spp (S. Stuen et al. 2003), A. phagocytophilum (de la Fuente et al. 2005; Bowen et al. 2003) as previously described.

Results: Six cats resulted infected by B. microti. As regarding the kind of samples three were whole blood and three FNA from different cats. All the samples were negative for the research of Rickettsia spp, Anaplasma spp, A. phagocytophilum and Coxiella burnetii.

Conclusions: Human babesiosis is an emerging zoonosis in Europe. Approximately 60 cases have been reported from Europe (Meliani et al., 2006). Most cases were caused by B. divergens at least 70%. Recently, a non-Babesia divergens organism causing zoonotic infection has been found in Italy and Austria (refered as EU1), most closely related to, but distinct from B. odocoi, parasite of deer. Over 300 human cases of babesiosis have been reported from the US, most caused by B. microti (Kjemtrup and Conrad, 2000). B. microti, once regarded as a single species, occurs as a world-wide species complex and although both phenotypic and genotypic features suggest that zoontic B. microti may occur in Europe, convincing medical evidence is lacking. Since the same parasite was detected also in wild foxes (Torina et al., 2007) it can be supposed that this parasite is subjected to some sort of changing, that make it capable of infecting non common hosts. Due to its nature, it can be possible that cats wander far from their homes and make contact with rodents, which are the natural reservoirs of B. microti. Since cats are often closely in contact with humans, they can act like a link between wild parasites and their owners.
Overall, kappa values were highly significant ranging between 0.763 ± 0.057) for the best ten models and 0.658 (± 0.108) for the worst ten models out of the 100 bootstrap samples. The equivalent figures for Sensitivity were 93.2% (± 1.76%) and 89.93 (± 3.48%) and for Specificity were 80.02 (± 2.94) and 70.77 (± 5.86%) respectively. Elevation of the sample sites was the best discriminator of HFRS presence and was selected in 99 of the 100 models, followed by the mean day-time Land Surface Temperatures selected in 96 of the models. These models show that in Sweden the disease is present in areas of lower temperature and at lower altitudes compared with areas of disease absence.

How this combination of low temperature and altitude determines successful PUUV circulation and/or bank vole numbers requires further investigation. PUUV viability outside the bank vole is known to benefit from lower temperature. Low altitudes in northern Sweden imply less snow-cover, as compared to higher altitudes. Lack of snow may drive bank voles to dispersal, which can result in the invasion of human dwellings.

**Conclusion:** We conclude that the better cooperation between clinicians, microbiologists and pathologist would result in diagnostics of more cases infected with rare helminthes.

**Figure 1. Indirect immunofluorescence of a patient serum with Capillaria aerophila antigen.**

**10.067 First Confirmed Case of Eosinophilic Meningitis Caused by Angiostrongylus cantonensis in the State of Espirito Santo, Brazil**

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**Background:** Eosinophilic meningitis caused by Angiostrongylus cantonensis is an endemic disease in Asian southeastern and in the Pacific islands. It was described just once in Brazil.

**Methods:** Confirmed case report of eosinophilic meningitis caused by Angiostrongylus cantonensis.

**Results:** A 40-year-old man ate a snail (Sarasinula marginata) after getting drunk and developed epigastralgia, headache, and itching 24-hours after. He presented, then, decreased strength in inferior limbs, nausea, myalgia and headache that has become worst and persisted for 2 weeks. Then he was admitted in an emergency room with intense headache, excitement, stiff neck and fever (38.3˚C). His cerebrospinal fluid showed: 1056 leucocytes, 20% of eosinophils, glucose: 33mg%, protein: 101mg%. ELISA IgG anti-Angiostrongylus in CSF: positive. He was treated with dexametasone 40mg/day and improved the neck stiffness and fever. He has used corticoid for 90 days and has a mild headache today.

**Conclusion:** The meningal syndrome, more than 10% of eosinophils in the CSF, positive serology and epidemiology support the statement that this is an eosinophilic meningitis caused by Angiostrongylus cantonensis, the first one described in the state of Espirito Santo, Brazil. This is an alert to the disease and to the necessity of thinking about the diagnosis mainly because the epidemiologic history may not be so clear.
Rotaviruses have been recognized as the major contributors of acute gastroenteritis in young children and are responsible for morbidity and mortality in children. Infection by rotavirus is the most common cause of acute dehydrating diarrhea in children under the age of 5 years. Dehydration causing Escherichia coli strains are the major pathogens associated with enteric disease worldwide. Among the six recognized patho-types of Escherichia coli, Enteropathogenic Escherichia coli are an important agent of pediatric diarrhea in both economically developed and underdeveloped countries. We reported a severe dehydrating diarrhea in a five month old male baby due to Group A rotavirus (G12P[6]) genotype co-infected with Enteropathogenic Escherichia coli (eeA-α). The severe dehydrating diarrhea with hypovolemic shock was successfully treated with intravenous fluid rehydration and antibiotic therapy. Sequence analysis of the VP7 gene from the neonatal strain indicated a high level of amino acid homology (98-99%) to other G12 strains reported world wide, suggesting introduction of novel rotavirus strain serotype into the community. The subtyping of the eae-a gene revealed atypical eaeA-α subtype.

Keywords: Rotavirus, Escherichia coli, Diarrhea, Gastroenteritis.

Geographical Races of Old World Screw-Worm Fly, Chrysomyia Bezziana Villeneuve, 1914, in South Western Iran

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The old world screw-worm fly (SWF), Chrysomyia bezziana villeneuve 1914 is an obligate parasite of warm-blooded animals, including humans. Infestation and damage of animal tissues by SWF larvae (myasis) causes serious livestock production losses in countries where the flies occur. It occurs throughout much of tropical and subtropical Africa, the Indian subcontinent and southeast Asia from southern china in the north to New Guinea in the south south (Norris & Murray, 1964, sutherst et.al. 1989).

Collections of the old world screw-worm-fly, Chrysomyia bezziana from different parts of its geographical rang have shown anatomical differences between populations from southeast Asia, Arabia and Africa.

It has been proposed that there are three geographical races of old world screw-worm: 1) an African race, 2) an Arabian race, and 3) a South East Asian race (spradbery,1991).

The present study concentrated on adult flies that cultured in the entomological research center of Razi Research Institute (Ahvaz-Iran). The larvae of chrysomyia collected from infested animals (Mostly sheep) and incubated under suitable substratum in the laboratory of entomology. In this study a total of 986 specimens (656 females and 330 males) were examined. All studies were carried out under a binocular microscope (X60 magnification). The characters used in the morphological assessment were all these previously reported to be of value in identifying distinctive geographical races of old world screw-worm by Spradbery, 1991.

The study of morphological characters of adult flies specially wings, frontale setulae and hairs of thorax showed that: 1) wing base is completely clear; 2) Soft hairs of the thorax (pleura ) are pale; 3) Lower(posterior) squama are brilliantly white, with long white hairs; 4) A few of setulae below vibrissa are black, and; 5) Genal groove below compound eye not heavily indented. These results are same with Arabian race characters, explained by Spradbery, 1991.

Assay-dependent Seroprevalence of Hepatitis E Virus (Hev) in Blood Donors from Brandenburg, Germany

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A new mite associated dermatitis (RMD) in humans in the Southern Italy from 2001 to 2008, where all cases were misdiagnosed by dermatologists. Eight cases involved citizens living in private apartments in six different cities and two cases from the Veterinary Service involving office employees in different cities. All mites collected were identified as D. gallinae, according to Baker, 1999. The source of the parasites were abandoned pigeons’ nests near the infested rooms. D. gallinae is one of arthropods most commonly found on feral pigeons. In today’s cities the hight pigeon population quickly increases due to an abundance of food and few enemies. This high population density allows the spread of diseases and parasites affecting the human population. Pigeons and others synanthropic birds build their nests close to human dwellings; when the avian hosts are not available, the mites can migrate into the nearest houses and bite humans. Red mites are well adapted to host absence and can survive months without a blood meal. It can lead to recurrent episodes of dermatitis (i.e. holiday homes). Our observations suggest that RMD in humans is an emerging public health problem but its misdiagnosis lead to underestimation of this epizoosnosis. Dermatologists should be more aware of RMD and include it in differential diagnosis of non-specific dermatitis. The possibility that D. gallinae can be a potential vector/reservoir of zoonotic agents needs further veterinary and medical research and a multidisciplinary approach to management and prevention.
Hepatitis E virus (HEV) is a major enterically transmitted pathogen in many developing countries, causing self-limiting, acute hepatitis. In developed countries hepatitis E has usually been seen in patients who have recently travelled to endemic areas. However, in these countries a number of cases of autochthonous (locally acquired) hepatitis E have been reported over the past few years. In most cases of autochthonous hepatitis E genotypes (GT) 3 HEV could be identified, which also has a high prevalence in pig populations world-wide.

The broad range of seroprevalence in low-incidence populations (from 3.2% in central France to up to 21.3% in US blood donors) might be dependent on the population tested and the assays used.

The aim of this study was to assess the HEV IgG seroprevalence in the German federal state of Brandenburg determined by two commercial ELISAs (Genelabs Diagnostics, Singapore; GL and Mikrogen GmbH, Germany: MK). Therefore, 202 serum samples from blood donors were screened for HEV IgG antibodies. The results were verified by a HEV strip-immunomassey currently being under development (Mikrogen GmbH, Germany), which uses recombinantly produced antigens of HEV genotypes 1 and 3. Additionally, IgG-reactive sera were tested for antibody-avidity by this strip-immunomassey.

Considering the results of both ELISAs, 30 sera (14.9%) were reactive for HEV IgG. The seroprevalence determined by MK ELISA was 12.9% (26) and by GL ELISA 6.9% (14). 100% (26) of the MK-ELISA and 78.6% (11) of the GL-ELISA HEV IgG reactive sera could be confirmed by strip-immunomassey with a high antibody-avidity.

Of 27 samples confirmed by strip-immunomassey, 10 samples were reactive in both ELISAs, 16 were MK-ELISA reactive but GL-ELISA negative and one was reactive in GL-ELISA but negative by MK-ELISA. Using a scanner and software for the automatic analysis and interpretation of the HEV IgG reactive test strips (recomScan, Mikrogen GmbH, Germany) 20 (74.1%) of these 27 samples were suspected GT 3 HEV and 7 (25.9%) GT 1.

Two of the HEV IgG reactive samples were also reactive for HEV IgM antibodies (determined by both ELISAs and strip-immunomassey) but negative for HEV-RNA. A sample, obtained 41 days from one of the blood donors was positive only for HEV-RNA (GT 3) verifying a recent HEV infection.

Our data will also be discussed with regard to age- and gender-distribution of the IgG reactive donors and to the necessity of an obligatory nucleic acid testing (NAT) for certain risk groups in addition to serology.

**10.074 Crimean-Congo Hemorrhagic Fever Induce Apoptosis**

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**Background:** Crimean-Congo hemorrhagic fever virus (CCHFV) causes severe and acute hemorrhagic fever in humans. Humans become infected through tick bites, nosocomial infections or by direct contact with virus contaminated tissue or blood. The mortality rate in humans is as high as 10-50%, fatal cases show a strong dysfunction in the coagulation system. CCHFV is the most widespread geographically of the medically significant tick-borne viruses and can be found in Africa, the Middle East, Asia, Central and Eastern Europe.

Up to today there are no effective antiviral drugs or vaccine available for the disease. CCHFV is a member of the Nairovirus genus within the Bunyaviridae family. The factors determining the virulence for this virus are completely unknown. Understanding of the molecular pathogenesis of this emerging virus is most important and necessary to design strategies for disease control.

Evaluation of the primary sequence of the nucleocapsidprotein of CCHFV demonstrated a cleavage site, for caspase-3 and -7. Caspase-3 prefers the sequence Asp-Glu-Val-Asp (DEVD) and believed to function as a linker between the prodomain and the catalytic domain of the enzyme. PARP is one of the main cleavage targets for caspase-3 and other caspases. In this project we will investigate if CCHFV can trigger apoptosis in different cell lines to gain further understanding regarding the pathogenesis of CCHFV.

**Methods:** CCHFV Np was cloned into a mammalian cell expression system (pSEV). Cells were infected with recombinant virus expressing Np and cells were harvested at different time points. Lysed cells were assayed for Np by Western Blot. To determine whether CCHFV infection induce apoptosis, different cell types were infected and treated with different caspase inhibitors.

**Results:** During infection with rSFV, PARP was degraded from 116 kDa to 85 kDa. Furthermore, we found that NP was cleaved to a smaller band (30kDa) in infected cells. We also found that PARP was cleaved in infected cells.

**Conclusion:** CCHFV Np serves a substrate through caspase mediated cleavage upon viral infection. By using a caspase-3 inhibitor, we could demonstrate that CCHFV induce apoptosis by the caspase-3 pathway. We will investigate which pathway uses by CCHFV to induce apoptosis.
**Background:** Crimean-Congo Hemorrhagic Fever Virus (CCHFV), a member of the Bunyaviridae family, is an important human pathogen causing a severe and often fatal hemorrhagic fever with a mortality rate of about 30–60 % in humans. It belongs to a group of viruses, which are prototypes of emerging or re-emerging pathogens and are classified as Biosafety Level 4 (BSL-4) agents. It is considered one of the most dangerous and likely agents to be used in a bioterrorist attack due to its ability to induce a fatal or seriously incapacitating illness, its relative infectivity in human patients, and the lack of measures available for its control. The requirement to handle the virus in a BSL-4 laboratory may explain the limited knowledge regarding the pathogenesis of the virus. However, identifying regulatory elements of virus replication and, accordingly, determinants of viral pathogenesis will be of pivotal importance in the development of an effective defense against CCHFV.

**Methods:** We used murine embryonic fibroblasts (MEF) derived from wt mice or PKR−/− or RNaseL−/− mice and infected them with CCHFV at a multiplicity of infection (moi) of 1. 24 and 48 hours post infection, we harvested supernatants and lysed the cells. Progeny virus titrations were performed on Vero cells and cell lysates were examined in Western blot analyses.

**Results:** Here, we describe the roles of interferon-inducible PKR and RNaseL in the inhibition of CCHFV replication. We have found that in wild-type (wt) mouse cells stimulation of PKR with dsRNA resulted in inhibition of CCHFV nucleocapsid protein (NP) expression and strong reduction of virus yields. In contrast, CCHFV-NP expression was not reduced after dsRNA stimulation of PKR−/− cells and the yield of progeny virions was less inhibited than in wt cells. We have also demonstrated that CCHFV infection does not induce the expression of PKR. Furthermore, there were no significant differences in CCHFV infection in wt cells and RNase L−/− cells.

**Conclusion:** Taken together, these findings indicate that PKR, but not RNase L, is an important mediator of the antiviral effect of interferons against CCHFV. Understanding innate immune defense mechanisms will lead to improved strategies to prevent and control this human pathogen.

**ABSTRACTS**

**International Meeting on Emerging Diseases and Surveillance 2009**

**10.076**

**Autochthonous HEV Infection of a German Plasma Donor**

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**Background:** Europe is considered to be non-endemic for Hepatitis E virus (HEV). However, in the recent past sporadic cases of autochthonous hepatitis E in European countries have been reported and are mainly caused by HEV genotype 3. Beyond this, studies in some European countries found high seroprevalences for anti-HEV in blood donors. Cases of transfusion-transmitted HEV infections by contaminated blood products were reported in Japan and even in Europe.

**Methods:** A highly sensitive quantitative HEV RT-PCR was established and used for the analysis of samples from a plasma donor. Long-range PCR assays generated overlapping fragments of the full-length genome. Serology was performed with two different commercially available ELISA kits.

**Results:** A plasma donor attracted attention due to high transaminases (ALT) in a voluntary testing at the phlebapheresis center and was hospitalized with acute self-limiting hepatitis. In this last donation high viremia (10E+6 copies/mL) was accompanied by anti-HEVIgM and increased ALT levels. Several plasma donations before onset of elevated ALT were screened and the sample two weeks before was already positive for HEV RNA (10E+4 copies/mL). The first European full-length genome sequence of human HEV was identified and showed divergent unique amino acid exchanges within ORF2 and ORF3. Phylogenetic analyses grouped the isolate within genotype 3, subtype 3f.

**Conclusion:** Sequence analyses and epidemiological data revealed that the origin of infection was most probably contaminated pork. This case raises the question of an additional HEV NAT testing of blood products for special recipient risk groups e.g., immunosuppressed patients and pregnant women.

**10.077**

**Learning the Lessons of the 2006/7 Rift Valley Fever Outbreak in Kenya and Tanzania**

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An outbreak of RVF in East Africa occurred in late 2006 through early 2007. This study documented lessons from the outbreak so as to inform future veterinary preparedness and response. The study consisted of focal group discussions and key informant interviews. Villages were the unit of analysis. Discussions took place in 15 Somali villages in North Eastern Province, Kenya, and 18 Maasai villages in Arusha Region, Tanzania. A variety of participatory epidemiological tools were used. The 2006/7 RVF outbreak incidence, case fatality and mortality rates in all livestock species in Kenya were higher than in Tanzania, but abortion rates were similar. Kenyan Somalis consistently identified signs such as abortion and froth/blood emanating from the nose as being indicative the disease sandik (literally ‘bloody nose’), but Tanzanian Maasai were less consistent. Risk factors associated with sandik in Kenya included heavy rain and swarms of mosquitoes. Sandik had last occurred during the floods in 1997/98, the last time RVF had occurred in the Kenyan study area. RVF was the disease found to have the highest impact on livestock-derived livelihoods in Kenya, ranking fourth or fifth in Tanzania, although it was not the most prevalent disease in either country. The mean time interval between the first retrospectively suspected RVF case in livestock and first veterinary service response was 61.7 and 113.1 days in Kenya and Tanzania. Between the first retrospectively suspected RVF case in livestock and the first public health service response it was 50.0 and 76.7 days in Kenya and Tanzania.

Livestock index cases were reported to be 30 November 2006 in Kenya and 13 January 2007 in Tanzania, but this study found that the disease was well established in livestock and humans by these dates. Because the disease was similarly well established by the time early warnings were issued and interventions put in place, they may have had limited impact. Early warnings are getting earlier, but timelines established by this study found the need for an early warning up to 6 months before rains start, pointing to the need to reassess the process of early warning distribution and their contribution to preparedness and prevention. This research found that RVF risk factors and signs were observed by pastoralists well before detection by veterinary services, and human cases well before detection by public health services. This shows the important role that livestock keepers can play in disease surveillance. ILRI and FAO have developed a tool to support East Africa veterinary decision makers in the prevention and control of RVF by facilitating timely, evidence-based decision-making. In September 2008 FAO warned that RVF could occur again in East Africa, to which the Kenyan veterinary service responded with multiple measures. ILRI is engaging in further research to determine what impacts the RVF Decision Support Tool had on Kenya’s 2008 response.

**10.078**

**A Preliminary Analysis of Leptospirosis Outbreak in 2008 in Sri Lanka**

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Leptospirosis was first reported in Sri Lanka in 1953 and the organism was isolated from human blood sample in 1959. For past decade or more, leptospirosis is endemic in some parts of the country with an outbreak situation once in every 4–5 years. Since the latter part of year 2007, Sri Lanka experienced an unprecedented outbreak with 7406 case notifications up to 26. 12. 2008. Districts with increased agricultural activity were
most affected. But not all traditional agricultural areas were equally affected. When compared with the year 2006, number of cases notified in 2008 was more than 400%.

A preliminary analysis of leptospirosis surveillance data available at the national centre for disease surveillance, Epidemiology Unit was performed to understand the epidemiological features of the current outbreak. 81% were males. Mean age was 40.2 years (SD 14.6) and 66% were between 25-54 years. Main presenting symptoms and signs were acute fever (99%), severe headache (90%), myalgia (91%), conjunctival suffusion (72%), prostration (37%), jaundice (24%) and anuria (33%). Case fatality rate was 2.9% with a wide district variation from 0.6 to 5.2%. 61% had a history of working in paddly fields while 24% exposed to marshy lands, 12% to other sources of water and 2% to other agricultural environment. Only 0.4% was exposed to animal husbandry. 51% of patients’ prolaphaxis history was unknown. Out of those with known history, only 3% were on prophyaxis.

In a subsample of 146 patients, 49% were positive (>800 titre level) for Microscopic Agglutination Test while 21 and 30% were equivocal or negative respectively. MAT was positive for L. pyrogen, australis, weerasingham, gem, and canicoila.

Available data are highly suggestive of that the current outbreak is linked with agricultural environment and the probable source of infection is rodents.

**Psittacosis Outbreak in Poultry Processing Workers in the East of England, 2008**

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Psittacosis (ornithosis) is an endemic disease of birds which can give rise to serious infections in humans, and outbreaks in UK poultry workers have been reported in the 1970s and 1980s. Following an outbreak at a local poultry factory, we conducted a cohort study to identify risk factors for infection.

Factory employees were recruited at two site visits. Exposures during the outbreak period (1st April to 5th June 2008) were assessed using a self-completed questionnaire and serum samples taken. Outbreak cases completing questionnaires were also included. Exposures included work areas and tasks, and exposures around and outside the factory. Serum samples were screened using complement fixation testing (CFT) and species specific antibody detected using whole cell immunofluorescence (WHIF). The primary outcome was evidence of recent infection (WHIF suggestive of recent infection or confirmed outbreak case), with any evidence of infection (CFT antibody>1/8 or confirmed outbreak case) being a secondary outcome.

65/215 (30%) employees were recruited of which 63 were included in the analysis. 8/63 (13%) had evidence of recent infection and 15/63 (24%) fulfilled the secondary outcome. Significant risk factors for the primary outcome included working in the killing (RR 3.73; 95% CI: 1.03-13.48) and evisceration areas (RR 4.00; 95% CI: 1.00-15.30), having contact with dead poultry (RR 6.52; 95% CI: 1.48-28.76) and having contact with contaminated hands (RR 5.88; 95% CI: 1.66-20.81) and having contact with contaminated water (RR 4.00; 95% CI: 1.11-14.43). A trematode (Dicrocoelid) was identified. For the first time, *Hymenolepis* is described in Bornean orangutans. Highest prevalence was found for *Strongyloides* (37% for individuals and 58% for groups), hookworms (41 and 58%), *Balantidium* (40 and 61%), *Entamoeba* coiI (29 and 53%) and *Trichosporon* (3 and 32%). Age and sex had the strongest influence on total parasite prevalence in individuals at 2 reintroduction centres and 3 field sites, respectively. In the centres, infants had a significantly higher prevalence for *Strongyloides* than adults, whereas in groups, the centres themselves had a significant influence on *Balantidium* prevalence. More wild males than females tested positive for hookworms. Wild male Bornean orangutans possibly serve as distributors for soil transmitted parasites. With respect to prevalence and pathogenicity, *Strongyloides*, hookworms and *Balantidium* represented the most important intestinal parasites in the investigated populations. Sequencing of rDNA suggests that a subspecies of *Strongyloides fuelleborni* with zoontic potential exists in Borneo orangutans.

**Screening of the Household Members and Contact Cases of Patients with Acute Brucellosis and Detection of Unrecognized Cases by ELISA**

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Background: Acute brucellosis among household members of an index case have been reported. The aim of this study was to determine active serological screening of the contact cases of patients with acute brucellosis can detect additional unrecognized cases.

Methods: A descriptive study was conducted among contact cases with an acute brucellosis patients in two provinces (Tehran and Lorestan) of Iran, between 2005 to 2007. An index case was defined as an individual with clinical syndrome of brucellosis and a positive history of epidemiological exposure that laboratory tests confirmed it. Contact cases were defined all of household and family members and their colleagues in slaughterhouse, husbandry or farm. 5 ml of venous blood samples were taken from index cases and contact cases then evaluated by using Brucella IgM, IgG, IgA ELISA kits.

Results: 36 Index cases with the mean of number of contact cases (4.5±2.5) with 117 contact cases [59 (50.5%) male, 58 (49.5%) female] were screened. Positive IgM, IgG, IgA ELISA titers were detected in [7(6%)], [ 25(21.5%)], [31 (26.5%)] of contact cases respectively. The seroprevalence rate was detected in 40 (34.2%) among contact cases. 38 (32.5%) of contact cases manifested various symptoms. Among 40 contact cases with positive serore prevalence, 14 (35%) had complaint, but among 77 contact cases with negative sero prevalence, 24 (31%) had complaint. There was not a significant correlation between positive sero prevalence with clinical complaint.

**Prevalence of Intestinal Parasites and Genetic Characterisation of *Strongyloides* in Bornean Orangutans (*Pongo pygmaeus*) in Central and East Kalimantan, Borneo, Indonesia**

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Fecal samples from 163 captive and 61 wild individual plus 38 captive groups of Bornean orangutans (*Pongo pygmaeus*) in Kalimantan, Indonesia, were collected during one rainy season in 2005/06 and screened for intestinal parasites by SAFC, sedimentation, flotation, Ziehl-Neelsen stain, McMaster and Baermann technique. In addition, the 18S and ITS1 rDNA genes of *Strongyloides* isolated from samples of 14 individuals and 19 groups were analyzed by direct PCR. The study aimed to determine factors that influence the infection risk for specific parasites in wild and captive orangutan populations and to get insight into which species of *Strongyloides* infect wild compared to captive orangutans and whether any anthropogenic influence can be determined. The findings may offer valuable clues as how to protect orangutans and humans from infections.

Six genera of protozoa (*Entamoeba, Eotrichomonas, Isodamoeba, Balantidium, Giardia, Blastocystis*), 4 nematode genera (*Strongyloides, Trichuris, Ascaris, Enterobius*), hookworms, a *Trichostrongylus*-like nematode and 1 trematode (Dicrocoelium) were identified. For the first time, *Hymenolepis* is described in Bornean orangutans. Highest prevalence was found for *Strongyloides* (37% for individuals and 58% for groups), hookworms (41 and 58%), *Balantidium* (40 and 61%), *Entamoeba* coiI (29 and 53%) and *Trichosporon* (3 and 32%). Age and sex had the strongest influence on total parasite prevalence in individuals at 2 reintroduction centres and 3 field sites, respectively. In the centres, infants had a significantly higher prevalence for *Strongyloides* than adults, whereas in groups, the centres themselves had a significant influence on *Balantidium* prevalence. More wild males than females tested positive for hookworms. Wild male Bornean orangutans possibly serve as distributors for soil transmitted parasites. With respect to prevalence and pathogenicity, *Strongyloides*, hookworms and *Balantidium* represented the most important intestinal parasites in the investigated populations. Sequencing of rDNA suggests that a subspecies of *Strongyloides fuelleborni* with zoontic potential exists in Borneo orangutans.
Conclusion: The high seroprevalence rate (34.2%) and symptomatic individuals among contact cases (35%) in this study showed the household members are not the single most important identifiable risk group, and screening of other contact cases is necessary.

Outbreak Control

10.082 – 10.113 Room: Bruckner/Mahler/Brahms – First Level

10.082 Effect of Solar Disinfection on Viability of Intestinal Protozoa in Drinking Water

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Background: Contaminated drinking water causes the majority of deaths and diseases all over the world, mainly in developing nations. The effect of solar disinfection on the viability of intestinal protozoa Giardia lamblia, Microsporidia sp., Cryptosporidium parvum, Cyclospora cayatenensis and Entamoeba histolytica in drinking water was studied as compared to chlorine disinfection.

Methods: The protozoa were collected from stool samples, to infect the distilled water. Chlorinated water samples were prepared at concentration of 4 ppm, and the parasites were incubated overnight at room temperature with the treated water. Sun treatment was applied for 2 exposures (6 and 24 hrs), in summer and winter. Sun treated water samples were put in tubes and exposed to sun. The 2 disinfection methods were tested in plastic and glass test tubes. Parasites viability was assessed by viability assay using trypan blue stain (0.4%), and bioassay infectivity tests in experimentally laboratory bred mice.

Results: Proved that all parasites’ viability was not affected by chlorine, following solar disinfection treatment, parasites became dark blue in colour and deformed by trypan blue stain. High parasites death was following solar disinfection treatment, parasites became dark blue in colour and deformed by trypan blue stain. High parasites death was followed by detection of significant reduction in mean number of all parasites in intestinal sections compared to controls. The best results were tubes exposure to sun for 24 hrs in summer, where G. lamblia, C. parvum and C. cayatenensis were inactivated or absent in intestinal sections. No statistically significant difference was found between the use of plastic and glass tubes, either in chlorine or sun treated parasites.

Conclusion: Solar disinfection proved to be a simple, cheap and effective means for improving water for human use, particularly in developing countries.

10.083 Efficacy of Bovine Colostrum Against Experimental Cyclosporiasis and Microsporidiosis

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Background: Colostrum is the specific first diet of mammalian neonates. It is comprised of immune and growth factors that have been designed by nature to protect against diseases and organisms. The bovine colostrum (BC) has been determined to be functionally identical to human colostrum (HC): even better, as it is richer in the immune factors. The efficacy of BC has been shown before for prevention and treatment of different disorders, mainly gut-associated diseases.

Methods: To evaluate the effectiveness of the BC as a prophylaxis for Cyclospora and Microsporidia infection, BC was administrated orally two days before infection, as well as to evaluate it as a management, BC was administrated one day following the infection in experimentally immunocompetent and immunosuppressed mice. Also BC was analysed to know its contents.

Results: Analysis of BC proved its contents of immunity and antimicrobial factors. Oral administration of BC two days before the infection eradicated the Cyclospora oocysts completely either from the stool or the intestine of both immunocompetent or immunosuppressed subgroups of mice. However, in case of Microsporidia, the spores were disappeared from the stool and the intestine of the immunocompetent subgroups only, and significantly diminished in immunosuppressed subgroups. On the other hand, when mice received the BC one day following the infection, the mean parasitic counts were significantly reduced in all subgroups either in case of cyclosporiasis or microsporidiosis. These results were coinciding with the histopathological changes detected in the intestine of the different subgroups.

Conclusion: BC proved its efficacy against the infection by both parasites, and it could have a vital role in the replacement of the life-supporting immune and growth factors and in stabilization of the person health especially in immunocompromised hosts.

10.084 Awareness, Possession and Use of Insecticide Treated Net for Prevention of Malaria in Children Under Five in Abeokuta, Nigeria

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A survey was carried out to assess awareness, possession and use of Insecticide Treated Net (ITN) by mothers in preventing malaria among children under-five. Malaria though was considered dangerous by almost all respondents (98.5%); the level of awareness of ITN as a malaria preventive tool was 75.1% whereas possession was 45%. Awareness and possession of ITN were positively and significantly influenced by high educational qualification of mothers and attendance of a public hospital for antenatal care. Hospitals were identified as the major source of awareness among respondents; Women that delivered their babies in Traditional birth home displayed least awareness (38.6%) and recorded low possession (10%). There was no significant relationship between ITN usage, birth order and age of child. Heat experienced while sleeping under ITN and problem of how to hang the net were major limitations identified in the use of ITN. The need to involve women receiving antenatal care outside the hospital in malaria control intervention is hereby recommended.

10.085 Aeromonas Hydrophila and Stenotrophomonas Maltophilia Outbreak in Intensive Care Unit Epidemiological and Microbiological Characterization

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Background: Is there any connection between the nosocomial outbreaks and the tap water?

Results: In an outbreak that occurred in January/February, 2008 in our ICU, we identified A. hydrophila in haemocultures from 9 patients. Five of them were also positive for S. maltophilia. Each of the 11 samples taken from the taps of the 3 hospital rooms and the hospital’s water tower was positive for Aeromonas spp. Further identification of the bacteria found in the water tower showed it was A. sobria. After decontamination of the water tower and fitting the taps with bacteria water filters they were neither positive water samples nor infection reports.

Methods: Disk susceptibility determination according to CLSI, PCR determination of 1-3 integron and tnpA gene, plasmid profile determination PFGE.

Conclusion: The identical PFGE patterns of the strains in the water samples and from the infected patients, respectively, suggest that water was the source of this outbreak. A. hydrophila and S. maltophilia strains can survive in hospital environment. Due to the variety of A. hydrophila’s virulence factors, it can produce diverse clinical presentations, especially in immunosuppressed patients. Modification of antibiotic susceptibility can be traced by typing the strains. Particular attention needs to be directed to the possibility of horizontal antibiotic resistance gene transfer between concomitant bacteria and to the increasing prevalence of high virulence A. hydrophila strains.
ABSTRACTS

**10.086** Concurrent Outbreak of Leptospirosis and Dengue in National Service Training Camp in Terengganu, Malaysia 2007

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**Background:** An outbreak investigation was initiated on September 2nd 2007 following notification of two cases of suspected leptospirosis with differential diagnosis of dengue involving trainees at a National Service Training Camp (NSTC).

**Methods:** A retrospective cohort study was conducted with case definition of “trainees or staff presented with fever with or without headache or cough or sore throat or leg pain or myalgia or abdominal pain or jaundice or skin rash from August 17th 2007.” The diagnosis of leptospirosis was confirmed by a positive microagglutination test (MAT) and dengue by a positive IgM or IgG antibody capture ELISA test. Environmental risk assessment were carried out.

**Results:** Of 236 exposed populations, 55 (23.3%) met the case definition. Sixteen cases were hospitalized. Eight percent (4/51) and 7.8% (4/45) cases were laboratory-confirmed leptospirosis and dengue respectively. The leptospirosis case fatality rate was 25% (1/4 cases). Headache was significantly associated with leptospirosis (OR 0.05; CI 95% 0.004, 0.55). No clinical features were significantly associated with dengue. The significant risk factors for leptospirosis were trainee (RR 1.80; CI 95% 1.50, 2.17), male (RR 1.35; CI 95% 1.10, 1.65), age below 21 years old (RR 1.68; CI 95% 1.40, 1.98), water confidence (RR 2.00; CI 95% 1.80; CI 95% 1.68, 2.20), kayaking (RR 1.59; CI 95% 1.23, 2.05), monkey rack (RR 1.98; CI 95% 1.25, 3.05). Of eleven lake water samples tested for leptospirosis, three (27.3%) were positive by polymerase chain reaction test. Aedes Index (AI) of 2.4 and Breteau Index (BI) of 2 were noted.

**Conclusions:** A concurrent outbreak of leptospirosis and dengue had occurred in a NSTC. Water activities were identified as risk factors for leptospirosis and high AI, BI indicative of dengue outbreak. Contaminated lakes were most likely the vehicles for leptospirosis transmission.

**10.087** First Reported Food-Borne Botulism Outbreak in Terengganu, Malaysia, 2008

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**Introduction:** On May 28th, 2008, 2 cases of suspected food-borne botulism were notified to the Terengganu State Health Department (TSHD). Another 2 cases with similar history of food consumption at the same food outlet as the first 2 cases were notified to TSHD by Selangor and Johore SHD. An outbreak investigation was initiated to determine the outbreak and institute control measures.

**Methods:** A case control study with case definition of “those presenting with gastrointestinal symptoms and one or more of neurological symptoms and isolation of Clostridium or detection of botulism toxin in stool or blood, or a clinically compatible case with an epidemiologic link with the laboratory confirmed case,” was conducted. The controls were among patient’s healthy co-workers or housemates who ate at the same food outlet. Laboratory investigations and environmental risk assessment were performed.

**Results:** Of 18 known exposed individuals, 4 (28.6%) fulfilled case definition. All were male, median age 24 years (range 23–28). All were mechanically ventilated with one death (case fatality rate 25%). Stool culture isolated Clostridium baratii in one case. Neurotoxin was not detected in all serum and stool samples. Left-over food samples were not available for laboratory investigation. Home made fermented durians (tompoyak) served on May 20th was significantly associated with this outbreak (p<0.05). Hazard analysis control and critical points (HACCP) of “tompoyak” revealed two critical points—during fermentation which is conducive for germination of Clostridium spores and production of toxin, and during serving of untreated “tompoyak” at the restaurant. Other contributing factors were unsanitary food outlet and unhygienic food handling.

**Conclusion:** This is an outbreak of food-borne botulism caused by Clostridium baratii. Type of toxin and source of the infection responsible for this outbreak could not be determined. The possible vehicle was “tompoyak” as ingestion of preformed toxin in the “tompoyak” might cause the illness.

**10.088** Outbreak of Scedosporium Prolificans in Haematological Patients

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**Background:** Scedosporium prolificans is a recently emerging opportunistic fungus that causes different degrees of infection depending on the route of infection and the immune status of the host. Infections by S. prolificans are not homogeneously distributed around the world, and are specially frequent in Spain, Australia and USA. The main aim of present study was to know the origin and risk factors causes of epidemic outbreak produced by S. prolificans in our hospital.

**Methods:** Environmental studies were carried out using MAS 100 instrument and plate Saboraud dextrose agar. A case control study was performed. Six cases and 24 controls were patients with leukaemia. The cases were defined as S. prolificans patients infected or colonized. The controls were patients during the same period of time, and selected in a random fashion.

**Results:** Five of six haematological patients affected died. The isolate of S. prolificans was manifested between February 2008 and April 2008 (Three cases per month in February, two cases in March, and one case per month in April) Only two patients were neutropenic for several weeks and the others four non. S. prolificans was isolated in sputum in five patients and endotracheal aspirate in one patient. The antifungal susceptibility test of six isolated was the same. S. prolificans was isolated from the air of the rooms used by some patients. Four patients had received chemoprophylaxis antifungal.

**Conclusion:** S. prolificans in leukemic patients is untreatable and leads to death. Risk factors for isolated was prophylaxis, and the age. The airborne origin of S. prolificans outbreak was confirmed.

**10.089** Swine Vesicular Disease in Lombardy Region: Pattern of Spread in a High Density Pig Area

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Swine vesicular disease (SVD) is a vesicular condition of pigs induced by an Enterovirus. Although the disease is frequently mild in nature, it was included in List A of the OIE for the similarity of its lesions to those produced by Foot-and-Mouth Disease (FMD). Even though compared to FMD, SVD is considered moderately contagious, morbidity is lower and the lesions less severe.

In Europe in the last decade, SVD has been persistently reported in Italy and for this reason surveillance and eradication activities are in place. In the period 2006–2007 SVD spread widely in the Italian Northern Regions, Lombardy, a densely populated pig area, was most affected and difficult to control. The outbreaks reported in the period may be grouped in two epidemic periods. During the first one SVD spread among the farms according to the typical pattern of transmission. In the second period, instead, the diseases showed an endemic trend in a small portion of the region (27 km²), where the main risk factor for outbreaks, was proximity to a previous outbreak. To achieve eradication in this area, it was necessary depopulate a group of pig farms, considered at risk of infection.
**Background**: Epidemic myositis (EM) is a rare syndrome following several viral infections, primarily affecting children. The characteristic severe muscle pain and walking difficulties can be misjudged as a severe neurological disorder. In February 2008, an increase of EM cases was seen by paediatricians in Northrhine-Westfalia. A nationwide investigation was started to verify the outbreak, its extent and identify whether influenza B contributed to this outbreak.

**Methods**: We performed active case search through public health authorities and a paediatric internet platform. Probable cases were defined as children clinical picture of EM developing after a febrile illness between 1.10.07-1.6.08. Confirmed cases were probable cases plus an influenza diagnosis confirmed by PCR or rapid test. Controls were children with confirmed influenza diagnosis, taken from the national reference laboratory and a sentinel registry. Nationwide, clinicians were asked to send back questionnaires on symptoms of cases, laboratory results and number of EM-patients seen in previous years.

**Results**: Altogether 288 cases were recorded from all regions in Germany, 50% of cases presented between 18.2. and 19.3.2008. Among cases were 210 (73%) boys, median age was 7 years. Symptoms resolved completely after a mean of 4 days, only one severe complication (rhabdomyolysis) was reported. 38% cases required a hospital visit, only 22% of paediatricians had seen EM in last 5 years. Influenza B-PCR was positive in 42 cases. Influenza A in 3 cases. OR for influenza B as risk factor for EM was 19 (95%CI:6,9;96,8), male sex 2,3 (95%CI:1,6;3,3) and positive in 42 cases, Influenza A in 3 cases. OR for influenza B as risk factor for developing EM was 19 (95%CI:6,9;96,8), male sex 2,3 (95%CI:1,6;3,3) and positive in 42 cases, Influenza A in 3 cases. OR for influenza B as risk factor for developing EM was 19 (95%CI:6,9;96,8), male sex 2,3 (95%CI:1,6;3,3) and positive in 42 cases, Influenza A in 3 cases.

**Conclusion**: An outbreak of EM could be verified. EM was associated with influenza B and male sex. Vaccination against influenza might be protective against developing EM. A substantial number of paediatricians had not seen EM cases in the last 5 years, therefore paediatricians should be informed about circulating strains during influenza season and reminded of influenza B’s potential to cause EM.

The impressive clinical picture but usually benign outcome should be communicated to physicians to avoid unnecessary anxiety among patients, parents and physicians.

Further studies into risk factors for developing the syndrome are needed to recommend prevention measures.

**Methods**: In the present study, we applied a novel genotyping method based on the analysis of 15 mycobacterial interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR) to characterize 50 M. tuberculosis strains isolated in Łódź, Poland, in 2006-2007. MIRU-VNTR method relies on PCR amplification of multiple tandem repeat loci. The size of amplicons reflects the number of targeted MIRU-VNTR copies in the chromosomal DNA of M. tuberculosis. Results are digitized. Related strains show the same MIRU-VNTR patterns. There are some variants of MIRU-VNTR typing, e.g. 12-loci based system which, combined with spoligotyping, provides adequate discrimination comparable to IS6110 RFLP. The new 15-loci based MIRU-VNTR typing system has been recently proposed.

**Results**: Molecular analysis using this method showed a high level of heterogeneity among the MIRU-VNTR patterns (that indicate their epidemiological independence) while 24% of strains was clustered into 5 groups with identical MIRU-VNTR patterns. The occurrence of 5 distinct clusters among the 50 strains tested suggests that these TB cases developed active disease from recent active transmission. However, this conclusion cannot be proved because of poor patients’ information. Discriminatory power of each MIRU-VNTR sequence was also determined. The highest discriminatory power was observed for MIRU-VNTR loci: VNTR4052, MIRU10, VNTR2163b, MIRU40, MIRU26 and VNTR1955.

**Conclusion**: The results indicate that the novel genotyping method could be a valuable tool for studying the genetic diversity of M. tuberculosis strains. However, to further confirm its utility in epidemiological studies of tuberculosis its evaluation on a larger number of strains and comparison with the reference IS6110 RFLP will be necessary.
ABSTRACTS

10.093 The Delivery of *Bacillus thuringiensis israelensis* to Adult Mosquitoes—Implications for Malaria and Dengue Control

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*Bacillus thuringiensis israelensis* (Bti) has been used successfully and safely to control mosquito larvae for decades. A new method for delivery of Bt to adult mosquitoes has been shown to be effective in significantly reducing the number of mosquitoes in laboratory trials. A plastic flower-like device was developed with visual, olfactory and chemical cues to target and deliver the Bti formulation to Anopheles and Aedes species. Field trials are being conducted to determine the reduction of mosquitoes in homes. This method may prove to be a "GREEN" and inexpensive tool in combating mosquitoes and the pathogens they transmit.

10.094 *Mycobacterium massiliense* Infections Following Video-Surgeries or Implant of Breast Prosthesis at Metropolitan Area of Vitoria (Brazil): 126 Cases Treated.

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Since Aug/2007, *M. massiliense* infections after video-surgeries or implant of breast prosthesis at different hospitals of Vitoria were referred to university hospital for at least 6 months of antimicrobial therapy; 2 cases of bariatric surgery or arthroscopy, 12 months. Option I: clarithromycin (Cl) 500 mg 12/12 h, ethambutol (E) 1.2 g and therrizidine (Th) 750 mg per os. Option II: Cl, E, per os, and amikacin (Am) 1g intramuscularly once a day 3x/wk. Since Feb/08, minocinoc (Min) 100 mg 12/12h and/or moxifloxacin (Moxi) 400 mg/day have been added to Cl and Am in place of Th and E, considering the probable resistance of *M. massiliense* (high minimal inhibitory concentrations).

Among the 88/126 pts (69.8%) treated with option I, all drugs were kept in 12 and changed for Am in 1 (side effects to Cl and E). Th and E were changed for Am in 75 pts (four pts submitted to arthroscopy [low levels of antimicrobials in joints], four bariatric surgery [limited intestinal absorption], 49 due to MIC results [probable resistance], 14 non-clinical response, three and one side effects of Th and E, respectively). Among the 31/126 pts (24.6%) treated with option II, E was interrupted in 25 due to high MICs, one vomiting and one visual turvation; Am was interrupted in one due to dizziness, two hypoaecusis, one neuropathy and one pregnancy, five to attend patient’s requirement, nine and five after six and three months of use, respectively.

Two pts (1.6%) were treated with Cl and Am; the last one was interrupted in one due to hypoaecusis and to attend patient’s requirement in the other. Two other pts (1.6%) were treated with Cl, Am and Moxi. Am was interrupted in one after 6 months of use.

Three pts were not treated since there was no evidence of active disease.

10.095 Comparing the effectiveness of Hantavirus outreach in Northwestern New Mexico, Panama, and Chile

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This research compared the effectiveness of Hantavirus Pulmonary Syndrome prevention outreach in southwestern New Mexico, the Los Santos region of Panama, and Region Araucania IX in Chile. Outreach effectiveness for hantavirus is not well understood, even though outreach in Chile appears to be more extensive than in southwestern New Mexico and Panama. Understanding the role of human demographics, disease ecology, and subsequent human behavior in the disease process is critical to the examination of community responses in terms of behavior changes. Through the implementation of a self administered 28 question survey instrument (n=600), I measured attitudes towards, and across public health conditions with respect to hantavirus within three human populations of similar disease ecology and assessed whether knowledge and behavior change with respect to hantavirus is greater in high prevalence areas vs. low prevalence areas.

Respondents in the high risk, rural poor sites in southwestern New Mexico and Panama continue to sweep and vacuum more than in Chile. Respondents in Chile have a tendency engage in proper cleaning methods of disinfesting and mopping than those in southwestern New Mexico and Panama. Levels of concern over contracting hantavirus were higher in New Mexico and Panama which indicates the public wants proper information on how to protect themselves and their families from exposure. While public health messages appear to be more effective in Chile, improper cleaning messages still affect behavior in all populations. Improved messages are needed on what to do to decrease risk of exposure to disease in all populations.

10.096 Control of Re-emerging Imported Measles in Albania

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Background: In 2000, Albania endorsed four strategies to eliminate Measles by 2007. Here we describe the status of measles control in Albania after implementation of catch up immunization strategies and analyse the importance of several factors on remerging measles and its control.

Methods: Data from enhanced measles case based laboratory surveillance of maculopapular rash syndrome were analysed with outbreak investigation and laboratory testing reports. The measles routine vaccination coverage of children 1-year-old and especially Roma population were further analysed. Supplementary immunization activities were implemented and the coverage during such activities and previous catch up and follow up campaigns were analysed.

Results: During measles catch up campaign of children 1–14 years old, 867,000 doses of measles-rubella vaccine were administered with an estimated coverage of 99%. The follow up campaign of vaccination of women of childbearing age in reached a coverage of 95%. The number of measles cases was reduced to zero from 2002–2005. 127 cases of maculopapular rash suspected of measles were investigated in 2006–2007 and out of them 72 cases were tested IgM positive by ELISA and 16 cases showed a significant increase of IgG antibody concentration between acute and convalescent specimens. The first outbreak was related to Roma communities in south of Albania with an index case from Roma population coming back from illegal immigration in Greece. Clusters of 5–10 people caused by D4 strain were identified mainly in Roma communities and other suburban areas in South Albania. Another outbreak during the last quarter of 2006 was seen in a kindergarden in a rural area in Northern Albania and spread into Roma community in Shkodra and caused by B3 strains. An Italian nun with similar clinical signs of measles coming from Italy had contacts with the infected children. The routine coverage of Roma comunity was 77% and supplementary immunization campaigns were implemented in 2006 and 2007 reaching a coverage of 95%.

Conclusion: Gaps in vaccination program and increased internal and temporary migration allow re merging of imported measles. All outbreaks should be investigated to identify the circulating measles genotype. Supplementary immunization campaigns are needed to sustain the high coverage of measles vaccition of 95% and elimination.
10.097 Outbreak of *Mycobacterium massiliense* Infections Following Video-Surgeries or Implant of Breast Prothesis at Espirito Santo State: Control and Prevention Measures

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Health care associated infections are frequent events and important causes of morbimortality. An outbreak of *M. massiliense* hospital infections involving 192 pts submitted to video-laparoscopies, -arthroscopies or implant of breast prothesis in 11 different private hospitals at Metropolitan area of Vitoria, capital of Espirito Santo in 2007 was notified. The epidemiologic features are described. The main measures adopted by the hospitals and the sanitary surveillance to control the outbreak and prevention of new cases included the modification of the reprocessing of the instruments, and the guidelines involving thermalizations and supervisions of cleaning and sterilization procedures of video-surgeries. The last case was notified in a video-surgery performed in Aug/07, one month after the first notifications of the outbreak and beginning of adoption of the measures. The authors conclude that this modification of the re-processing of the instruments used in video-surgeries led to the control of the outbreak in these hospitals.

10.098 Food-Borne Listeriosis Outbreak: Are Current Thresholds Sufficient for Susceptible Populations?

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Background: Listeria monocytogenes (L.m.) is a food-borne pathogen. Immunocompromised patients are at higher risk of developing invasive listeriosis with high fatality rates. In Europe L.m. concentrations up to 100cfu/g food are permissible. In October2007, notificationsof two *M. massiliense* cases/Universidade Federal do Espirito Santo, Vitoria, Brazil, 6Centro de Referencia em Tuberculose/Universidade Federal Espirito Santo, Vitoria, Brazil

Health care associated infections are frequent and important causes of morbimortality. In 2007, 263 patients submitted to video-laparoscopies, -arthroscopies or implant of breast prothesis in 11 different private hospitals at Metropolitan area of Vitoria city (capital of Espirito Santo state) were notified to the Secretaria Estadual de Saúde as suspected cases. Among them, 192 (73%) cases were confirmed based on the presence of AFB, isolation of rapidly growing mycobacteria (RGM) or presence of granuloma in histopathology of the biopsy. As soon as the diagnosis was made, each patient was referred to the clinics or mycobacterial disease located at Hospital Universitário Cassiano Antonio de Moraes for treatment. These RGM were first identified as M. abscessus type II (PCR-restriction analysis) and, later on, as *M. massiliense* (molecular sequence). The most frequent surgeries performed were cholecystectomy (33.4%), bariatric surgery (20.8%) and arthroscopy (16.2%). Female sex (75%) was more affected than male (25%). Age ranged between 8 and 81 years old (median=41 years) The minimal and maximum values of the incubation period were 1 and 142 days, respectively (median=23 days). The main clinical complaints included spontaneous drainage of fluid (57%), hyperemia (45%) and nodules (40%). Most patients denied underlying diseases.

10.100 An Outbreak of *Salmonella* Infections Following Video-Surgeries or Implant of Breast Prothesis

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Background: *Salmonella* is a leading cause of bacteriological gastroenteritis in humans. However, in Sweden a control program covering the whole production chain has been in place since more than 50 years. In case of *Salmonella*, farms are put under restrictive measures and sanitized. In addition, birds on poultry farms are killed and destroyed.
The prevalence of Salmonella in the Swedish animal production is very low (<0.5%). Feed is considered an important source of Salmonella in animals but few reports have been published on contaminated feed as a source of Salmonella in humans. S. Reading, a rare serotype in Sweden, caused an outbreak in 2007-2008.

Methods: An outbreak investigation was performed. Samples were analyzed using standard microbiological procedures. PFGE analyses were performed using published methods.

Results: A cluster of human cases of S. Reading was notified with six cases in 2007 and five in 2008. S. Reading was also isolated from four cattle farms, one sheep farm, from five horses and from wild birds in a small area in the county of Skåne, southern Sweden. The same serotype was repeatedly isolated from a river running close to these farms. In addition, S. Reading was also isolated at one duck and one turkey farm in Skåne, from one feed factory and from minced meat at a cutting plant.

In 2007, the human cases were spread throughout the country and all except one had eaten minced meat. In 2008 all the patients were living in Skåne, with connections to the area of the infected herds. All isolates were indistinguishable in the PFGE.

Conclusion: This outbreak highlights the importance of feed and the environment as a source of infection.

A Food-Borne Outbreak of Shigellosis

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Background: In Sweden about 500 cases of shigellosis are reported every year and the yearly incidence is approx. 5/100,000 inhabitants. However, the majority of the cases have acquired the disease abroad, often from Egypt or India. About 20 % of the cases are indigenous which results in a yearly incidence of approx. 1/100,000 inhabitants. Shigella sonnei accounts for 60 % of all reported cases.

In late August 2008, an outbreak of gastroenteritis was detected among people visiting a lunch restaurant in Stockholm, Sweden. An outbreak investigation was launched.

Methods: An alert was sent out to hospitals and other health care facilities with a request to report possible cases to our department. Faecal samples were collected from possible cases and analysed for bacteria, viruses and parasites. A retrospective cohort study was undertaken to find out a possible source of the outbreak. A web-based questionnaire was sent to the head of administration of the 20 different companies which personnel often had their lunch at the restaurant with a request to pass it on to all people in their respective company. About 500 people are estimated to have received the questionnaire.

Results: Shigella sonnei was isolated from 70 faecal samples. The strain did not ferment mannitol which is highly unusual. 340 people responded to the questionnaire and 145 of them had developed gastroenteritis after having lunch at the restaurant. Among all the dishes served, including items from the salad buffet, only grated raw carrots turned out to be significantly associated with disease in the analyses. RR=2.47 (95% CI 1.50-4.07). Five persons belonging to the kitchen staff fell ill at the same dates as the restaurant guests. All other kitchen workers were sampled twice with negative result. Food samples including grated raw carrots were collected by environmental health officers and analysed with PCR technique for Shigella bacteria with negative results. The carrots were grown in Sweden and were delivered to the restaurant rinsed. The restaurant peeled and grated them in the room used only for raw vegetables which is separated from the rest of the kitchen.

Conclusion: This outbreak shows how easily a microorganism with a low infectious dose is spread to a high number of people. How the carrots became contaminated and acted as a vehicle in this outbreak is still unknown.

Fluorescence-tagged Influenza Virus for Tracking of Infection Cycle

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Introduction:

The virus will be useful for following the fate of individual virus particles and monitoring dynamic interactions between viruses and cellular structures. And also, the results obtained using FIIV can be used as important information when developing influenza antivirals.

Conclusion:

The virus will be useful for following the fate of individual virus particles and monitoring dynamic interactions between viruses and cellular structures. And also, the results obtained using FIIV can be used as important information when developing influenza antivirals.

A Nationwide Outbreak of Salmonella Panama in Children Due to Mini-Salami Sticks, Germany, Summer 2007


Background: In August 2007, increasing notified infections with Salmonella (S.) Panama were noticed by the Robert Koch Institute, Germany's national infectious disease surveillance institution. Cases were spatially scattered, young children most affected. Together with food safety authorities, an outbreak investigation was conducted to identify the outbreak's source and to devise interventions.

Methods: A case-control study focused on a specific food vehicle hypothesis. Cases were aged 20 months to 20 years, with onset in July/August (first case per household). Controls were randomly selected from municipal registries, frequency matched on age. Participants or their parents were questioned on food consumption the week before onset (cases) or interview (controls). Case isolates of S. Panama were compared microbiologically (PFGE and MLVA). Food safety authorities investigated production and distribution of the incriminated food vehicle.

Results: In exploration, two infections had direct links to a factory producing various types of salami products (an asymptotically infected worker and the grandchild of another employee). Other cases had consumed miniature salami. Analyzing 22 cases (of 34 in total) and 63 controls, consumption of small caliber mould-ripened salami sticks (MSS; OR=29.9, 95% CI: 8.2-112.7), and among salami eaters, of MSS likely produced by the implicated factory (OR=12.0, 85% CI: 1:0.141.3), were strongly associated with disease, explaining >70% of cases (100% of those <29 months of age). Microbiologically, case S. Panama strains, including those directly factory associated, constituted an outbreak clone. Food safety investigations found no general faults at the production site and failed to demonstrate S. Panama in factory and retail product samples.

Conclusion: MSS containing S. Panama likely caused the outbreak. Parents should be aware, that short fermented raw meat products like MSS may harbor viable enteropathogens sufficient to produce infection in children and plausibly those with compromised immune systems. MSS in Germany are frequently marketed specifically to children.
Background: The national reference centre for Measles/Mumps/Rubella, Robert Koch-Institute, Berlin, Germany, *Local Health Authority, Berchtesgaden, Germany, *Local Health Authority, Traunstein, Germany, *Local Health Authority, Rosenheim, Germany, *Local Health Authority, Munich*am Inn, Germany, National Reference centre for Measles/Mumps/Rubella, Robert Koch-Institute, Berlin, Germany, *Bavarian Health and Food Safety Authority, Department for Epidemiology, Oberschleissheim, Germany

Germany is committed to the WHO goal of eliminating indigenous measles transmission in Europe by 2010. Since 2001, measles are notifiable in Germany. In March 2008, two Bavarian local health authorities bounded by Austria reported 15 measles cases. Among those, 14 attended an Austrian school with an ongoing measles outbreak. In 2006, vaccination coverage for two doses of measles vaccine was 76% among Bavarian first graders. An investigation was initiated to describe the outbreak’s extent in Bavaria and to find reasons for non-immunisation against measles. We defined a case as a resident of one of the four most severely affected Southern Bavarian districts diagnosed with measles according to the WHO clinical case definition and onset of disease between March 14 and July 15, 2008. We used the national electronic surveillance system and a questionnaire to collect and analyse data on demographics, clinical symptoms, measles vaccination status and reasons for non-immunisation against measles. The national reference centre for Measles/Mumps/Rubella provided genotype information.

In total, 217 cases fulfilled our case definition. Cases were 0-57 years old (mean 11 years), 47% male. 51 cases could be epidemiologically linked to the measles outbreak in Austria. 26 cases were hospitalized. 193 (97%) cases with known vaccination status were unvaccinated. Four vaccinated cases had received one measles vaccine dose, one two doses. Exclusively genotype D5 was detected in 26 of 29 tested cases. 160 (74%) completed questionnaires were obtained. 14 cases reported otitis media, six pneumonia and one paralysis. Main factors for non-immunisation were fear of vaccine-related adverse events (32%) and the perception measles was not a severe disease (18%).

Health care workers and the general population need to be continuously educated on the highly protective effect of measles vaccination to increase vaccination coverage. Immunisation is much safer than accepting the possible complications of measles disease.

**Results:**

1. There wasn’t confirmation of infection with Brachyspera hyodisenteriae, so farm was free of Dyserenteriae suum.
2. There was the improvement of all performance characteristics: daily weight gain was higher, the number of raised piglets per sow and the number of slaughtered pigs was higher, too. Although, there was a lower percentage of death, in group of treated animals.

**Conclusion:** After the three months of medical therapy and veterinary and sanitary procedures of suppressing and eradication of Dyserenteriae suum, the results of this trial shown:

- **Methods:**
  - During a 21 month period (starting on Sept 1st, 2006) the UIHC EMR system recorded roughly 19.8 million login records which we use to construct contact networks of varying density (based on the definition of ‘contact’). By combining EMR login data with the detailed model of the UIHC facility, we are able to infer the ‘mobility’ of each individual. We compared several different targeted vaccination policies including: the random vaccination policy in which individuals are chosen uniformly at random for vaccination, the degree-based vaccination policy which first vaccinates individuals with a large number of contacts in the contact network, and the mobility-based policy which first vaccinates individuals with greatest mobility within the hospital.

**Results:** The contact networks constructed from the UIHC EMR login data exhibit many of the structural properties observed in social contact networks arising in other contexts including the small-world property of having a “small degree of separation,” which allows disease to spread rapidly through the network. Our experiments provide strong evidence that the targeted degree-based and mobility-based vaccination policies perform substantially better than the random vaccination policy and are comparable in effectiveness. This follows our expectation that highly mobile individuals provide the “long distance” contacts that make the networks “small world.”
Conclusions: Our results provide the first conclusive evidence, based on large-scale actual data, that targeted vaccination policies are substantially better than the random vaccination policy in a large hospital setting. We believe that the targeted vaccination policies, especially the mobility-based policy, can be implemented with limited hospital resources.

Methods: The film was produced for a Congolese audience in Lingala and French. It features doctors and affected residents from Likouala region. Portable projection equipment is used to facilitate outreach to resource-poor areas accessible only by foot or boat. In November 2008, we piloted the intervention in 5 villages. Over 200 individuals attended; 28 were randomly selected to participate in pre- and post-intervention evaluation surveys to capture changes in knowledge and attitudes of monkeypox prevention and care and 22 completed the survey.

Results: Twenty (91%) respondents had previously heard of monkeypox, 4 (20%) of them reported a neighbor was infected with it more than 1 year ago. Prior to the film 45.6% of respondents cited both rash and fever as symptoms of monkeypox; 45% said they would seek hospital care at the first signs of monkeypox. Respondents would also seek care from witch doctors and traditional healers (9%), or would self-treat (41%). After the film knowledge of these symptoms increased to 100%; 73% said they would seek hospital care; and 73% would isolate infected household members. Half of respondents reported handling or eating animals they found dead in the forest. Prior to the intervention 25% said that diseases in general can be prevented by avoiding animals found dead in the forest; after the intervention this increased to 91%. Themes from the discussion were; concern for food insecurity if animals found dead can not be eaten, and what to do if the hospital is far.

Conclusion: This is the first video-based public health intervention targeting local individuals in this region. It was effective in increasing knowledge of monkeypox prevention and care in the target population. Scaling up the intervention should be explored to prevent further monkeypox outbreaks in the Congo-Basin region.

Impact of a Video-based Intervention on Knowledge and Attitudes of Monkeypox Prevention and Care in Rural Republic of Congo (Likouala Region), November 2008

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Background: Monkeypox is an endemic zoonotic disease in parts of Africa. The first human infections were identified in 1970 during the smallpox eradication campaign. Similar to smallpox, it is characterized by fever and disseminated rash. Mortality is roughly 10%. About 95% of all human cases since 1981 have occurred in the Congo Basin. An intervention package consisting of an educational film followed by a structured discussion was developed to improve knowledge, attitudes, and behaviors for prevention and care of human monkeypox infection. The materials were piloted in a remote region of the Republic of the Congo previously affected by monkeypox.

Methods: The video was produced for a Congolese audience in Lingala and French. It features doctors and affected residents from Likouala region. Portable projection equipment is used to facilitate outreach to resource-poor areas accessible only by foot or boat. In November 2008, we piloted the intervention in 5 villages. Over 200 individuals attended; 28 were randomly selected to participate in pre- and post-intervention evaluation surveys to capture changes in knowledge and attitudes of monkeypox prevention and care and 22 completed the survey.

Results: Twenty (91%) respondents had previously heard of monkeypox, 4 (20%) of them reported a neighbor was infected with it more than 1 year ago. Prior to the film 45.6% of respondents cited both rash and fever as symptoms of monkeypox; 45% said they would seek hospital care at the first signs of monkeypox. Respondents would also seek care from witch doctors and traditional healers (9%), or would self-treat (41%). After the film knowledge of these symptoms increased to 100%; 73% said they would seek hospital care; and 73% would isolate infected household members. Half of respondents reported handling or eating animals they found dead in the forest. Prior to the intervention 25% said that diseases in general can be prevented by avoiding animals found dead in the forest; after the intervention this increased to 91%. Themes from the discussion were; concern for food insecurity if animals found dead can not be eaten, and what to do if the hospital is far.

Conclusion: This is the first video-based public health intervention targeting local individuals in this region. It was effective in increasing knowledge of monkeypox prevention and care in the target population. Scaling up the intervention should be explored to prevent further monkeypox outbreaks in the Congo-Basin region.
Conclusions: Our results support the exclusion of healthcare workers for 5 days following the onset of symptoms. Increasing isolation to 9 days produces a modest but statistically significant reduction in the spread of the disease, especially when vaccine effectiveness is lower (i.e., 75%).

Results: The effective reproduction number (the average number of secondary infectious cases produced by a typical infectious case in a given population) for the 1918 influenza virus during the grow phase of cases was 2.79 (95% CI: [2.32, 3.27]) in Montreal. It was 1.65 with the range 1.53-1.78 in the area of Winnipeg.

Conclusions: When the pandemic influenza made its appearance in Montreal during late of September, in a very short time it had spread all over the city. The effective reproduction number is much higher than one for Winnipeg, where the pandemic influenza was transmitted from the eastern part of Canada with the speed of human travel from a city to another city. With the knowledge the disease was to reach the Winnipeg, one of methods were considered of controlling the pandemic was to use a vaccine as prepared from virus infecting the respiratory tract of those suffering from the disease. The majority of the "inoculations" were given in the early stages of the pandemic in Winnipeg. Our study shows that the methods of controlling the disease in Winnipeg reduced 40% secondary cases for an infectious case. These findings confirm the relatively low transmissibility of the 1918 (Spanish) influenza virus in the Winnipeg area, because of early preparedness and control measures.

10.110 Rabies Control Post Invasion Iraq

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In an effort to protect military personnel from Rabies two approaches were taken depending on situations. 1. Trap and euthanize all animals on the bases. 2. Selectively vaccinate, neuter and vaccinate animals. Five dogs diagnosed with Rabies via DFA gained access to the bases in #1 exposing over 45 personnel to Rabies. In situation 2 no rabid dogs emerged. This observation took place over 5 years euthanizing over 15,000 animals. In addition to testing animals for Rabies randomly and when indicated by the situation, necropsies and blood work revealed an array of diseases not otherwise documented in Iraq.

On bases where all mammals captured were euthanized the threat of Rabies continued to be uncontrolled and human and other animal exposures continued. On bases where vaccination and animal control via surgical sterilization took place, there was control of Rabies indicated by the lack of positive dogs of all tested. The conclusion that vaccination and stray animal control via neutering is far superior to attempting to eliminate the canine species or other mammals harboring Rabies.

10.111 Understanding Transmission of the 1918 Influenza in Canada: Implications for Pandemic Control Strategies

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Background: The 1918–1919 Spanish influenza pandemic killed about 50 million people worldwide. There have been several studies of the transmissibility of the 1918 (Spanish) influenza virus. Many of the analyses to date have involved fitting predictions from a transmission model to the observed epidemic curves based on data from cities in Europe or United States. It was reported that the 1918 “Spanish Flu” had been brought to Canada from United States. During the period of three months from September 15 to December 15, 19.1% of troops in Canada were infected. The mortality varied from 15.4% to 76% among the reported areas. For general population, there were 323 deaths due to the pandemic flu per 100,000 people for the period of three months.

Methods: We obtained surveillance data that were compiled daily, during 1918–1919, in some Canadian cities; the records included medically treated influenza-like illnesses (ILIs), hospitalizations, and deaths. We used regression model to estimate the growth rate in the grow phase of the pandemic, then, we estimate effective reproduction number. We contrast the use of several different methods for estimating the reproduction number and we compare the results obtained from other cities in the world.

10.112 Neuro-Nocardiosis in an 11-year-old Boy

P. Nikbakht. Azad University, Tehran, Iran (Islamic Republic of)

Introduction: Nocardiosis occurs as sporadic cases. Nocardia species are the etiologic agents of Nocardiosis that is found wide world in soil and dust. The respiratory throat and skin are the sites of entry in most of the Nocardiosis. Nocardia are gram positive organism partially acid Fast positive bacilli. Meningitis due to Nocardia were reported in addicts, immuno suppressant host and the people who had had surgical operation to different reasons.

Case Report: An 11-year-old boy presented to the emergency department of Mofid Pediatric hospital with 80 days history of headache and vomiting. He got involved paresis of left extremities. During the period of the disease, the patient had not fever, CT scan findings revealed intracerebral hemorrhage, cerebral and cerebellum edema. Due to clinical diagnosis of hydrocephaly + pseudo tumor cerebri peritoneal ventricular surgical shunt was performed for the patient. He involved diplopic with decrease level of awareness associated with cerebella bleeding after surgery. The patient treated with Estazolamid, Furosemide, Dexamethasones, Acetaminophen codeine.

Para Clinical Positive Findings:

13th April 2003, WBC: 7, RBC: 0, Glu: 20, Pro: 20
19th April 2003, WBC: 1, RBC: 15, Glu: 132, Pro: 28

Microbiologic Findings: Few budding yeast identified on CSF direct smear which suggested the presence of Cryptococcus. CSF was cultured on L. Jensen and Brucella agar media. Some rough-irregular colonies were identified on these two media. Many typical long Acid Fast Bacilli were identified by Ziehl-Nelson staining. They were catalase and unase positive. The other tests such as; casein, gelatin and hypoxanethine were positive. These findings were confirmed the Nocardia asteroides.

Treatment: The choice drug is Sulfamethosone-Trimepropim-Mino cyclin combination. Sometime surgical drainage or resection is required.

10.113 Histoplasmosis in the Oral Cavity: A First Case Report in Turkey

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Histoplasmosis is a rare but serious fungal infection commonly presenting as mucosal ulceration of the oral cavity. We report a case of histoplasmosis in the oral vestibule. Histological examination and culture of purulent discharge was consistent with histoplasmosis, and the patient responded favorably to treatment with oral itraconazole. In some cases; oral lesions appear primarily or only manifestation of disease; and we have been able to find only few case reports in the literature. In this study, we describe the first case of histoplasmosis in oral cavity in Turkey. 21 year-old male was admitted the oral diagnose and dental surgery clinic with complain-
ing of swelling and pain in his left hemifacies. The diagnosis of histoplasmosis was confirmed by a positive culture for Histoplasma capsulatum at 28–30 °C, the organism grows as a white mould, with typical tuberculated macroconidia on microscopy. Following the diagnosis of the histoplasmosis, itraconazole (Itaspor-Janssen-Cilag) 200 mg daily had been given per orally. Antifungal treatment had been continued for three months. In this time, infection had been resolved totally.

Avian Influenza

2. Pyrulandischargeisseenattheoralvestibule(linearrow)

Figure 1. Histologic section stained with gram dye revealed prominent macrophages containing yeast cells (line arrow).

Avian Influenza

An Early Warning System for Low Pathogenic Avian Influenza (LPAI) in Commercial Egg Laying Flocks

D. Beltrán Alcrudo, T.E. Carpenter, C. Cardona. University of California, Davis, Davis, CA, USA

Although low pathogenic avian influenza virus (LPAIV) infections are sometimes clinically inapparent, they often result in a moderate egg production decline and low increased mortalities in egg producing birds. The aim of this study was to develop and evaluate an early warning system (EWS) for commercial egg laying flocks to detect the sometimes very subtle mortality and egg production changes that characterize LPAIV infections. The EWS was based on daily data collected from flocks affected by the 2000-2004 H6N2 LPAI epidemic in California. Three EWSs were evaluated: 1) EWS1, which is triggered when the observed mortality increase or production decrease exceeds more than "x" times the expected daily value, 2) EWS2, which is triggered when the observed mortality increase or production decrease exceeds more than "y" times during each of two consecutive days the expected values, and 3) a combination of the two. Results showed that an egg production-based EWS added no benefit to a mortality-based system, because LPAI-related egg production decreases always occurred after increased mortality. In addition, combining the two EWSs resulted in a reduced detection delay and no missed outbreaks, but at the expense of a slight increase in the number of false alerts triggered. The system presented in this study, when compared with existing EWSs, resulted in shorter detection times of real outbreaks, lower number of false alerts and no missed outbreaks. The proposed EWS, if used as part of a poultry cooperative program and combined with a rapid laboratory diagnosis, could be a very useful tool in the detection and control of LPAI outbreaks and other poultry diseases. Built in a spreadsheet, the system could be inexpensively, easily and quickly incorporated into a commercial egg production farm decision support system. In addition, instead of using fixed trigger values as with other EWSs, the proposed system can be quickly adjusted to changing epidemic situations, and easily customized to individual flocks.

10.115

Human Influenza Virus and Mitochondrial Respiration

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Studies of interaction between viruses and mitochondria have shown that they can affect the mitochondria. Mitochondria are the powerhouse of the cell; their dysfunction underlies the pathology of several diseases. Many viruses induce changes in morphology and location in these organelles. However there have been relatively few investigations of how such processes might affect the basic mitochondrial function of energy generation. This study was sought to investigate the effect of influenza virus on mitochondria. MDCK Cells were maintained using MEM modified with 10% FCS, antibiotics and non-essential amino acid supplements, incubated in humidified incubator with 5% CO2 in air at 37°C until confluent. To determine that the cell respiration is attributed to the mitochondria, antimycin A (AA), an inhibitor of complex III was used. After addition of antimycin A during recording the basal respiratory rate of uninfected cells, the cellular respiration was completely blocked that indicates almost all of the cellular consumption of oxygen is related to mitochondria. To search for any virus induced effects on cell respiration, MDCK cells were infected with influenza virus strain A/Puerto Rico/8/34 (10 ID50/cell), and tested at intervals post infection. Control and infected cells were recovered by scraping, both were counted and viability was identified using trypan blue exclusion. Then cells were transferred into the oxygen electrode (OE) to measure oxygen consumption (OC). It was shown that following IVA infection of MDCK cells, total cellular respiration (TCR) was significantly decreased. The present study demonstrates that after IVA infection of the MDCK cells TCR is decreased that indicates a significant viral effect on mitochondria.

10.116

Molecular Characterization of a Highly Pathogenic H5N1 Influenza A Virus Isolated From a Tiger In China

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Background: In July 2005, zoo tigers in Shanghai, P.R. China showed typical signs of respiratory infection. Virus was isolated from lung tissue samples of dead tiger and H5N1 influenza A virus infection was confirmed. Complete genome sequence analysis was performed to understand evolution and origin of the isolate.

Methods: Viral RNA was extracted from the allantoic fluid using TRizol LS reagent. All gene fragments were amplified by using RT-PCR and cloned into pMD18-T vector. The gene sequences were determined using...
an automated sequencer and were compared with other related H5N1 isolates in GenBank using software Clustal W and MEGA.

Figure 1. Phylogenetic tree based on the nucleotide sequences of the HA1 gene.

Results: Sequence analysis revealed that tiger influenza virus was highly identical to H5N1 virus isolated from migratory duck at Poyang lake, Jiangxi in May 2005. Genotyping results showed that Shanghai tiger virus belonged to genotype K,G,D,S,J,F,1,J,F,1E and phylogenetically it was a clade 2.2 virus (Fig. 1). Sequence analysis revealed that HA gene harbored substitution of R to G at position 323 in cleavage site, which might be a host adaptive signature and an important factor in evolution. NA gene showed deletion of 20 amino acids in stalk region, same as found in other H5N1 isolates from hosts like humans, cats, dogs, leopards, tigers and variety of domestic and wild birds. PB2 gene contained E at position 627 and evidences indicate that PB2 gene having no substitution of K at position 627 can also cross species barrier and replicate in mammalian hosts like humans and tigers. NS gene showed deletion of 5 amino acids at position 80-84. Sequence ESEV at C terminal of NS gene indicated virulence of the isolate. These results may contribute to efforts to increase knowledge about the genome of influenza A viruses and highlights the necessity for continuous surveillance.

Conclusion: In summary, molecular characterization of all the gene segments revealed characteristics of highly pathogenic influenza A viruses. Molecular determinants like substitution of amino acid at cleavage site of HA gene, shortening of stalk region in NA gene and presence of sequence ESEV at C terminal of NS gene indicated virulence of the isolate. These results may contribute to efforts to increase knowledge about the genome of influenza A viruses and highlights the necessity for continuous surveillance.

10.117 Sequence Variation in Molecular Diagnosis Target of Influenza Virus Type A

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Avian Influenza (AI) is a significant infectious disease caused by Influenza virus type A, which belongs to the Family Orthomyxoviridae. Its genome consists of eight single-stranded segments, negative-sense RNA, which encodes 10 or 11 proteins. The high nucleotide substitution rate for this type of viruses allows them to rapidly adapt to new environments and hosts, even crossing the species barrier. This could explain, at least in part, the high prevalence of these agents and their definition as emerging or re-emerging pathogens. Moreover, this high genetic variability could affect molecular diagnosis methods.

Among all type A influenza virus, matrix (M) protein gene is considered to have a highly conserved sequence, so it is extensively used as a diagnosis target for the detection of AI by Reverse Real Time Polymerase Chain Reaction (RRT-PCR). Nowadays a large number of influenza virus sequences are available in GenBank; this allowed us to evaluate the usefulness of the primers and probe more widely employed in RRT-PCR and recommended by the OIE, using in silico methods. The multiple alignments of more than 300 M-gene sequences of different type and subtypes of AI viruses, using MEGA 4.1 and BLAST software, were carried out. The study includes sequences from different host species (birds, pigs, horses and humans) and widespread geographical regions. The specificity of the assay was confirmed including other viruses like B and C influenza virus types, Newcastle virus or Avian Infectious Bronchitis virus. On the other hand, the sensitivity of this PCR could be impaired due to the great number of single nucleotide polymorphisms observed, especially in the region corresponding to the target sequence of reverse primer M-124. In this sequence a large number of multiple mutations were detected, including 228 isolates showing changes at three nucleotides and 115 isolates showing double mutations at different positions. These mismatches between gene-specific oligonucleotides and the target sequence could yield false negative results under certain conditions.

In summary, sequence heterogeneity may have affected the efficiency of the amplification or detection steps of the RRT-PCR assay. Due to the high and rapid genetic variation of AI viruses, it is essential to perform a constant revision of published sequences of AI viruses to make sure the target sequences of molecular techniques remain stable, as recommended by the OIE.


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Background: The reproduction ratio (R0) is an indicator of the magnitude of disease transmission. High values of R0 are likely associated with the presence or absence of factors that, respectively, promote and prevent disease spread. The objective of this study was to compare the values of R0 estimated at different clusters of outbreaks reported during the H5N1 avian influenza (H5N1 AI) epidemics that affected Europe from January 2006 through January 2008.

Methods: Date and location of H5N1 AI outbreaks reported in Europe from January 2006 through January 2008 were obtained from the Office International des Epizooties. For each outbreak, the nearest neighboring wild waterfowl was assumed to be H5N1 AI-infected. The Poisson model of the time-space scan statistic was used to identify regions and periods of time at high risk of being H5N1 AI-infected, using European wetlands as the population at risk. The within-cluster R0 was computed for each cluster using R0 = 1 + (D/td) ln 2, where D is the duration of infectiousness of each case and td is the time interval within which the number of detected
cases doubled. A baseline value of $D=7$ days, which is an average number
assumed by others, was also assumed here. The relative R0 (RRi) was estimated for each cluster i as the ratio of the median value of R0 (MR0) in cluster i to the smallest value of MR0 estimated in the study. Therefore, the cluster with the smallest value of MR0 represents the base-
line, which is indicated by RRi=1, to which values of MR0 estimated for other clusters are compared. Spatial autocorrelation of R0 was explored by
use of the Moran’s I test.

**Results:** Twelve clusters of H5N1 AI outbreaks were identified. The clus-
ter with the smallest value of MR0 (MR0 =1.2; RRi=1) occurred from
March 1 through April 30, 2006 and encompassed wetlands located in
Germany and Switzerland. The largest value of MR0 (MR0 =3.4; RRi=2.9) was estimated for two clusters that occurred from June 1 through July 31,
2007 in Germany. The median value of MR0 estimated in the 12 clusters was 1.9. No evidence of spatial clustering was found (Moran’s I = -0.03, P=0.62).

**Conclusion:** The R0 of H5N1 outbreaks in Europe between January 2006 and January 2008 was spatially homogeneous. The value R0 =1.9 estimated here is similar to other estimates obtained for H5N1 AI epidemics in poultry. Future efforts will be directed to quantify the association between R0 and demographics and environmental features of the clusters.

**Development of a Small Scale Facility to Evaluate the Efficiency of Commercially Available Masks and Respirators Against an Aerosol of Influenza A Virus.**

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Only limited data is available measuring the efficiency of filtration of available
masks and respirators against air-borne viruses using an in vivo test. We will describe here the small-scale facility we have developed for this purpose. In this context, we established an infection model by aerosolisa-
tion of influenza A/H1N1 virus adapted to Balb/c mice and realised a phys-
ical characterisation of generated aerosol.

Our institute has a nose-only apparatus for air-borne delivery initially
designed to measure the protection factor (PF) of gas masks. This appar-
ratus was optimised in order to evaluate viral filtration efficiency of the fil-
ter material used in these types of masks and also their factor of protection. Indeed mask-face interface is a key issue when evaluating the level of protection of a mask.

The device was adapted here to comprise two respiratory chains, each one composed of an half-head, an artificial breather and a mice exposi-
tion unit. These two units were installed inside the aerosol chamber. In a typical assay, only one unit is equipped with the mask, the second unit acting as control. PF is the ratio of the number of viral units detected in mice directly exposed to air-borne viruses to the number of pulmonary
viral units detected in the protected mice. After exposure of masked and unmasked mice against an aerosol of Influenza A virus, weight loss, mor-
tality rate, mortality period and initial viral pulmonary load were monitored and compared between both groups of mice.

Using the aerosol infection procedure with Influenza A H1N1 virus, viral
infection was 100% lethal in eight days for an initial viral load equivalent
to 100 pfu per animal. The size distribution of influenza loaded particles ranged between 0.5 and 15 μm of equivalent aerodynamic diameter with a mean value close to 1 μm. Viral particles were detected in lungs. This air-borne delivery system is able to induce the pulmonary infectious process in mice. This retention efficiency in lungs is in accordance with the aerodynamic size of generated particles.

**Predictions of Structural and Functional in the Hemagglutinin Protein of Selected Strains of Influenza Virus**

R. Liam, M.M. ElEhewani

The genome of the Influenza A virus consists of eight RNA segments that encode 1-2 proteins each. The lipid layer of the virus contains molecules of Hemagglutinin (HA) and molecules of Neuroaminidase (NA), two

surface antigens with protective antibody responses and which constitute the basis for subtyping the influenza A virus. There are 16 hemagglutinin and 9 neuroaminidase subtypes currently known. Three distinct hemagglutinin proteins (H1, H2, H3) and 2 neuroaminidases (N1,N2) have been found in human viruses. Experimental characterization of the of the HA1 region of the hemagglutinin gene from different hosts has increased in the last three decades, in response to most recent pandemics such as the 1918 Spanish flu, the 1957 Asian flu, and the 1968 Hong Kong flu. Within increasing reliance on bioinformatics and computational biology, structural bioinformatics, on par with experimental approaches, are used to map locations of epitopes and antigenic sites on the hemagglutinin protein as potential targets for antibody, vaccine and drug design. In the last few years, bioinformatics has been used extensively to predict mutation rates of the HA gene and elucidate mutation patterns. In conjunction with sta-
tistical analysis, evolutionary trees and whole-genome alignments have been used to identify mutation locations in H3N2, predict their yearly fre-
quency, and determine modes of antigenic drift and positive selection.

Our previous study identified hyper variable motifs in the hemagglutinin protein named motifs 7, 2, and 3 (Gendoo et al., 2008). We focus this study on motif 7 hyper variable sites exhibit the greatest occurrence within the Hong Kong data set from 1968-1999. Interestingly this motif contained the greatest number of post-translational sites. We observed that amino acid substitution positions in 1980 were most implicated in post-translational sites. The CAMP site, was solely implicated within MEME 7. An analysis of each PROSITE motif revealed that many of the post translational modification sites occur within this motif, including our previously reported Myristyl sites appear, and several ASN glycosylation sites. We identify hot spots within this motif and correlate this finding with co-occurring mutations in other regions of the protein. The Myristyl motif/site was most frequently correlated with the amino acid substitutions, followed by the PKC and ASN motifs. Interestingly the PKC motif exhibits the greatest entropy within MEME motif 3, ASN exhibits the greatest entropy in MEME motif 1, and CK2 exhibits the greatest entropy with MEME motif 2. Based on these different correlations, MEME motif 2 is highly correlated with amino acids substitutions, post-translational sites, and entropy values of key amino acids.

**Project “Constanze”—Tri-National Surveillance Program of Avian Influenza in Sentinel Mallards at Lake Constance**

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**Background:** After nine Austrian cases of highly pathogenic avian influenza (HPAI) in spring 2006 at the Lake Constance, the question arose how the epidemiological status of HPAI could develop. Lake Constance is one of the most important wild and aquatic bird habitats in Central Europe. The bordering countries Austria, Germany and Switzerland set up a three-
years research project called “Constanze” (www.projekt-constanze.info). This multi-disciplinary project aims to investigate changes of Avian Influenza prevalence, modes of transmission and risks of infections for poultry in this region. Therefore sentinel spots were established. We pres-
ent here the preliminary results of the Austrian sentinel animals.

**Methods:** Since Feb 2007, ten mallards (Anas platyrhynchos) have been kept in an enclosure accessible by water fowl. Cloacal and orpha-
nygeal swabs have been taken periodically every two weeks, while serum samples every four weeks.

Viral RNA was isolated from the swabs and an Influenza A virus specific Real-time RT-PCR was performed (Spackman et al., 2002). Initial genetic
typing of AI positive samples was done by using specific H5, H7 and N1 Real-time RT-PCRs. Pathotyping of H7 positive swabs was performed by
sequencing the HA cleavage site (SANCO, 2006). Hemagglutinin (HA) and neuroaminidase (NA) subtyping of non H5/H7 virus strains was per-
formed by Hoffman et al. (2001).

Serum samples were tested for specific antibodies against the viral nucleoprotein of AI by a blocking ELISA (Pourquier) which reacts for all AI subtypes.
ABSTRACTS

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10.122 Preparation for Pandemic of Influenza in Republic of Serbia

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Introduction: Pandemic of Influenza is necessity and represent world wide Influenza outbreak that get after creation of new A Influenza virus subtype, which is transferable among people. From historical point of view, pandemics of Influenza have been periodically repeated and regularity of theirs appearance were known to scientific public. It is consider that world is close to new pandemic of Influenza more than ever.

Aim: The main aim was to assure realization of Republic of Serbia Government Activity Plan before and during the pandemic of Influenza; to achieve the highest possible readiness of health and society for absolute response till the year of 2010; to decrease direct effects of pandemic and to relieve recovery of society after pandemic ends.

Methods: It was used descriptive method.

Results: On the Serbian Government session in October 2005, Activity Plan before and during the pandemic of Influenza was adoptive as a major document for all activities of Government or non-Government sectors. The Operation plans have been made on the municipals level. Guidelines for implementation of the Influenza Preparedness Plan before the Pandemic (Phases 1, 2 and 3), have been written and distributed in 25,000 copies. The priorities in laboratories and anti infective departments’ fixings; supply of personal protective equipments as well as storage of antiviral drugs, have been defined. Checkouts of readiness in case of appearance of Avian Influenza (of mobile epidemiological teams; of primary health care; of section statistic in anti-infective departments/clinics quarantine units and of emergency transport capacities for diseased) were accomplished. Questionnaire that was used had 39 variables. Instructions for creating action plans in all environments and structures of society were prepared. Additional education and trainings of health care employees; employees that directly educate others; employees in local autonomies and in media, were managed. In the pipeline there is establishing of communication strategy, before and during the pandemic of Influenza. Among the period of 2005-2008, coordination of all activities and participants, together with the support to the work of experts with international co-operation, was executed.

Conclusion: Minister of Health of Republic of Serbia said (cite): "Speak- ing about pandemic, there is none of the rational reasons to be believed that in the first years of 21th century will be any difference compared with the past. When pandemic starts, it will come in Serbia, too."
Schaerding could not be phylogenetically clustered into any of the two Austrian subclusters and are closest to a virus found in Bavaria, Czech Republic and Italy, and therefore we called this third subcluster “Bavaria.”

**Conclusion:** Our data suggest the introduction of at least three distinct H5N1 HPAI variants into the wild bird population of Austria in 2006.

**10.125 Serological Survey of Influenza Type A Viruses in Dogs Living in Poultry Farms in North-Eastern Italy**

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Our study reports on a serological survey for antibodies to influenza type A viruses in dogs living in areas at high risk of introduction and spread of influenza viruses in North-eastern Italy, where LPAI and HPAI outbreaks have been frequently reported. The serosurvey was aimed to provide further information about the possibility of dogs being infected and spreading of avian influenza viruses (AIV). In 2008 a total of 59 serum samples were collected from dogs kept as companion animals in 38 poultry farms located in the provinces of Padua and Rovigo (Veneto region). Ten farms had been affected by HPAI H7N1 outbreaks and 4 by LPAI H7N3 in 2000 and 2003, respectively. Furthermore, 7 canine sera were collected in a dealer farm (ornamental birds), where there was an ongoing outbreak of LPAI H7N3 in the birds. Screening of serum samples, using a Mab-based competitive ELISA for anti-nucleoprotein A (NPA) antibody detection of influenza viruses, showed that none was positive. This finding seems to support the hypothesis that dogs are at very low risk of AIV infection and that their role into the natural transmission cycle of influenza viruses is very limited.

**10.126 Detection of Avian Influenza Viruses in Wild Birds in Catalonia (North-Eastern Spain) from 2006 to 2008**

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**Background:** In 2006, as part of the European initiative, an Avian Influenza (AI) surveillance system in wild birds was established in Spain. The purposes of this program were to detect the circulation of influenza A viruses in wild birds, to improve the knowledge of their pathogenesis in wildlife, and to provide an early warning system to prevent the transmission of these viruses from wild birds to domestic poultry. The present study describes the results of the AI surveillance in wild birds in Catalonia (North-Eastern Spain) between 2006 and 2008. This region, which has important resting sites for waterbirds along the Western Eurasian and African migratory flyways and concentrates more than 10% of the wintering census of waterbirds in Spain, also holds 25% of the poultry industry production in the country.

**Methods:** AI viruses were detected by real time RT-PCR with and internal positive control, and inoculated in SPF embryonated eggs. The AI isolates were subtyped by IHA and INIA, and then sequenced. To understand the relationship between Spanish isolates and other AI viruses circulating around the world, phylogenetic analyses were performed.

**Results:** Between July 2006 and November 2008, a total of 1161 birds, belonging to 54 species, were tested. Fifty-five samples belonging to 6 different species were positive (4.7%). The highest proportions of positive tests were detected in Anas crecca (12.3%) and Anas platyrhynchos (9.6%). Twenty-two of the positive samples could be subtyped, and 13 different subtypes combinations were found. The most common combinations were H4N6 and H1N1. An H1N6 subtype was isolated from a Larus michahellis. One H5 and two H7 were detected and characterized as low pathogenic. Phylogenetic analyses showed that all the Spanish isolated viruses belonged to the Eurasian cluster. The H7 gene of the Spanish H7N9 strain, isolated from a Eurasian teal, had a 99% of similarity at protein level with the H7N3 isolated from a mallard in Italy in 2003, progenitors of the virus which caused a low pathogenicity avian influenza epidemic in Northern Italy.

**Conclusion:** Although no outbreaks of AI have occurred in Spain in domestic poultry, the results show that the proportion of wild birds detected as positive to influenza A viruses in North-Eastern Spain is similar to other regions of Northern Europe that have been recently affected by the disease.

**10.127 Detection of Avian Influenza Virus in Surface Water**


Avian influenza virus (AIV)-infected waterbirds shed high amounts of virus in feces and therewith, in the water they inhabit. Low pathogenic AIV is transmitted oral-fecally, and ingestion of water is considered to be a major route of transmission among waterbirds. Several AIV variants of subtypes H5, H7, and H9, have been shown to be directly transmissible from poultry to men. For such variants the possibility of water-borne transmission to men should be considered. Moreover, AIV might be transmitted to poultry via drinking water, with substantial economical consequences if it concerns H5 or H7 subtypes. Because of possible risks of interspecies transmission of AIV via water, it is relevant to develop efficient methods to detect AIV in water.

We have compared two methods to concentrate viruses from large volumes of water, negative membrane filtration at low pH and hemoflow filtration. Optimization of the RRT-PCR on hemoflow-derived surface water concentrates yielded recovery rates as high as 60–100%. The lowest virus concentration we have tested was 10^5 PCR-detectable units (PDU) of AIV per liter of surface water. In this case, AIV was still detectable in 100-fold dilutions of RNA extracts from hemoflow-derived water concentrates, indicating a detection limit of approximately 10^5 PDUL of surface water. Whether this sensitivity is sufficient to detect AIV in surface water needs to be confirmed by analysis of natural waters populated with AIV-infected birds.

**10.128 Mutations of Public Health Relevance in Contemporary H5N1 Viruses Isolated in Africa and the Middle East**

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**Background:** Since 2006, several H5N1 outbreaks have been reported in Africa and the Middle East. The extensive circulation of the H5N1 virus in these geographical areas renders of fundamental importance the monitoring of its molecular evolution to identify rapidly mutations that confer unique properties, such as a wider host range or a modified sensitivity to antiviral drugs. This study provides a comprehensive set of information on the occurrence of mutations, which may have an impact on public health, in the sequences of the H5N1 viruses isolated in Africa and the Middle East.

**Methods:** 443 sequences of 54 African and 13 Middle Eastern H5N1 viruses were generated and included in the present study. In addition, publicly available DNA and protein sequences of Middle Eastern and African H5N1 viruses were downloaded from the Influenza Virus Resource at NCBI and from the EpiFluDatabase at GISAID. All the sequences were aligned and translated using MEGA 3 programme.

**Results:** Several molecular markers that discriminate human influenza viruses from avian influenza viruses, have been recognized in the sequences of the Middle Eastern and African H5N1 isolates. Of particular
interest is the identification, in the sequences analysed, of two PB2 mutations which are 100% conserved in the viruses responsible of 1918, 1957 and 1968 pandemics (1). A rare NS mutation, previously described in the 1918 “Spanish” pandemic virus, was identified for the first time in H5N1 strains belonging to the 2.2 lineage (2). Mutations associated with modified sensitivity toward antiviral drugs have been recognized in 10 African and 7 Middle Eastern H5N1 viruses.

Conclusion: The analysis performed on the sequences of the Middle Eastern and African H5N1 viruses allowed us to identify specific amino acid substitutions which are believed to modify the efficacy of the replication in non-avian species and to alter the sensitivity to antiviral drugs. The results of this study highlights how whole genome analysis is instrumental to recognize rapidly any molecular change of public health relevance and underpin the importance of ongoing monitoring programmes to follow the molecular evolution of influenza viruses.

References:

10.129 Achievements, Failures and Future Strategies Related to Capacity Building and Training Efforts in African Countries Following the H5N1 Global Crisis

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Since early 2006, HPAI viruses of the H5N1 subtype have been circulating in Africa posing an unprecedented threat to animal and public health. Since 2004, international efforts have supported an increasing number of training activities and significant funds to develop veterinary infrastructure in Africa. As a result of this the number of veterinary laboratories able to diagnose avian influenza has increased, and overall competence has improved.

However, in 2007, a UN-FAO survey on Western African region has recognised that, although the majority of veterinary laboratories under study were operational, the quality of their facilities and diagnostic performances was heterogeneous and in certain instances inadequate.

The result of the survey indicated that in a number of cases, poor coordination had led to the duplication of efforts and wastage of resources. Some laboratories had received sophisticated equipment that was not fully exploited due to the lack of local supplies, technical assistance and poor maintenance. In addition, in many countries reagents are difficult to source due to the lack of local suppliers, the high added costs and the administrative bureaucracy related to importation.

Dedicated staff had been sufficiently trained on AI diagnosis (average of 4 training courses per person/year) in all countries under study, but the benefits of training are limited by the scarce number of samples submitted routinely.

These findings highlight the need of intervention strategies aiming at sustainability and capitalisation on to existing infrastructure. In order to maximise the outcome of international efforts, on-going support/maintenance projects should be implemented with international reference laboratories. Initiatives such as OIE twinning projects and the EU funded project FLUTRAIN are developing partnerships in this direction.

In addition, international support should shift focus on the development and implementation of surveillance/monitoring programmes which include the participation of the local veterinary services and laboratories.

The development of functional networks identifying regional laboratories and the availability of funds enhancing collaborative efforts between existing laboratories is a crucial step to the improvement of public health in the African continent. Motivation of local staff through the implementation of sustainable work programmes, will generate scientific data for the international community and avoid the deterioration of equipment and resources, enabling capitalisation on the investments made so far.

10.130 Development of a RT-PCR and a Real Time RT-PCR Assay for the Detection of Avian Influenza Virus

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Background: Influenza A viruses (IAV) are responsible for major disease problems in birds, as well as in mammals including humans. The conventional RT-PCR recommended in the OIE Manual for avian influenza virus (AIV) detects false positive in samples from different bird species1. According to this, it could be recommended to implement more than one diagnostic method at the laboratory. In this study a conventional and a real time RT-PCR assays were developed targeting different genome segments for the detection of avian influenza (AI) virus and compared with the OIE reference RT-PCR techniques.

Methods: A total of 14 complete gene NS1 and 24 complete gene NP sequences of AIV were downloaded from Genebank and aligned using ClustalW. Conserved regions were identified and a TaqMan probe and primers were designed for real time RT-PCR (NP) and a set of primers for RT-PCR (NS1). The specificity was confirmed doing a Blast search. A panel of 21 AIV (including all subtypes described of IAV), 1 Equine I, 1 Newcastle disease virus (NDV) and a total of 159 oropharyngeal and cloacal swabs from pigeons and seagulls were employed. The sensitivity of the RT-PCR assays were determined using 3 replicates of 10-fold dilutions of viral suspension of A/Vk/t/2676/99 H7N1 (6.02x104 EID50/ml), and compared with the sensitivity of the generic influenza A (M1)2, 3.

Results: Conventional and real time RT-PCR methods showed a detection limit of 1.806x10-3 EID50/ml for AIV H7N1, similar to the sensitivity obtained with the referred methods in the OIE Manual. Analytical specificity of the developed RT-PCR assays was confirmed with the amplification of the 22 influenza A strains analysed. No amplification was detected with the strain of NDV tested. As expected, all avian field samples analysed by both RT-PCR methods remained negative, whereas some false positive results were obtained using the conventional RT-PCR referred in the OIE Manual.

Conclusions: Although conventional and real-time RT-PCR methods are described for the pan-AIV detection, 3, both are designed to amplify a DNA fragment within the M gene. In this work, a conventional and a real-time RT-PCR methods have been developed for generic detection of AIV targeting NS1 and NP genome regions, respectively. Both assays have proved to be sensitive and specific methods, and could be useful tools for the molecular diagnosis of AIV.

References:

10.131 At Least Four Separate Introductions of H5N1 in Nigeria Between 2006 and 2008

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Background: Nigeria was the first African country reporting a Highly Pathogenic Avian Influenza of the H5N1 subtype outbreak in February 2006 in Kaduna State. Since then, the infection has spread to 25 of the 36 Nigerian states and to the Federal Capital Territory (FCT). The aim of this study is to provide a better understanding of the epidemiology and evolutionary dynamics of the H5N1 viruses circulating between 2006 and 2008 in Nigerian.

Methods: 104 representative H5N1 viruses from different AI Nigerian outbreaks were selected and then characterised by sequencing of the HA and NA genes. Phylogenetic analysis was conducted using Bayesian
methods implemented with the computer program MrBayes version 3.1.1. Rates of nucleotide substitution and the times of the most common ancestors (IMRCA) were estimated using BEAST version 1.4.7. DnaSP version 4.0 was used to examine selection in HA sequences.

Results: The HA and NA sequences of the Nigerian viruses are distributed in four separate genetic clades of the Bayesian trees. The co-circulation of distinct sublineages allowed the occurrence of reassortment events. The substitution rate calculated for the HA-NA concatenated genes of the 104 Nigerian H5N1 viruses was 5.56X10^-3 (HDP 95%) substitutions per site per year. IMRCA, the substitution rates and dN/dS values obtained for the HA-NA concatenated genes of the Nigerian viruses varied according to the sublineage. Specifically, for the two most representative sublineages, the parental one and the reassortant virus, the IMRCA were July and October 2005 respectively and the mean evolutionary rates were 6.58X10^-3 (HDP 95%) and 4.93X10^-3 (HDP 95%) substitutions per site per year. Analysis of the selection pressure showed that the HA of the two major sublineages were subject to purifying selection (dN/dS of 0.13 and 0.62).

Discussion: This study classified H5N1 HPAI viruses circulating in Nigeria into four distinct sublineages and discovered a group of naturally occurring reassortant virus between two sublineages. Our preliminary evolutionary analysis suggested that the most representative parental sublineage and the reassortant viruses co-circulating in Nigeria in the same population and geographical area evolved at different rates.

10.132 Predicting Altered Glycan Binding by Avian Influenza Hemagglutinin Mutants

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Background: One major concerns in avian influenza surveillance is monitoring for the emergence of strains that are readily transmissible between humans. Binding to cell-surface glycans found in the human upper respiratory tract is believed an important permissive factor for such transmission. The fundamental basis for this specificity switch is unknown, and laboratory tract is believed an important permissive factor for such transmission. We have developed physics-based simulation and analysis methods to predict important sites of mutation and analyze their effects on glycan-binding by influenza hemagglutinin. Here, we report a proof-of-concept application to predict mutations that destabilize binding of a 2,3-sialylactose by hemagglutinin from H5N1 viruses.

Methods: To predict important sites of mutation for hemagglutinin ligand-binding specificity, we combine analysis of protein-ligand interaction dynamics with sequence data. We perform molecular dynamics simulation of H5N1 hemagglutinin bound to avian-type glycans and identify residues dynamically related to the ligand via a mutual-information-based analysis. As a first step towards predicting specificity mutants, we predict point mutations that may disrupt ligand binding. We evaluate predicted mutants by running large-scale molecular dynamics simulations of mutant-ligand complexes and calculating dissociation rates.

Results: We have identified 6 mutation sites both at and distant from the ligand-binding site, generating 17 point mutants. Based on our simulations, we predict significantly decreased binding stability for 8 mutants. Our top-scoring mutation, S136A, has been experimentally characterized on the H3N2 X-31 background and substantially reduces hemagglutination in this context. We have also tested two mutations that do not disrupt binding of a 2,3-sialylactose by H5N1 viruses and find no significant change.

Conclusion: We present a proof-of-concept study for prospective prediction and computational evaluation of glycan-binding mutations in influenza hemagglutinin. Our methods successfully predict mutations that disrupt binding of a 2,3-sialylactose by H5N1 hemagglutinin; further work to predict changes in specificity to a2,6 glycans is now in progress.

10.134 The Spread and Evolution of Highly Pathogenic Avian Influenza (HPAI) H5N1 in Africa

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Background: In Africa highly pathogenic avian influenza (H5N1) virus was first detected in northern Nigeria and since then in 10 other African countries. Phylogenetic analysis and substitution rates of complete genome sequences of Nigerian strains showed that three sublineages were present in Nigeria as early as February 2006 suggesting three independent introductions of H5N1. All three sublineages belong to clade 2.2, including all African strains with a distinct geographic distribution. Here we describe the genetic diversity of HPAI H5N1 viruses identified in 2006 in Burkina Faso and in 2007 in Nigeria and present spread and evolution of these viruses.

Methods: In 2006 we have characterized the first avian influenza strains from Burkina Faso found in wild birds. Additional, in 2007 eight full length sequences of HPAI H5N1 from four different states in the southwest of Nigeria were analyzed. Phylogenetic trees were calculated implemented in PAUP with the maximum-likelihood method.

Results: In 2006 H5N1 was firstly identified in hooded vultures found dead or sick throughout Ouagadougou. All viruses were related to a distinct sublineage (sublineage C) and were similar to viruses found in poultry in the same period. In 2007 all gene sequences were most closely related to either one of two genetic sublineages (A and C) which were found in Nigeria in 2006. Six viruses had evolved by at least three reassortments from sublineages A and C. In all but one of the Nigerian reassortants, hemagglutinin and neuraminidase genes originated from...
different sublineages (C and A), suggesting compatibility between phenotypes of both sublineages. Interestingly, in all reassortants nonstructural genes were derived from sublineage C with two characteristic amino acids, which were never identified in sublineage A viruses.

**Conclusions:** Our results suggest that highly pathogenic avian influenza H5N1 viruses initially imported into Nigeria in 2006 have been gradually replaced by various reassortant strains. Further, these viruses have most probably evolved from sublineage A and C viruses initially imported into the country in 2006. Moreover, those reassorted viruses possessed a distinct bias of preferred genes, which may suggest a higher fitness of these viruses. Since the high prevalence of reassortants was typical for West Africa in 2007, the absence of such reassortants anywhere else suggests that reintroductions of influenza A (H5N1) from Africa into Eurasia must be rare.

**10.135 Cross-Neutralization Activity Against the Hemagglutinin of Influenza A**

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**Background:** The hemagglutinin (HA) glycoprotein plays an important role in receptor-binding and membrane fusion of influenza virus entry into host cells. The HA is a target for neutralizing antibodies, changes in the HA structure could prevent antibody binding and escape from protection. In this study we performed a neutralization assay using polyclonal antibodies against the full length HA and a monoclonal antibody towards the N-terminal of the HA from an Asian isolate of H5N1. The virus used in this study was highly pathogenic avian influenza H5N1 belonging to sub-clade 2.2.2 and isolated in Sweden 2006.

**Methods:** A neutralization assay was performed. Sera were diluted 1:10 and inactivated at 56°C for 30 min, and diluted serially two-fold. Virus was added approximately 170 50% tissue culture dose (TCID50) to wells containing the serum dilutions. The mixture of sera and virus was incubated at 37°C for 60 minutes and was then transferred to 96 well plates with MDCK cells. After 72 hours the neutralization titers were deduced by the TCID50 calculated from the cytopathic effect caused by the virus in presence of the different dilutions of antibodies.

**Results:** Both the polyclonal antibody directed towards the full length HA and the monoclonal antibody against the N-terminal of HA showed good neutralization titers.

**Conclusion:** Antibodies directed towards the HA of an Asian isolate were able to produce good neutralization titers against a highly pathogenic avian influenza H5N1 isolate from Sweden.

**10.136 From Avian Influenza to One Health: A Connected Governance of Emerging Diseases**

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The Health and Environment Program of the French Institute for International Relations (Ifri) conducted a research on the global governance of the fight against avian influenza. Documentation and field research—more than 40 interviews in Indonesia, United States, Italy, Switzerland, Belgium and France—allowed for the drafting of a first peer-reviewed report (to be published in January). The research highlighted the importance of the connected efforts by national and international actors to respond to emerging diseases. The report recommended the strengthening of those networked efforts, as they were seen as important and efficient, and it considered that creating a new international institution would only endanger the current efforts to promote a One Health approach. As the global debate on the future of avian influenza and the framing of a One World One Health effort is unfolding, our research contributes to the debate by highlighting some strong policy options.

**10.137 One Medicine Approach to Avian Influenza Prevention and Control in the Live Bird Marketing Systems in the U.S.A. and the Western Hemisphere**

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USDA APHIS in collaboration with OIRSA and IICA conducted a model 3-day training workshop program on Biosecurity in the Live Bird Marketing Systems (LBMS) in 2007-2008 to address the prevention and control of Avian Influenza (AI) in 6 Central American countries, the Dominican Republic, Mexico, and several Andean countries in South America. Officials from the Ministries of Agriculture and Public Health, local municipalities, poultry industry, and local LBMS participated. Presentations on their HPAI Response Plans, poultry industry structure, live bird markets, biosecurity, public health, and AI epizootiology were followed by visits to local live bird markets. Participants discussed their findings and presented their reports and recommendations on the final day of the workshop. Working Groups of interagency and industry representatives were formed at the conclusion of each workshop to continue discussions on development of LBMS inspection and surveillance programs, and on biosecurity education. All the Central American countries and the Dominican Republic convened for a joint meeting in March 2008 to address common identified public health and animal health issues. Results of these workshops and the regional meeting will be presented.

**Antibiotic Resistance**

10.138 – 10.180 Room: Bruckner/Mahler/Brahms – First Level

**10.138 Outbreak of Infections Due to Klebsiella Pneumoniae Carbapenem (KPC)-producing K. pneumoniae in a Hospital in Greece**

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**Background:** The last decade nosocomial outbreaks due to KPC-producing strains have occurred in Eastern USA and Israel. We describe a KPC-producing K. pneumoniae outbreak in a hospital in Crete during 2007–2008.

**Methods:** A K. pneumoniae suggestive of (s/o) KPC production was defined as an isolate displaying an MIC to imipenem>1µg/ml, a positive Hodge test, and a negative EDTA test. Strains were identified through a computerized database system of the hospital, searching from January 2007 through May 2008. From November 2007, such isolates were transferred for molecular typing at the Department of Microbiology, National School of Public Health.

**Results:** From May 2007, an a KPC-producing K. pneumoniae outbreak involving 22 patients, none of whom had travelled in a country with known high prevalence of such isolates. KPC-producing K. pneumoniae strains were mainly isolated from patients in the Intensive Care Unit (72.7%), on mechanical ventilation (63.6%), with prolonged hospitalization (mean: 20.18 days), and prolonged administration of antibiotics (72.7% for a mean of 13.3 days). Diagnoses were pneumonia (62%), surgical site infection (19%), bacteremia (9.5%), urinary tract infection (4.7%), and peritonitis (4.7%). Overall, 61 KPC-producing K. pneumoniae isolates
were recovered, mainly from the respiratory tract (59.1%), catheter tip (22.7%), surgical site (18.2%), and blood (18.2%). Among 16 patients for whom therapeutic data were available, 14 (87.5%) were treated with a combination of colistin, tigecycline and/or garamycin. Clinical failure was noted in 22.2% of 18 patients available for assessment of clinical outcome, and microbiologic failure in 87.5% of 8 patients available for assessment of microbiologic outcome.

Discussion: The emergence of KPC-conferring resistance in a Greek hospital is alarming since it is added to the already high burden of resistance in Greece. Efforts are required to promote rational use of available antibiotics, increase adherence to infection control measures, and enhance active antibiotic resistance surveillance.

**10.139**

**A Novel Extended-Spectrum β-Lactamase, SHV-104, from Klebsiella pneumoniae**

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Background: Since first reported in Germany in 1983 and in the United States in 1989, extended-spectrum β-lactamases (ESBLs) have spread worldwide. These enzymes are mostly plasmid-encoded derivatives of TEM-1, TEM-2, and SHV-1 by one or more base pair changes or are from a rapidly evolving class called CTX-M.

Methods: K. pneumonia ML2011 was collected on July 2004, from intensive care unit of Military hospital in Tunisia. Identification of strains was performed by using both API 20 E and the VITEK automated system. Minimal inhibitory concentrations (MICs) were determined by E-test Strips for the strain on Mueller-Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI) (CLSI/National Committee for Clinical Laboratory Standards [NCCLS], 2006). Transfer of resistance phenotypes was performed by transformation and conjugation experiments. The ESBL was identified by double-disk synergy test, by isoelectric focusing, β-lactamase assay and sequencing of PCR products. The free forms of SHV-1 and SHV-104 were modeled to investigate the role of residue Arg202Ser in ceftazidime and cefotaxime hydrolysis.

Results: MiCs for K. pneumoniae ML2011 showed that this strain was resistant to all β-lactams tested expect imipenem. K. pneumoniae ML20011 exhibited a high level of resistance to oxyimino cephalosporins. The strain was also resistant to kanamycin, chloramphenicol, ciprofloxacin, nalidixic acid, tetracycline and streptomycin. The disk diffusion method showed synergy between ceftazidime, cefotaxime, aztreonam, ceftriaxone, and amoxicillin-clavulanic acid against the strain and its transformants and transconjugants, suggesting plasmid-mediated production of an ESBL enzyme. PCR analyses confirmed the presence of blaSHV in parent strain K. pneumoniae ML2011, and its transformants E. coli DH5α ipML2011 and transconjugants E. coli HB101 X pML2011 indicating that this gene is located on transferable plasmid with estimated molecular size of 50 kb. Nucleotide sequence was performed on the coding region 861bp used to predict the amino acid sequence. This sequence was compared with strain K. pneumoniae Kp297 (DDBJ/EMBL/GeneBank accession no. EF035567) for nucleotide sequence homology and predicted amino sequence. Two amino acid substitutions were found at position 5 and 202, resulting respectively in a Met (ATG) to Leu (TTG) and an Arg (CGT) to Ser (AGT) changes. As theses substitutions not showed by other known SHV β-lactamases, the pl 7.3 from K. pneumonia ML2011 appears to be a novel ESBL and has been designated SHV-104 (http://www.lahey.org). Specific activity analysis of this β-lactamase partially purified from the crude extract of the strain K. pneumoniae ML2011 indicated that SHV-104 hydrolyzed ampicillin, cephalothin, ceftriaxone, cefotaxime and cefazidime. The specific activities varied from 6 to 97.7 U/mg of proteins. For the extended spectrum cephalosporins, the cefotaxime was the most hydrolyzed. Compared to SHV-1, SHV-104 has only one interesting change: Arg202Ser. Ser-202, on the upper surface of the enzyme, located at the N-terminal of α helix ∂9, is exposed on the opposite side of active site. Ser 202 is very far from the binding site. However, the C-terminal end of the helix ∂9 forms the upper edge of the binding site. On the other hand, at the helix ∂9 end, in SHV-1, we could imagine a possibility of interaction between Arg-202 and Glu-92, located at the C-terminal end of the helix β2, for a saline bridge. The distance is extremely long, 5.7 to 6 Å but we can imagine hydrogen bonding via the water molecule HOH582. The interaction between Arg-202 and Glu-92 can be intervening in the good positioning of the helix β2 which carries the active serine.

Conclusion: The modification at position 202 may result in a change of the pl to 7.3 and the ability to hydrolyze the cefotaxime and the cefazidime.

**10.140**

**Detection of Resistant Van-positive, Negative Enterococci and Determination of Their Antibacterial Susceptibility Patterns to the Newly Introduced Antibiotics**

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Background: Vancomycin is the antibiotic of choice for the treatment of infections. However, the emergence of vancomycin-resistant enterococci (VRE) during last decade became an important issue in hospitals worldwide. This study was conducted to characterize clinical features of Van -positive and negative nosocomial VRE. In addition, antibacterial susceptibility patterns of them to newly introduced antibiotics were also determined.

Methods: Totally 297 enterococcal strains were isolated from patients’ specimens. VRE were isolated based on agar screen method and the phenotypes were confirmed by tube dilution method. MICs of resistant isolates to vancomycin and teicoplanin were determined by E test method. Simultaneous detection of Van genes and species identification was performed using multiplex PCR assay. Sensitivity patterns of VRE isolates to several antibiotics including new ones were determined by disk diffusion method.

Results: One hundred and four (34%) of isolates were vancomycin resistant. Enterococci were detected in 12.5 % of urine, 10.5% of blood and 7% of stool samples. E. faecalis was more prevalent resistant isolates (62.5%). Thirty seven (35.5 %) of resistant isolates were VanA -positive, predominantly consisting of E. faecium strains. VanB and VanC2/C3 were not detected, while VanC1and VanC1+B were observed only in one (1%) of the specimens. Resistant isolates were sensitive to tigecycline and linezolid and resistant to ciprofloxacin and amikacin. Significant differences in sensitivity patterns of Van-positive and negative isolates to teicoplanin and gentamicin were noticed. Our data indicate that quinupristin/dalfopristin is not effective based on in vitro activities. All isolates which were resistant to ciprofloxacin also showed cross-resistance to the other tested antibiotics. Almost all amikacin and gentamicin resistant isolates also showed cross-resistance to the tested antibiotics.

Conclusion: Consistent with previous reports, VanA is predominant gene of Van -positive isolates in Iran frequently detected in E. faecalis. In contrast, prevalence of Van-negative VRE in E. faecalis is markedly increased. These findings lend support to the hypothesis that due to frequent vancomycin administration in our clinics the acquisition of Van -genes as well as selection of resistant mutant isolates could be facilitated. Rational prescription of vancomycin concordant with prudent and wisely administration of newly introduced antibiotics like tigecycline and linezolid is warranted.

**10.141**

**Multidrug-Resistant Bacteria Isolated from Patients’ Samples in Intensive Care Units**

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Background: The aim of this study was to determine epidemiological aspects and bacterial resistance patterns of isolated bacteria from patients’ samples in Intensive Care Units.

Methods: During a 10-month-period (from June 2006 to March 2007), 812 samples of blood, urine and CSF from 553 hospitalized patients in ICU wards including pediatrics surgical, neonatal, adult surgical I, adult surgical II, general pediatrics, neurosurgical I, neurosurgical II, and internal medical were collected. Minimum Inhibitory Concentration (MIC) of antibiotics for isolated bacteria was determined by the E-test method.

Results: Internal ICU with 28.7% admissions was standing in the highest rank. Coagulase Negative Staphylococci with 66.7%, 36.5% and E. coli with 20.9% frequencies were the bacteria isolated from the blood, CSF and urine sequentially. Samples taken from patients with ages between 20-40 years were at the highest level (32.2%) while for the patients over sixty years this figure standing at the lowest level (18.5%). Both gram positive and negative isolates expressed resistance to the majority tested penicillins and cephalosporins.

Conclusion: Combined therapy with vancomycin and meropenem or imipenem showed the most effective treatment against gram positive and negative isolates when empirical therapy needs to be considered. High multi-resistant bacteria in ICUs warn us to administer a few effective antibiotics in our hospitals more wisely to reduce pressure on sensitive strains. This could be beneficial for lifesaving of ICU patients and prevention of spread of resistant isolates in these critical wards. Due to continuous change of antibacterial susceptibility patterns, periodical antibacterial sensitivity assessment in ICUs seems mandatory.

10.142 Antimicrobial Resistance Patterns of Gram-Negative Bacteria from the Surgical Department of a General Hospital

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Objectives: To determine the antimicrobial resistance of Gram-negative bacteria isolated from patients of the Surgical Department of “G. GENNHMATAS” Thessaloniki General Hospital, Greece.

Methods: From January 2006 to November 2007, a total of 333 non-repetitive positive cultures from the Dept of Surgery were studied. All specimens were routinely inoculated into culture media. The blood cultures were incubated in Bactec 9250 system (Becton Dickinson®) in aerobic plus, anaerobic plus and mycosis vials. Identification of microorganisms and susceptibility test was performed with the Vitek 2 system (BioMerieux®, France), the susceptibility disc diffusion method according to CLSI directions and the E-test method (Solnà, Sweden). The infections were characterized as nosocomial according to CDC criteria.

Results: From 333 positive cultures 17 were positive with two microorganisms. Gram-negative bacteria more frequently isolated from surgical patients were: 67 (20.12%) Escherichia coli, 26 (7.81%) Klebsiella pneumoniae, 25 (7.51%) Proteus mirabilis, 22 (6.61%) Acinetobacter baumannii, 20 (6.01%) Pseudomonas aeruginosa and 10 (3%) Enterobacter cloacae. The resistance rates (%) of Escherichia coli, Klebsiella pneumoniae and Proteus mirabilis strains were: amikacin 81/6/5, gentamicin 7/8/6, ceftazidime 12/60/56, ciprofloxacin 13/64/52, imipenem 0/36/14, piperacillin/tazobactam 11/27/0, colistin 0/0/0. The resistance rates (%) of Acinetobacter baumannii were: amikacin 91, gentamicin 89, cefepime 88, imipenem 95, ampicillin/sulbactam 55, doxycycline 30, minocycline 20, colistin 0. The resistance rates (%) of Pseudomonas aeruginosa were: ceftazidime 32, cefepime 22, ciprofloxacin 37, amikacin 26, aztreonam 50, piperacillin/tazobactam 29, imipenem 32, colistin 0.

Conclusion: Escherichia coli was the predominant pathogen followed by Klebsiella pneumoniae. High resistance rates to all antibiotics, under study were observed for all Gram-negative bacteria except E. coli. Colistin was active against all microorganisms. Continuous monitoring to recognize new patterns of antimicrobial resistance is mandatory in our Hospital.

10.143 Antimicrobial Resistance of Gram-Negative Pathogens Isolated from Intensive Care Unit Patients with Bacteremia

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Objectives: To determine the frequency Gram-negative bacteria of pathogen isolated from ICU patients with bacteremia and to investigate their susceptibility patterns.

Methods: The blood cultures were incubated in Bactec 9250 system (Becton Dickinson®) in aerobic plus, anaerobic plus and mycosis vials. Identification of microorganisms and susceptibility test was performed with the Vitek 2 system (BioMerieux®, France), the susceptibility disc diffusion method according to CLSI directions and the E-test method (Solnà, Sweden).

Results: A total of 2519 blood cultures were screened from January 2006 to December 2007. Bacteremia was detected in 350 ICU and 132 non-ICU patients. Of the 482 pathogens Gram-positive bacteria were the predominant isolates (50.62%), followed by Gram-negative (47.93%) and fungi (1.45%). Gram-negative bacteria more frequently isolated from ICU were: A. baumannii 49, K. pneumoniae 46, Ps. aeruginosa 36, Enterobacter cloacae 37, E. coli 9. The resistance rates (%) of A. baumannii were: amikacin 87, gentamicin 70, imipenem 80, meropenem 34, piperacillin/tazobactam 84, ampicillin/sulbactam 46/46, colistin 0/0. The resistance rates (%) of Ps. aeruginosa were: ceftazidime 69, ciprofloxacin 72, amikacin 48, aztreonam 91, piperacillin/tazobactam 48, imipenem 69, meropenem 71, colistin 0/0. The resistance rates (%) of K. pneumoniae were: amikacin 33, gentamicin 32, ciprofloxacin 5, imipenem 80, piperacillin/tazobactam 67, colistin 0/0.

Conclusion: A very high incidence of multidrug resistant Gram-negative strains causing bacteremia were isolated from ICU patients. There is necessary to limit the overuse of antibiotics and implementation of a new antibiotic policy.

10.144 Frequency and Antimicrobial Susceptibility of Gram-Negative Bacteria Isolated From Makkah Hospitals-Saudi Arabia

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Background: Antibiotic resistance among Gram-negative bacteria has become a major clinical concern worldwide. This study is aimed to estimate the prevalence and antibiotic susceptibility of the Gram-negative bacteria isolated from Makkah hospitals.

Methods: This study was undertaken in the two main tertiary care hospitals in Makkah: Al-Noor specialist hospital (560 bed) and Hera hospital (283 bed) during the period of six months, starting on October 2005. A total of 1137 Gram-negative bacteria were identified in non-duplicate clinical specimens obtained from 965 patients of various body sites infections. Demographic data, type of organisms and their antimicrobial resistances were collected from medical and laboratory records for each patient.

Results: The most prevalent Gram-negative bacteria were E. coli and P. aeruginosa, which accounted 359 (31.6%) and 355 (31.2%), respectively. Subsequently, A. baumannii (123/10.8%), K. pneumoniae (94/8.3%), S. aureus (70/6.2%), H. influenzae (42/3.7%), Proteus spp. (37/3.3%) and Enterobacter spp. (22/1.9%). Results demonstrated that, Gram-negative bacteria have high rates of resistance to commonly used antibiotics. Above 50% of resistance were reported for E. coli and K. pneumoniae against Ampicillin (83.9%, 95.3%), Piperacillin (59.3%, 54.2%), Levofloxacin (52.5%, 50%) and Nalidixic acid (49%, 42.3%). A high rate of resistance also reported for E. coli against Cephalothin (58.4%), Augmentin (51.6%), and Ciprofloxacin (54.6%). On the other E. coli and K. pneumoniae showed a high susceptibility to carbapenems. P. aeruginosa and A. baumannii also showed high rates of resistance ranging from 50–100% to most antimicrobial agents. A moderate resistance rates were found among carbapenems ranging from

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25–46%. Multi-drug resistance among Gram-negative isolates was also common in this study.

**Conclusion:** Our data showed a high rate of resistance among Gram-negative pathogens. The implementation of monitoring and surveillance programs is an important part of the prevention strategy against the development of resistance.

### 10.145 Antimicrobial Resistance Patterns of *A. baumannii* Isolated from Intensive Care Unit

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**Objectives:** The aim of this study was to evaluate the antimicrobial resistance of *A. baumannii* strains isolated from intensive care unit throughout a two-year period.

**Methods:** A total of 199 *A. baumannii* strains were selected from January 2006 to December 2007. 175 strains were obtained from intensive care unit and 82 from other departments of our Hospital. Identification of microorganisms and susceptibility testing was performed with the Vitek 2 (BioMerieux, France) and the susceptibility disc diffusion method according to CLSI directions and the E-test method (Soln, Sweden). For the phenotypic confirmation of extended-spectrum-b-lactamase producing isolates, inhibition zones were compared by using cefazidime, ceftipime, aztreonam, cefotaxime with and without clavulanic acid (DDST test). To screen for metallo-b-lactamase production (MBL), a synergy test using an imipenem and EDTA-containing discs was employed.

**Results:** Bloodstream infections were most frequent followed by urinary tract infections, central venous catheters infections, respiratory tract infections and surgical site infections. Resistance rates to antibiotics tested were as following: amikacin 88%, ampicillin/subactam 42%, aztreonam 100% cephepine 96%, cefotaxime 100%, ceftriaxone 100%, ceftazidime 100%, ciprofloxacin 99%, colistin 0%, levofloxacin 97%, imipenem 76%, meropenem 88%, moxifloxacin 94%, netilmicin 86%, norfloxacin 100%, pefloxacin 100%, piperacillin/tazobactam 82%, tobramycin 78%, gentamicin 63%, minocycline 12%, ige cycline 0%. All of the isolates were positive in the DDST test and 47.8% of them were MBL producers (EDTA test: positive).

**Conclusions:** The emergence and rapid spread of multidrug resistant *A. baumannii* isolates are of a great concern worldwide. The majority of isolates were resistant to 10 or more antibiotics tested and some strains were defined by resistance to all antimicrobial agents except colistin and tigecycline. The resistance to imipenem rose dramatically. There is a need to limit the overuse of antibiotics and implementation of a new antibiotic policy.

### 10.146 Re-conceptualizing Interventions in Drug-Resistant TB Among Poor Communities in Africa

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Presently, TB is the second greatest contributor among infectious diseases to adult mortality causing approximately two million deaths a year worldwide. WHO estimates that one-third of the world’s population is infected with Mycobacterium tuberculosis. Efforts to control the spread of TB have been going on with limited progress. This is mainly for two reasons. First, the emergence of HIV/AIDS in the last couple of decades has further exacerbated the TB infection by compromising the immune system of HIV infected individuals. In high poverty-stricken settings, HIV is the main reason for failure to meet TB control targets. Second, Poor TB control programs have largely contributed to not only the steady spread of TB, but to the emergence of drug-resistant TB. This is especially so among the poor communities. Poor health care facilities and laboratory systems have meant that TB control programs either collapse or perform below average expectation. The drugs we are using to treat TB all date from the 1940s. We are treating this disease with very old technology. The DOTS strategy has failed to meet its set objectives due to a lack of well-thought out pro-poor collaborative TB/HIV framework.

Such a framework would reduce the burden of TB among HIV affected populations and the burden of HIV among TB patients. The following strategies are thus suggested for TB control programs;

- Deliberate targeting of mobile populations and other high risk and difficult-to-reach groups which are at considerable risk of both TB and HIV
- Community involvement of persons infected/affected in TB control
- TB/HIV collaborative activities
- Emphasis on Advocacy communications, Social mobilization
- Improved Monitoring and Evaluation systems
- Involvement of all health care providers
- A deliberate emphasis on TB Treatment Literacy to encourage adherence
- Development of new rapid tests (like existing ones for HIV and malaria) for TB.

### 10.147 The Correlation Between Antimicrobial Consumption and Resistance Pattern of Pathogens Isolated from Patients with Nosocomial Infections from ICUs of a Teaching Hospital

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**Background and Objectives:** The purpose of this study was to compare the pattern of bacterial resistance in association with antibiotic usage among different ICUs.

**Methods:** During a one-year-period, we collected culture and susceptibility results from patients with nosocomial infections from medical ICU (MICU), surgery ICU (SICU) and neurosurgery ICU (NICU) of a teaching hospital. Corresponding demographic data and risk factors of these cases were recorded. Minimum Inhibitory concentration (MIC) of the bacteria isolated from samples was determined by E-test method. Antibiotic consumption data was calculated as defined daily doses (DDD) per 1000 patients-days for each unit.

**Results:** In the MICU 33.3% of *Acinetobacter baumannii* isolates were susceptible to amikacin and gentamycin whereas in NICU 12.5% of them was susceptible to these aminoglycosides. *Acinetobacter baumannii* has no carbapenem resistance in MICU. However in NICU 75% of them was susceptible to imipenem and meropenem. Statistically significant positive correlation between antibiotic consumption and pattern of microorganism resistance were found in *Acinetobacter baumannii*.

**Conclusion:** The antibiotic susceptibility of microorganisms is different among ICUs. Concomitant surveillance of both antimicrobial resistance and antimicrobial use is necessary to design empiric therapy and ICU antibiogram for each ICU. Although, the role of variables other than antimicrobial use for interpreting the reasons of antimicrobial resistance should be determined.

### 10.148 Risk Factors for Nosocomial Bacteremia Due to *Pseudomonas aeruginosa* and Multiple Drug-Resistant *P. aeruginosa*

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**Background:** Nosocomial bloodstream infections due *P. aeruginosa* are still a prevalent and life-threatening condition. The increasing frequency of multiple drug resistance is a matter of concern. The identification of potential risk factors is essential to improve the appropriateness of empirical therapy and design preventive strategies.
Objective: To determine risk factors for nosocomial bacteraemia (NB) due to *P. aeruginosa* (Pa) and to multiple drug resistant *P. aeruginosa* (PaMR).

Methods: Retrospective analysis of patients with nosocomial bacteraemia diagnosed during an 11-year period (1997–2007) in a 700-bed, University Hospital. Variables evaluated included demographics, underlying diseases, and exposure to selected procedures (surgery, urinary tract catheter, mechanical ventilation), corticosteroids and antibiotics within the previous month. Multiple drug resistance was defined as resistance to at least cefotaxime, carbapenems (imipenem or meropenem) and ciprofloxacin.

Results: Out of 4,799 episodes of NB, 362 (8%) were due to Pa and 47 (1%) to PaMR. Logistic regression analysis selected male gender (OR 1.25, 95% CI 1.01-1.56), COPD (OR 1.56, 95% CI 1.12-2.19), solid organ cancer (OR 1.41, 95% CI 1.09-1.82), solid organ transplantation (OR 2.02, 95% CI 1.39-2.93), mechanical ventilation (OR 1.61, 95% CI 1.23-2.1), use of corticosteroids (OR 1.42, 95% CI 1.12-1.8), and exposure to any non-antipseudomonal antibiotic (OR 1.54, 95% CI 1.23-1.92), carbapenems (OR 1.54, 95% CI 1.16-2.05) and aminoglycosides (OR 1.6, 95% CI 1.18-2.16) as the best predictors of Pa NB. For PaMR NB, independent risk factors included male gender (OR 4.29, 95% CI 2.02-9.09), COPD (OR 4.52, 95% CI 2.11-9.68), chronic renal insufficiency (OR 3.01, 95% CI 1.4-6.46), solid organ transplantation (OR 5.4, 95% CI 2.58-11.3) and exposure to quinolones (OR 3.83, 95% CI 2.04-7.18), carbapenems (OR 3.52, 95% CI 1.86-6.64) and aminoglycosides (OR 4.88, 95% CI 2.45-9.74).

Conclusions: In addition to some well known underlying conditions and previous administration of non-antipseudomonal antibiotics, the exposure to carbapenems and aminoglycosides may be associated with nosocomial *P. aeruginosa* bacteraemia. Multiple drug resistance is associated with previous exposure to antipseudomonal agents, particularly quinolones, carbapenems and aminoglycosides.

**10.149**

Antimicrobial Susceptibility and Resistance of MRSA-Methicillin Resistant Staphylococcus aureus

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Staph infections, including MRSA are among the most common hospital-acquired infections of the skin, nostrils, lungs, surgical wounds, bloodstream worldwide, and are now endemic in most hospitals. In addition to causing therapeutic problems for patients, especially in immunocompromised hosts, infection can be fatal as most strains are multi-drug resistant. Colonized medical personnel and infected patients serve as the major reservoir of MRSA in affected hospital worldwide. Consequently, the therapeutic options for treatment of staphylococci infections are limited. Conventional methods for identifying MRSA, such as disc susceptibility testing, are insufficiently sensitive. The goal of this work is to test in vitro antimicrobial susceptibility of MRSA, and disk diffusion testing on mannitol salt agar, screening method for detection of oxacillin resistance in MRSA. Antimicrobial susceptibility based on the agar disc diffusion method on Mueller Hinton agar was the standard of the NCCLS method. The antimicrobial tested were: ampicillin, penicillin, teicoplanin, azitromycin, ampicillin/subbactam, cefoxitin, ciprofloxacin, clindamycin, chloramphenicol, mupirocin, erythromycin, gentamicin, linezolid, oxacillin, norfloxacin, rifampin, tetracycline, vancomycin, trimethoprim, and trimethoprim/sulfamethoxazole. Staphylococcus species were clinically isolated from different patient samples in the routine hospital microbiological work and identified by standard microbiology methods. Samples included swabs of: nostrils, skin, wounds, ear, drain, navel, catheter, bloodstream, urine and stool. In the study, a panel of 128 clinical MRSA isolates was tested. The Mueller-Hinton agar was inoculated with MRSA species isolates and antimicrobial discs applied. Within 30 minutes of application of the discs, the plates were incubated at 370C for 18 to 24 hours. MRSA refers to strains that are resistant to many antibiotics. The results indicate that vancomycin, linezolid, teicoplanin, rifampin, tetracycline, trimethoprim, and trimethoprim/sulfamethoxazole are antibiotics that can be used in the treatment of MRSA infections. Oxacillin disk diffusion on mannitol salt agar is an excellent method for surveillance cultures of MRSA, with a zone diameter <16 mm as oxacillin resistant.

**10.150**

Parallel Evolution of Multidrug-Resistance in Pathogenic Bacteria isolated from an Asymptomatic Livestock Reservoir

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Background: The increase in frequency of *Salmonella enterica* resistant to antibiotics in food producing animals is of great concern to public health. Determining the rate at which different resistance phenotypes are generated and maintained in the environment is thus of great importance. However, most of our estimates of drug resistance and genetic diversity come from studies of epidemic outbreaks or disease-related isolates; much less attention has been paid to bacterial carriage populations, which inhabit hosts without producing disease. Since new virulent strains that cause disease can be recruited from the carriage population of bacteria, our understanding of infectious disease and antibiotic resistance evolution is seriously incomplete without knowledge on the population structure of pathogenic bacteria living in asymptomatic host.

Methods: We report the first extensive survey of the abundance and diversity of a human pathogen in asymptomatic animal hosts. The distribution and evolution of antibiotic resistance and multidrug-resistance in 362 *Salmonella* stains as part of a cross-sectional study of the Canadian swine industry was investigated. The susceptibility of all isolates to 12 antimicrobial agents was tested and the statistical and phylogenetic distribution of resistance among strains characterized via multilocus sequence typing was studied to test the origin of multidrug-resistance in *Salmonella*.

Results: We have found that asymptomatic swine from livestock productions frequently carry populations of *Salmonella enterica* with a broad range of drug-resistant strains and genetic diversity greatly exceeding that previously described. More than 25% of all isolates were multidrug-resistant, with predominance in serotype Typhimurium, a serotype of vital importance to public health. The strong associations between resistance phenotypes, which differ among serotypes and which is supported by the phylogenetic relationship of the different serotypes, was indicative of the independent acquisition of multidrug-resistance in at least two different serotypes, i.e. Typhimurium and Derby. Conclusion: The independent origin of multidrug-resistance in *Salmonella* indicates that strong selective pressures are present in the environment of the bacteria and that statistical and phylogenetic studies of antibiotic resistance are an essential part in the understanding and the control of the epidemic. This study shows how agricultural practice and human intervention may lead and influence the evolution of a hidden reservoir of pathogens, with important implications for human health.

**10.151**

Treatment of Bacterial Vaginosis: A Comparison of Oral Metronidazole and Metronidazole Vaginal Gel

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Introduction: Bacterial vaginosis is the most common cause of vaginitis. It is well recognized that serious forms of BV can induce several complications among women undergoing gynecologic or obstetric surgery, having pelvic inflammatory diseases, temporary or absolute infertility, miscarriage and abortion. The purpose of this study was to compare the efficacy of oral metronidazole with metronidazole vaginal gel 75% for the treatment of bacterial vaginosis (BV).

Methods: Seventy women with a clinical diagnosis of BV were treated with oral metronidazole and metronidazole vaginal cream (group, n=36; cream group, n=34). Assessments included pelvic examination and diagnostic testing. Primary efficacy endpoints were a resolution of two of three diagnostic criteria at the first follow-up visit and three of three diagnostic criteria at the second.

Results: Cure rates in the evaluable patient population were similar between oral metronidazole 72.2% for the cream group 66.7% (p <0.05). The most commonly reported medical event, vulvovaginal pruritus, had
similar incidence in both treatment groups using traditional clinical and laboratory criteria. Post treatment vulvovaginal candidiasis was experienced by 11.1% of subjects treated with oral metronidazole, and 23.5% of subjects treated with metronidazole vaginal gel.

**Conclusion:** Oral metronidazole and metronidazole vaginal creme nearly equivalent cure rates for the treatment of bacterial vaginosis. Patients treated with these agents experienced similar rates of posttreatment vulvovaginal candidiasis.

### 10.152 Alternative Therapies for Combating Antimicrobial Resistance and Reduction of Severe Nosocomial Infections

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**Background:** Worldwide, researches are carried over for the antimicrobial resistance phenomenon, in the following directions: fundamental research, for better knowledge of the natural and acquired resistance mechanisms, development of epidemiology surveillance networks, establishment of the antibiotic sustainability of the isolated bacterial and fungal strains and conception of drugs with an increased antimicrobial effect for alternative therapy.

**Objectives:** The aim was to specify the most efficient drug and natural product, but also to compare those products efficiency with that of some antibiotics.

**Methods:** A study concerning the capacity of some bee products and vegetal extracts under different presentation forms to inhibit microorganism development (bacteria, fungi, algae), as alternative therapy of infections with antimicrobials were tested. Comparatively were tested the effect of honey on Staphylococcus aureus strains, isolated from animals and humans collected samples. The bee products used were honey, apireved, propoderm, meltonic, royal jelly and propolis tincture. Vegetal extracts used in experiment were essential oils obtained from plants found in Romanian flora, as well other countries with subtropical climate. The tested extracts were: polioel 3, coconut, eucalyptus, rattle marigold, fir, aloe, and savoy essential oils.

**Results:** The bee product with the highest efficiency were propolis tincture, followed by royal jelly, meltonic and propoderm. The honey has been active on S. Aureus strains. From the vegetal extracts, the most efficient was first essential oil, followed by savoy, coconut and polioel 3 essential oils.

**Conclusion:** An important issue in therapy is the occurrence of the resistant strains, both to common antibiotics drugs and to those recently discovered. Our future goal is to study the susceptibility of some bacteria and fungi to natural products and vegetal extracts in order to struggle against these wide spread infections. The findings emphasize the need for continuous surveillance and further clinical investigational studies.

### 10.153 Investigation of Vancomycin Resistance Among Different Clinical Isolates of Staphylococcus aureus in Tehran

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**Staphylococcus aureus** is one of the most common causes of both hospital and community acquired infections worldwide, and vancomycin has been used to treat many *S. aureus* infections that lead to the emergence of vancomycin intermediate and resistant *S. aureus* (VISA and VRSA) in various parts of the world. Therefore, the present study was carried out to confirm (or not confirm) the possible presence of VISA and VRSA in Tehran-Iran.

First, 100 clinical samples of *S. aureus* were collected from 6 month in 2008. Then, the minimum inhibitory concentration (MIC) of vancomycin was carried out according to guidelines of the Clinical Laboratory Standard Institute (CLSI) using the agar dilution method. Disk diffusion method was used for determination of susceptibility of strains to other common antibiotics.

The frequency distribution tables, diagrams ( applying SPSS computer programs ) were used to describe and analyze the data. Out of 100 *S. aureus* isolates, none of isolates were resistant to vancomycin (MIC < 1 µg/ml) and one strain have show to be vancomycin intermediate (MIC=8 µg/ml). All the isolates were resistant to penicillin while the lowest resistance (15 %) was seen to co-trimoxazole.

According to the results of this study, one isolate of VISA was found in Tehran that call for further epidemiological studies.

### 10.154 Frequency of Staphylococcus Species Among Nosocomial Infections from Sina Hospital in Tehran, Their Resistance to Usual Antibiotics and Identification the Beta Lactamase Producers by Starch Rapid Paper Strip Test

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**Introduction and Aim:** Due to importance of nosocomial infections, in different studies, the role of staphylococcus sp, the cause of their resistant to usual antibiotics and the effect of new antibiotics against them is doing each year. Then, this study was done in Sina hospital in Tehran to evaluate the frequency of Staphylococcus sp and determine their resistance to usual antibiotics and their ability to produce beta-lactamase.

**Methods:** From July 2007 to April 2008 (9 months), 100 staphylococcus sp. isolated from 100 different samples by standard bacteriologic tests. The frequency of isolated staphylococci and their resistance to amoxicillin, tetracycline, erythromycin, chloramphenicol, cotrimoxasol, gentamicin, cephalotin, vancomycin, cloxacillin and cephalozin disks determined by disk diffusion in Kirby- Bauer method. Beside, beta lactam production determined by rapid penicillin paper strip test among isolated staphylococcus sp.

**Results:** The frequency of isolated staphylococci strains were 63% *Staphylococcus aureus*, 19% *Staphylococcus saprophyticus*, 16% *Staphylococcus epidermidis* and 2% other sp. The most resistance was against amoxicillin 93% and 62% in coagulase positive and negative respectively. In addition, 73% of both coagulase negative and positive staphylococci strains were beta lactamase producer.

**Conclusion:** Beta lactamase production is the main cause of resistance to beta lactam drugs.

### 10.155 The Effect of Grapefruit Aqueous Seed Extract Against Candida albicans Resistant to Floconazol and Clotrimazol

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**Introduction:** With considering that high prevalence of vaginitis in all communities including Iran, which can be a infectious or non infectious, inflammatory disease and caused by bacteria, fungi and etc. With attention to drug resistance of infective agents, it was determined to study grapefruit seed extract effect that contains various bioflavonoids.

**Methods and Materials:** Our sampling was from vaginal secretions of 100 women with vaginitis that were referred to health care. After transferred to laboratory, carried out differential tests for microbial diagnosis and studied common drug resistance of microbial samples and after preparation of grapefruit seed from Tonekabon and supply the hydroextract with decoction method in various concentrations, we exposed this extract on samples with MIC method.

**Results:** The most women with vaginitis were 25–30 years old and the greatest reason (40%) was Candida albicans. Candida samples mostly resisted to Clotrimazol. After exposing hydroextract to samples, we could not see any antifungal effect. We prepared alcoholic extract with maceration method in various concentration and we exposed this extract on samples with MIC method and good effect was seen.
**Conclusion:** So we hope that in future we use the alcoholic grapefruit seed extract for treatment of bacterial and fungal vaginitis.  
**Keywords:** Grapefruit seed; antibiotic; antifungal-vaginitis

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**10.156**

**The Role of Aerobic and Anaerobic Bacteria Impotant of Foot in Diabetic People**

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**Objective:** Diabetic foot infections are a potentially severe complication of diabetes. Diabetic foot infections can sometimes lead to long-term debilitation and, in the most severe cases, amputation. They are the most common infections in patients with diabetes whose weakened immune systems put them at an increased risk of acquiring antibiotic-resistant infections.

**Method:** In order to determine the microbiological characteristics of diabetic foot infection, 60 diabetic patients (12 women, 48 men; age between 45 and 65 years with a duration of diabetes from 0.5 to 37 years) were investigated. Immediately after the hospitalization specimens from infected foot lesions were taken using Thio and BHI as transport medium. Aerobic cultures were done in all cases according to conventional methods while anaerobic cultures were carried out when clinical signs indicated to perform it. After identification of agents, susceptibility tests were performed on isolated microorganism.

**Result:** Among all diabetic patients in this study, we found the frequency of polymicrobial infections were 75% and monobacterial infections were 25%. We isolated gram positive cocci 95%, gram positive bacilli 35%, gram negative 45% and 10% mycobacterium. Also we found that 12.83% of our bacteria were anaerobic and 87.18% were facultative aerobic bacteria. In antimicrobial susceptibility testing Rifampin was the most effective antibiotic against S. aureus and peptostreptococcus. Surprisingly E.coli was resistant to all the antibiotic we used.

**Conclusion:** Diabetic foot infections have a polymicrobial nature. Antibiotic treatment of infections should be based on the results of microbiological investigation of diabetic foot.

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**10.157**

**Questionable Use of Fluoroquinolones for the Treatment of Enteric Fever in Nepal**


**Background:** In Nepal, ciprofloxacin and ofloxacin are the most commonly prescribed drugs for the treatment of enteric fever, the most common clinical diagnosis among febrile cases presenting to Nepalese hospitals.

**Methodology:** During April through October, 2008, a total of 41 isolates of S. enterica serotype Typhi (60.97%) and Paratyphi A (39.03%) were isolated from the blood samples of clinically diagnosed enteric fever cases (n=443) visiting National Public Health Laboratory, Nepal. All isolates were identified by standard microbiological techniques including serotyping. Antibiotic susceptibility to 8 antibiotics (Ampicillin, chloramphenicol, cotrimoxazole, tetracycline, nalidixic acid, ciprofloxacin, ofloxacin and ceftriaxone) was performed by using CLSI approved disc diffusion method and interpretive criteria. MIC to ciprofloxacin, ofloxacin and nalidixic acid were determined by agar dilution method.

**Results:** We found that 95.02% of the isolates were susceptible to all the antibiotics tested. Two S. Typhi isolates (4.88%) but none of the S. Paratyphi A isolates were found to be multi-drug resistant. To the best of our knowledge the isolates with full resistance to ciprofloxacin and ofloxacin with additional resistance to cotrimoxazole and tetracycline has not been previously reported from Nepal, which we have reported. Ceftriaxone was found 100% susceptible. We also found that 33 (80%) isolates were resistant to nalidixic acid, with higher resistance rate toward S. Paratyphi A (87.5%) compared to S. Typhi (76%). One encouraging trend that we report is the re-emergence of strains susceptible to ampicillin, chloramphenicol, cotrimoxazole and tetracycline (95.12%) in our region.

In the present study, the decreased susceptibility to fluoroquinolones in S. Typhi and S. Paratyphi A has been strongly correlated with resistance to nalidixic acid with sensitivity and specificity of 100%.

**Conclusion:** Ciprofloxacin can no longer be considered as the drug of choice in treating Salmonella infection. Though first line drug may still have a role to play in the treatment of enteric fever, ceftriaxone remains the sole defense against ciprofloxacin resistant Salmonella infection which is also resistance to other first line drugs.

**Keywords:** Enteric fever, Reduced susceptibility to fluoroquinolones, Nepal

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**10.158**

**Isolated of Staphylococcus Epidermidis to Vancomycin and Oxacillin from Nosocomial Infections**

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**Background:** Staphylococcus epidermidis isolated from hospital specimens often is the most multiple difficult drug resistant, therefore treatment is problematic and drug prevention of infection is the greater concern. This microorganism has become a major cause of nosocomial infections. The aim of this study was to determine S. epidermidis resistance to Vancomycin and Oxacillin in nosocomial specimens.

**Methods and Materials:** In this descriptive study that performed for 6 months, 109 S.epidermidis Strains isolated from various nosocomial specimens including blood, urine, wound, throat swabs, cerebrospinal fluid, corneal ulcer, catheter and dialysis fluid of hospitalized patients in Imam Hossein Hospital Tehran-Iran. There organisms were tested in vitro for their resistance to Vancomycin (30 mcg per disc) and Oxacillin (1 mcg per disc) by disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations.

**Result:** Among 109 strains of S.epidermidis 37.6% (41 strains) isolated from blood, urine 33% (36 isolates), wound 14.6% (16 isolates), throat swabs 5.5% (6 isolates), cerebrospinal fluid 2.7% (3 isolates), corneal ulcer 2.7% (3 isolates), Catheter 1.8% (2 isolates) and dialysis fluid 1.8% (2 isolates). Rate of ncomycin resistant and Oxacillin was 11% (12 isolates) and 86.2% (94 isolates), respectively (p<0.00001).

**Conclusion:** Our data demonstrate that incidence of oxacillin resistance, however, is so high and resistance to Vancomycin that the antimicrobial choice for treatment of staphylococcal infection is major problem and continues to increase Speedily.

**Keywords:** Antimicrobial resistance, Vancomycin, Oxacillin, Staphylococcus epidermidis.

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**10.159**

**Nosocomial Staphylococcal Infections and Their Colonization in Different Wards of a Tertiary Care Hospital in Pakistan**

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**Background:** Outbreaks of hospital-acquired infections caused by different species of Staphylococcus are being recognized with increasing frequency at global level. All living and non-living objects contribute towards the bacterial load of hospitals. Hence a study was planned to assess the nosocomial staphylococcal infections and their colonization in various wards of a public hospital in Lahore.

**Methods:** A total of 4502 positive cases were selected out of a total 32,620 indoor patients and processed for bacteriological analysis and the antibiogram of the isolates.

**Results:** In this study upon bacteriological examination of 4502 samples, 1277 isolates of Staphylococcus were recovered. In medical unit there were found 17.8% (n=227) staphylococcal isolates while in surgical unit 42.2% (n=539), ICUs 6.2% (n=79), cardiology 3.4% (n=43), cardiac surgery 5.1% (n=65), chest surgery 6.2% (n=79), orthopedics 4.5%
ABSTRACTS

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10.160

Antibiotic Susceptibility of Staphylococcus aureus from Blood Cultures: Medical vs. Surgical Wards

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Background: Staphylococcus aureus is one of the most common causes of bacteremia and sepsis of hospital origin. The aim of the study was to establish the total number of Staphylococcus aureus isolated from blood cultures of patients hospitalized at tertiary care hospital during period 1999-2007; to evaluate their antimicrobial susceptibility and distribution by departments of their origin: medical or surgical wards.

Methods: Blood cultures have been processed on the Bactec 9240 automatic system (Becton Dickinson), and positive samples have been streaked onto solid media. The cultures have been identified by standard methods of identification and/or by Vitek 2 automatic system. Antimicrobial susceptibility was tested by disc diffusion method, according to the CLSI guidelines, and/or by Vitek2 automatic system.

Results: A total of 298 isolates of Staphylococcus aureus have been isolated from blood cultures from January 1999 to the end of December 2007. Of the total of 298 strains, 157 (52.68%) have been isolated in medical and 141 (47.32%) in surgical wards. Methicillin resistant Staphylococcus aureus (MRSA) was detected in 42.35% (range between 14.29% and 66.67%) strains originating from Medical wards and in 87.65% (range 77.78% and 100%) strains originating from Surgical wards. Antibiotic resistance on Medical wards was: to penicillin 93.35%, erythromycin 39.11%, clindamycin 39.63%, amikacin 37.44%, gentamicin 50.59%, ciprofloxacin 39.38%, trimethoprim-sulfamethoxazole 29.24%, rifampicin 36.78%, fusidic acid 6.94% and vancomycin 0%. Antibiotic resistance on Surgical wards was: to penicillin 99.50%, erythromycin 82.12%, clindamycin 76.02%, amikacin 66.54%, gentamicin 89.34%, ciprofloxacin 82.67%, trimethoprim-sulfamethoxazole 43.03%, rifampicin 54.41%, fusidic acid 2.26%. All of the strains were vancomycin susceptible.

Conclusion: Very high rate of MRSA in surgical wards (87.65%) does not allow the use of beta-lactam antibiotics in surgical wards patients suffering from staphylococcal bacteremia. S. aureus strains originating from Medical wards were more susceptible to other antibiotics: macrolides (erythromycin and clindamycin), aminoglycosides (amikacin and gentamicin), ciprofloxacin and trimethoprim-sulfamethoxazole compared to S. aureus strains originating from Surgical wards. The frequency of MRSA is high compared to the countries of Northern Europe, but similar to the frequency of MRSA in the surrounding countries. Vancomycin is still the drug of choice for therapy of severe staphylococcal sepsis.

10.162

The Prevalence of Antibiotic Resistance in Anaerobic Bacteria Isolated from Patients with Skin Infections

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Summary: Antibiotic resistance in anaerobic bacteria and the lack of proper outline to treatment of anaerobic infections have been increased in recent years. In this study 100 patients with skin infections (10-60 years old) were considered. Specimens were collected in the sterile condition and transported and cultured in the Thio Glycolate media. After growing and staining of bacteria (gram staining) from selective media, bacteria were cultured in the differentiated media. Strains that were isolated, undergone antibiogram test (Kirby bauer method). Skin infections are usually polymicrobial involving aerobic and anaerobic bacteria. Common aerobic and anaerobic facultative bacteria contained: Staphylococcus aureus (37.3%), non coagulase Staphylococci (8.5%), group A streptococci (16.3%), group D enterococci (5.7%), E. coli (15.8%), enterobacter-spp (5.6%), citrobacter-spp (0.8%), Pseudomonas aeruginosa (6.9%), proteus-spp (2.7%), others (0.8%). Predominant anaerobic bacteria contained: Peptostreptococcus-spp (42.5%), pigmented prevotella and Porphyromon-spp (5.4%), Fusobacterium (7.6%) Bacteroides-spp (23.2%), Clostridium-spp (18.4%), Propionibacterium acres (2.1%), others (0.8%). Atbiogram test was done on aerobic-anaerobic facultative bacteria. Susceptibility of these bacteria were as following: Cefoxitoxin100%, Ciproflox- cin 98%, Cefazidim 82%, Tobramycin 47%, and Amikacin 33%. And their resistance to Gentamycin was 97%, Penicillin 93%, Cloxacin 86%, and Erythromycin 62%. In anaerobic bacteria, susceptibility to Ciprofloxacin was 100%, Cefoxitoxin 100%, Cefazidim 91% Rifampin 76%, Colistin 67%, and their resistance to Penicillin was 95%, Erythromycin 83%, Cloxacin 85%. Susceptibility of both aerobic and anaerobic bacteria to Cefoxitoxin was 100 %, so we suggest this drug for treatment of many skin infections.

10.161

Enterococci from Blood Cultures-Antimicrobial Susceptibility before and after Occurrence of Vancomycin Resistance

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Background: The aim of this survey is the retrospective study of the enterococcal strains isolated at the Bacteriology laboratory during the period 2002-2007, before and after the occurrence of first vancomycin resistant enterococci (VRE) in blood cultures.

Methods: A total of 35 enterococci were obtained from patients’ blood cultures in the period of 6 years. Blood cultures have been processed on Bactec 9240 system. The enterococcal strains were further identified by the use of API Strep and Vitek 2 systems. Susceptibility testing was performed by disk diffusion method, according to the CLSI guidelines, and MICs were determined using Vitek 2 system.

Results: In the period of 2002-2005, 17 enterococci were collected and in the period 2006-2007 that number was 18. The identified species were: E. faecalis (23; 65.72%), E. faecium (10; 28.57%) and E. durans (2; 5.71%). The first VRE from blood culture was isolated in 2006 and it was the only VRE (16.66%) from this kind of specimens. In 2007 the VRE number increased to 5 (50%). Comparing sensitivities of enterococci in the 2 periods we found that the sensitivity to ampicillin fall (88.2 to 55.56%), ciprofloxacin (29.40 to 22.22%) and vancomycin (100 to 61.11%). Sensitivity of E. faecalis remained almost the same to ampicillin and ciprofloxacin while it slowly decreased to imipenem (100% to 90%) and vancomycin (100% to 80%). E. faecium sensitivity to ampicillin and imipenem decreased (33.33% to 0%) as well as to vancomycin (100% to 28.57%) while it remained 0% to ciprofloxacin. The both strains of E. durans were sensitive to all antibiotics tested. The average sensitivity to gentamicin high doses was 37.09%, and to streptomycin high doses was 31.36%.

Conclusion: Our results prove that ampicillin is still efficacious antibiotic for treatment of infections caused by E. faecalis and E. durans, but it can not be used for treatment of infections caused by E. faecium. Vancomycin was efficacious for the examined strains of E. durans, but other enterococci were less susceptible to this antibiotic. The decrease of sensitivity was especially evident for E. faecium, like in many other surveys. Increasing number of E. faecalis compared to E. faecalis in blood cultures led to decrease of the average susceptibility to vancomycin, as well as to ampicillin, imipenem and ciprofloxacin.
Antimicrobial Activity of Various Antibiotics Against Pathogens of Nosocomial Origin

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Background: The era of antibiotic therapy is marked with continuous developments and introduction of new antimicrobial agents. These drugs are highly effective against a variety of microbes and are therefore known as Broad Spectrum antibiotics and life saving drugs. On the basis of survival of the fittest, the bacteria has also developed an inverse response against these drugs, and evolved into the multi drug resistant strains. The irrational use of antibiotics may lead to the suppression of drug sensitive strains and selection of drug resistant bacterial strains.

Aims and Objectives: A prospective in-vitro antimicrobial susceptibility testing study of various hospital acquired pathogens was performed to evaluate the efficacy of various antibiotics including Amoxicillin, Augmentin, Cefotaxime, Cefoperazone, Ceftriaxone, Ciprofloxacin, Avelox, Sparaxin, Levofloxacin, Doxycyclin, Klaricid, Amikacin, Tazocin, Tienam, Meropenam, Fucidin, Vancomycin, Linezolid and a newly introduced drug Tigecyclin. This study was carried out in a large tertiary care hospital of Lahore, Pakistan.

Methods and Materials: During a period from January 2008, up to November, 2008 A total of 1788 nosocomial pathogens were collected and subjected for antimicrobial sensitivity testing using disc diffusion technique. These microbial isolates were identified from morbidity samples including pus, wound swabs, blood, pleural fluid, peritoneal fluid, cerebrospinal fluid, urine, sputum endotracheal secretions, burn swabs, fecal and drainage tube material from the admitted patients.

Results: All of the isolates including Staphylococcus, Streptococcus, Enterococcus, Pseudomonas, Enterobacter, Acinetobacter, Klebsiella, Proteus, Escherichia, Serratia and Haemophilus, presented variable resistance (10–80%) against the tested antibiotics. Most of the isolates were found resistant (>30%) to Ampicillin, Augmentin, Cefotaxime, Cefoperazone, Ceftriaxone, Ciprofloxacin and Doxycyclin. The maximum antimicrobial activity among oral antibiotics was observed for Avelox, Sparaxin, Levofloxacin, Klaricid and Fucidin and among injectables Tienam, Meropenam, Fucidin, Vancomycin, Linezolid and a newly introduced drug Tigecyclin. This study was carried out in a large tertiary care hospital of Lahore, Pakistan.

Conclusion: The result of this study would be helpful for Pakistani clinicians/consultants to select the appropriate antibiotics therapy for the treatment of infections associated with resistant bacterial strains.

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I. Tayyaba1, M.A. Khan2, S. Ijaz2, M.K. Shahzad2. 1Mayo hospital Lahore Pakistan, Lahore, Pakistan, 2UVAS Lahore Pakistan, Lahore, Pakistan, 3Services Institute of medical Sciences, Lahore, Pakistan, 4Department of Pathology UVAS, Lahore, Pakistan

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Conclusion: The result of this study would be helpful for Pakistani clinicians/consultants to select the appropriate antibiotics therapy for the treatment of infections associated with resistant bacterial strains.

Keywords: antibiotics, infections, Pakistan

Comparative Minimum Inhibitory Concentration (MIC) and Mutant Prevention Concentration (MPC) Values of Enrofloxacin (ENR) Against E. coli (EC), Pasteurella multocida (PM) and Actinobacillus pleuropneumoniae (APP) Bacteria Prevalent in Swine Infections

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Background: EC, PM and APP are prevalent bacterial pathogens associated with swine infections. MPC defines the antimicrobial drug concentration threshold blocking growth of mutant sub-populations from high density bacterial inocula. We tested clinical isolates of EC, PM and APP by MIC and MPC against ENR in vitro and define the MPC potential of ENR against each pathogen.

Methods: For MIC testing, the recommended procedure of the Clinical and Laboratory Standards Institute was followed with microbroth dilution utilizing 10⁵ CFU/ml tested against doubling drug dilutions in appropriate media with optimal incubation temperature and time. For MPC testing, ≥10⁵ CFUs were applied to agar plates containing drug concentrations, incubated under optimal conditions and screened for growth at 24 and 48 hours. The lowest drug concentration preventing growth was either the MIC or MPC depending on method.

Results: For EC, PM and APP isolates, MIC (µg/ml) values were as follows: 0.016, 0.016, 0.063. The MPC (µg/ml) values were as follows: 0.25, 0.125, 0.25. For APP strain, no isolates had an MPC >0.5 µg/mL. Considering parameters such as Cmax/MIC, AUC/MIC, AUC/MPC, the following ratios (based on serum drug concentration for 5 mg/kg dose) were determined for EC, PM, APP respectively: 84.4, 5.4, 856, 84.4; 10.8, 856, 110; 21.4, 5.4, 218, 55; ratios based on urine drug concentration and E. coli were 844, 54, 8050, 512.

Conclusion: MIC and MPC values were low for ENR against EC, PM, APP strains. Based on published serum drug concentrations, ENR had serum drug concentrations in excess of the mutant selection window for 21-26 hours of the dosing interval and provided AUC/MPC values from 55-110 (512 for urine and E. coli) for 90% of the strains. An AUC/MPC ≥22 was previously shown to prevent resistance selection. As such, ENR shows a low propensity to select for fluoroquinolone resistance with EC, PM and APP based on achievable and sustainable drug concentrations in swine.

Comparative Killing of Bovine Isolates of Mannheimia haemolytica (MH) by Enrofloxacin (ENR), Florfenicol (FL), Tilmicosin (TIL) and Tulathromycin (TUL) Using the Measured Minimum Inhibitory (MIC) and Mutant Prevention Concentrations (MPC) and Maximum Serum (Cmax and Tissue (Tmax) Drug Concentration Values

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Background: MH is the most prevalent bacterial pathogen associated with bovine respiratory disease and ENR, FL, TIL and TUL are used for therapy. Bacterial eradication has been shown to positively impact clinical
outcome and may reduce resistance selection. MPC defines the drug concentration required to block the growth of the least susceptible cell present in bacterial population ≥10^6 colony forming units (CFU). We compared 4 drugs for killing clinical isolates of MH using the measured MIC, MPC, Cmax and Tmax drug concentrations.

**Methods:** For MIC testing, the CLSI recommended procedure was followed using 10^5 CFU/ml; for MPC testing ≥10^9 CFU/ml were applied to drug-containing agar plates with doubling drug dilutions in appropriate media, and incubation under optimal atmosphere/temperature. For kill experiments, 10^7–10^10 CFU/ml were exposed to drug and triplicate aliquots sampled at 0, 20, 30 min, 1, 2, 3, 4, 12 and 24 hours, plated, incubated and log_{10} reductions percent kill in viable counts recorded.

**Results:** MIC/MPC (µg/ml) values for ENR, FL, TIL, TUL against 4 MH strains respectively were: 0.016/0.125-0.5, 0.25-2/2-8, 0.5-4/4-64, 0.2-2/2-4. Exposure of 10^4-10^9 CFU/ml to MIC drug concentrations gave a +0.16 to -2.6 log_{10} reduction in viable cells by 4 hrs for FL compared to +0.2 to -0.2, +0.7 to -0.52, +0.7 to -0.35 log_{10} reduction for ENR, TIL & TUL respectively. Exposure of 10^4-10^9 CFU/ml to MPC drug concentrations gave a -1.5 to -4.9, -0.2 to -2.9, -0.7 to -2.1 and -0.5 to -1.6 log_{10} reduction in viable cells; -2.1 to -4.5, -0.14 to +2.2, +0.42 to -0.38, +0.45 to -0.27 for Cmax drug concentrations; -2.0 to -4.0, ND for FL, -0.48 to -0.92, -0.06 to -1.4 for Tmax following 4 hours of exposure to ENR, FL, TIL, TUL respectively.

**Conclusion:** Killing of MH strains was less efficient at MIC drug concentrations but was more complete and efficient at MPC drug concentrations with ENR > FL > TIL > TUL. As well, killing by the Cmax and Tmax drug concentration was faster for ENR and this likely relates to the observation that MPC values for some strains were above the maximum drug concentration achievable with the other agents but within achievable concentrations with ENR. Dosing to achieve MPC minimizes resistance selection and ensures more efficient and rapid killing provided MPC values are within clinically achievable drug concentrations.

**10.167 Emerging Escherichia coli Clone ST131 is Highly Prominent Among Extrainestinal Pathogenic Isolates from a Private Hospital in Northern India**

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We have analyzed a collection of 45 consecutive clinical E. coli isolates from the urine or bloodstream of patients treated at a private hospital in Mohali in the state of Punjab. Multiple locus sequence typing indicated that the phylogenetic group distribution of the collection was typical of extraintestinal pathogens: 67% from group B2, 15% from group D, and 18% from groups A and B1. However, 24/30 (80%) of group B2 isolates represented the emerging ST131 clone, which has achieved international notoriety for carriage of extended-spectrum beta-lactamase CTX-M-15 in a high intrinsically virulent host background. The ST131 strains were diverse in resistance properties (only 71% were CTX-M positive by PCR), certain virulence properties (e.g., only 58% carried P. mirabilis and 17% carried hly, while 100% carried iha and sat), and pulsed field gel electrophoresis fingerprints. This degree of genetic diversity is consistent with an extended period of endemic circulation in India prior to international detection as an emerging resistant strain.

**10.168 An Emerging Infection by Carbenapenem-resistant Acinetobacter baumanii in Adult Intensive Care Units**

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**Background:** Acinetobacter baumanii has become one of the major causative pathogens of nosocomial infections (NIs), particularly in intensive care units (ICUs). The resistance of A. baumanii develops rapidly to many available antibiotics, for which even higher than that of other non-fermentative gram-negative bacilli, including Pseudomonas aeruginosa. The objectives of this study were to investigate the incidence of nosocomial infections caused by A. baumanii and the proportion of drug resistance.

**Methods:** The Centers for Disease Control and Prevention’s definitions and methods were used to collect the NIs data in adult ICUs of an 1100-bed university-affiliated hospital in central Taiwan from 2003 to 2006. The isolated micro-organisms and their antibiotic susceptibility were recorded for all microbiologically or clinically documented infections. Antimicrobial susceptibility tests were performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

**Results:** The overall NI incidence was 14.0 per 1000 patient-days, and incidence of A. baumanii causing NI was 2.4 per 1000 patient-days. The leading causative pathogens in ICUs were Candida spp. (18.2%), Pseudomonas aeruginosa (15.1%), Acinetobacter baumanii (13.9%), Staphylococcus aureus (8.9%), Escherichia coli (7.6%), and Klebsiella pneumoniae (7.4%), coagulase-negative Staphylococci (4.9%) and Enterococci (4.5%). The proportion of nonfermenters was significantly higher in 2006, compared with 2003 (p<0.05, by χ² test for trend), and that of Staphylococcus aureus decreased significantly (p<0.05, by χ² test for trend). There was no significant change in trend for other pathogens during study period. The ratios of carbapenem-resistant A. baumanii were 9.8% in 2003, 48.6% in 2004, 53.8% in 2005 and 65.3% in 2006, respectively.

**Conclusions:** Nonfermentative gram-negative bacteria, particularly A. baumanii and P. aeruginosa, were the most important causative pathogens of NIs in ICU settings, and the prevalence was on the rise. A. baumanii has been one of the special concern because Acinetobacter is likely to be resistant to many antimicrobials. Since rapidly developing high proportion of carbapenem-resistant A. baumanii in ICUs was observed, which limits the treating options and then may cause excess mortality.
C. glabrata infection diagnosis, cans Candida albicans initially required mechanical ventilation. Although one-quarter of cases were diagnosed based on cultures drawn in the ED. Candidemia. Biology, and outcomes in patients with HCAC compared to persons with nosocomial candidemia. Results: During the study period, there were 218 cases of candidemia. One-quarter of cases were diagnosed based on cultures drawn in the ED. Nearly all patients with ED and positive cultures met at least one criterion for interaction with the healthcare system. These patients were as severely ill as those with nosocomial candidemia (median APACHE II score 16 vs 14, p=NS). Half of patients admitted via the ED with candidemia initially required mechanical ventilation. Although Candida albicans was the most common pathogen irrespective of location at time of infection diagnosis, C. glabrata was noted twice as frequently in persons presenting to the ED (p<0.05). Fluconazole resistance was also more common in individuals initially presenting to the ED (7% vs. 2%, p=0.04). The median length of stay in HCAC was 12.5 days. Mortality in the HCAC cohort was 35% vs. 37% in nosocomial candidemia (p=NS). Conclusions: HCAC appears to be a distinct entity and to account for a significant proportion of candidemia. Persons with HCAC are often critically ill requiring care in the ICU and consume significant medical resources. These patients face substantial risk for mortality.

Comparison of Clinical Outcomes Between Candidemia Caused by Candida albicans and Candida glabrata

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Background: With the increase in more procedures being done as outpatient, and in health care associated infections, there has been a trend towards an increase in candidemia caused by non-C. albicans species. This has been associated with an increase in fluconazole resistance, likely due to inappropriate fluconazole use, especially in species like C. glabrata. Therefore, we conducted a study to analyze the differences in epidemiology and clinical outcomes between C. albicans and C. glabrata.

Methods: All patients admitted to our hospital between January 2004 and December 2006 with at least one blood culture positive for candidiasis were considered for inclusion in our study. We compared those with C. albicans infection to those with C. glabrata infection to determine if there is any difference in mortality and other secondary outcomes between the two groups. Data was collected from patient charts about baseline characteristics, fluconazole resistance, and percentage getting effective treatment and clinical outcomes for these two groups.

Results: During the study period, we identified 94 cases of C. albicans and 28 cases of C. glabrata. 78.72% of the cases with C. albicans were hospital acquired, whereas this was 60.71% for C. glabrata (p=0.09). Patients in both groups were severely ill, with high APACHE II had high apache scores (15.8 vs. 14.6, p=0.4). Those with C. albicans did not show any fluconazole resistance, whereas the resistance was 35.7% in the C. glabrata group. p<0.001. C. glabrata had higher rate of effective treatment, although this was not statistically significant (25% vs. 15.96%, p=0.28). The inpatient mortality between the two groups was similar (40.4% vs. 39.3%, p=0.91). Even after adjusting for effective treatment and APACHE score, no difference in mortality was found between the two groups (OR=1.03, p=0.94). The time to first dose of antifungal was high for both groups (58.6 vs. 57.8, p=NS).

Conclusion: Although C. glabrata is associated with considerably higher rate of fluconazole resistance, it shows similar mortality rates when compared to C. albicans.

Phenotypic and Genotypic Characterization of Antimicrobial Resistance in Uropathogenic Escherichia coli in Lebanon

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Improper monitoring of antibiotic usage has hastened the development of antimicrobial resistance among uropathogenic Escherichia coli (UPEC). The rapid dissemination of antibiotic-resistance is mediated by gene transfer mechanisms involving mobile genetic elements including the recently characterized gene cassettes in integrons. Integrons are conserved DNA sequences associated with multi-drug resistance. The aim of this study was to generate data on the prevalence and molecular basis of antimicrobial resistance in UPEC. PCR assay was used to screen a total of 100 UPEC isolates for the presence of the class 1 integron variable region (VR) containing the gene cassettes. Positive isolates were further tested for antimicrobial susceptibility using the agar diffusion test. The VR amplions were then characterized by direct partial sequencing and restriction digestion with AluI. 30% of the isolates were positive for the Class 1 integron VR, with a size ranging from 0.7 to 2 Kbp. They carried the genes dfrA7, dfrA17-aadA5, dfrA1-aadA1, dfrA12-aadA2 and biaOXA-30-aadA1. Resistance to trimethoprim-sulfamethoxazole (SXT) was detected in all class I integron positive isolates, to streptomycin (S) in 73% of the isolates, and to oxacillin (Ox) in 1% of the isolates. The predominant resistance genes were dfrA17 and aadA5 (47% of the isolates) for SXT and S, respectively. Five different restriction patterns were detected; all isolates with the same class 1 integron VR ampiclon size had the same restriction pattern. Characterization of class I integrons from UPEC isolates by direct sequencing revealed that those isolates exhibit a wide repertoire of genetic elements to sustain antimicrobial pressure. Additionally, the study provided basal information for future pursuit and comparison especially with respect to epidemiologic distribution, antimicrobial resistance and evolution of these important pathogens.
Utility of Real Time-PCR in the Diagnosis of Drug-Resistant Tuberculosis in Veracruz, Mexico

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Globaly 3.2% of the tuberculosis (TB) infected people have a drug-resistance-TB (DR-TB), Mexico is one of the most important generators of DR-TB in Latin America and Veracruz contribute annually with the 35%. The identification of single nucleotide polymorphism (SNP) in genes associated to DR by the real time polymerase chain reaction (PCR-RT), is considered a new and attractive tool for the diagnostic of DR-TB. So the goal of this work is to development by PCR-RT a diagnostic procedure for the resistance to Rifampin (rpoB) and Isoniazid (katG) in patients with TB from Veracruz, Mexico.

15 DR-TB isolates were Rifr, 17 Isor and 15 were MDR-TB. Real time analysis show that 5 Rifr isolates had the SNP531 and 8 Isor isolates the SNP315. Concordance with the ADN sequences was 80% for rpoB and 100% for katG. In the field test only 5 sputum samples show SNP531, 4 SNP315 and 3 both.

In conclusion the specificity, sensibility, positive and negative predictive value show that the participation of this technique in a diagnostic algorithm will be in the order of the confirmation more than screening. Finally, the molecular characteristics of rpoB and katG show several new mutations and a high heterogeneity of DR-TB isolates circulating in the south of Mexico, a region highly producer of immigrant workers to the United States.

New Antimycobacterial Substances for MDR and XDR Mycobacterium Species

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Tuberculosis (TB), one of the oldest diseases, is still a global threat. It is estimated that one third of the world population is infected with TB caused by Mycobacterium tuberculosis and other non-tubercle species. Increase of multidrug-resistant TB (MDR) and emergence of extremely drug-resistant ant-TB (XDR) provide the rationale to search for new antmycobacterial drugs. Plants are considered as rich source of new therapeutic agents. The aim of this study was to evaluate antmycobacterial activity of plants commonly used and easily available in Pakistan. Aqueous and methanolic crude extracts of three different indigenous plants including Camellia sinensis (Green Tea—commonly used beverage), Juglans regia (dried bark of walnut tree, very famous teeth brightening agent in Pakistan) and Hippophae rhamnoides (Sea buckthorn berries—famous shrub, widely consumed as food product in Northern areas of Pakistan) were screened for activity at concentrations ranged from 0.75 to 5 mg/mL. Reference strain of Mycobacterium tuberculosis H37Rv and clinical strains of mono-resistant Mycobacterium tuberculosis, Mycobacterium avium and Mycobacterium bovis were used. All extracts were found to be effective against Mycobacterium tuberculosis H37Rv with MIC <0.75mg/mL. Methanolic extract of Camellia sinensis was found to be active against all species of Mycobacterium whereas the activity of its aqueous extract was restricted to Mycobacterium tuberculosis. It is interesting to note that aqueous crude extracts of all plants exhibited inhibitory activity against reference and clinical strains of M. tuberculosis but no activity against other species. In contrast, methanolic extracts of Juglans regia and Hippophae rhamnoides inhibited all species of Mycobacterium tested. Our initial observations indicate the potential of these indigenous plants to serve as source of affordable antimycobacterial drugs. Further investigations towards the purification of active components are in progress.

10.177 Antibiotic Susceptibility Testing and PCR-RFLP Analysis of Protein A Gene (spa) of Methicillin Resistant Staphylococcus aureus Isolates

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Background: Methillin resistant Staphylococcus aureus infections are a particular problem in hospitals. Recently increasing numbers of cases of MRSA infection in the community have been seen in many countries around the world. Thus, genotypic surveillance of these strains in patients with staphylococcal infections is important to control the community and hospital acquired infections caused by this bacterium. The aim of this study was antibiotic susceptibility pattern and spa typing of MRSA isolates.

Methods: One hundred clinical isolates of MRSA were obtained from Tehran university hospitals. The source of samples was: wound (27%), blood (19%), respiratory tract (28%), urine (14%), and other sites (12%). Antibiotic susceptibility pattern of isolates were determined using a panel of 14 antibiotics (clindamycin, rifampin, ciprofloxacin, ceftriaxone, oxacillin, vancomycin, telithromycin, gentamicin, tetracycline, erythromycin, chloramphenicol, cephalotin, tobramycin and cotrimoxazole). After DNA extraction, spa gene was amplified using specific primers. Then, spa PCR products were digested by Haell enzyme.

Results: Antibiotic susceptibility testing showed 26 antibiotics (Ab1-Ab26). Ab8 (22%) and Ab4 (20%) were the most common antibiotics. Six patterns (S1-S6) of spa gene were identified according to PCR results. Seventy (70%), 4 (4%), 13 (13%), 11 (11%), 1 (1%) and 1 (1%) of isolates showed patterns S1-S6, respectively. PCR-RFLP of spa gene by Haell

10.176 Bacterial Resistance to Ciprofloxacin in Tripoli Medical Center: An Overview of Four Months Surveillance Study

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Objective: This is study was aimed to assess the antibiotic resistant of 544 isolates to ciprofloxacin in hospital microbiology laboratory during the period of four months at Tripoli Medical Center.

Methods and Materials: From February 2006 to April 2006 a total of 544 isolates were enrolled in this study. The identification of microorganisms were carried out using conventional methods and the routine procedures such as colony appearance, gram stain reaction, morphology, and biochemical reactions as well as API 20 E and API 20 NE (bioMerieux, France). All Bacterial isolates were tested for susceptibility to Ciprofloxacin on Mueller Hinton agar using Kirby-Bauer disk diffusion methods in accordance with the National Committee of Clinical Laboratory Standard (NCCLS). The isolates were categorized as sensitive or resistant using the criteria described by NCCLS in the guide to sensitivity testing.

Results: During the period of this study, the most common bacteria isolated was Klebsiella pneumoniae (123 isolates), Staphylococcus epidermidis (89), Staphylococcus aureus (84 isolates) E. coli (81 isolates), Non hemolytic Streptococcus (48 isolates), Acinetobacter species (47 isolates), Pseudomonas aeruginosa (35 isolates) and other bacteria (57 isolates). Among the tested isolates, the highest percent of resistant rate to ciprofloxacin was observed with Non hemolytic Streptococcus (45.8%), Acinetobacter species (44.7%), Staphylococcus aureus (29.7%), Klebsiella pneumoniae (20.3), Staphylococcus epidermidis (14.6) and Pseudomonas aeruginosa (14.3). The resistance rate to ciprofloxacin by type of ward, clinical specimen and bacterial species were also investigated.

Conclusion: This study indicates emerging of ciprofloxacin resistance among most bacterial isolated pathogens. Increasing resistance against ciprofloxacin demands coordinated monitoring of its activity and rational use of the antibiotics.
An outbreak of Panton-Valentine leukocidin-Methicillin-Resistant Staphylococcus aureus (MRSA) infections among health-care workers (HCWs) in a long-term care facility (LTCF)

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Background: To describe the epidemiologic and microbiologic investigations during an outbreak of community-acquired MRSA infections that occurred in a LTCF among HCWs only. There were 228 residents and 209 HCWs at the time of notification of the outbreak.

Methods: An unmatched case-control study was conducted among HCWs to identify risk factors for the development of infection. Nasal swabs were collected from all cases and controls and from 30 randomly selected HCWs to identify risk factors for the development of infection. Nasal swabs were collected from all cases and controls and from 30 randomly selected HCWs to identify risk factors for the development of infection. Nasal swabs were collected from all cases and controls and from 30 randomly selected HCWs to identify risk factors for the development of infection.

Results: From November 2006 through December 2007, 8 cases were identified among practice nurses, for an attack rate of 10% for this profession category. All developed furuncles and small abscesses. All isolates were identified as MRSA Panton-Valentine leukocidin (PVL) producing Staphylococcus aureus (S. aureus). The cases were more likely to be colonised with PVL-positive S. aureus than controls (37.5% versus 3%, P-value=0.018). Nasal S. aureus carriage was also detected in 16.6% of screened residents; none of them carried a PVL-positive strain. Multivariate analysis revealed that working in a specific department and being a practice nurse were statistically significant risk factors for infection. Intranasal mupirocin was administered concomitantly to all HCWs and residents. Nasal swab cultures were repeated one month later and showed a 95% eradication rate. Nine months after the intervention no new case was reported.

Discussion: The current outbreak indicates that HCWs may serve as vehicle for the entry of PVL-positive MRSA strains from the community in LTCFs, whereas deficient hygiene practices and unrecognized carriage may facilitate their spread. Given the increasing prevalence of PVL-positive MRSA infections worldwide, guidelines for the eradication of PVL-positive MRSA carriage within closed communities should be established and efforts to obtain cultures from compatible infections should be made.

The effect of Galbanum (from Ferula Gummosa) against mycobacterium bovis

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Objective: Over the last decades, great advances in treatment and prevention of infectious disease have fostered complacency about infection in a society which has access to antibiotics. However infections and also adverse reaction of such drug remain. Also due to increasing demand for less toxic and more potent antibiotics, it is necessary to find new sources.

Method: In this research, anti-mycobacterial activity of the extract of Ferula gummosa was investigated effects of the extract of the root and fruit was tested against Mycobacterium bovis. Extract of fruit was achieved using maceration method the effect of different concentrations of extract on the growth of mycobacterium compare to the 1 MC Farland standard and quantity of colonies, was investigated by microscopic method. Strep-tomyein used for the control of anti-mycobacterial test systems.

Result: Showed the Galbanum of 0.03 and 0.04 g/ml have protection effect and the other concentrations of extract have decreasing effect on the growth of weak mycobacterium bovis. (BCG).

Conclusion: We can use the Galbanum against mycobacterium. Some studies are needed about other microorganisms.

Effect of Hyperproduction of SHV Extended-Spectrum Beta-Lactamases on the Duration of Postantibiotic and Post-Beta-Lactamase Inhibitor Effect of Ceftazidime Combined with Clavulanic Acid

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Background and Aim: Postantibiotic effect (PAE) is a delay of bacterial growth after short exposure to antibiotics. The phenomenon of continuing suppression of bacterial growth after removal of β-lactamase inhibitors is termed post-β-lactamase inhibitor effect (PLIE). It is a well known fact that β-lactam/ inhibitor combinations are not recommended for the therapy of infections caused by extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae due to incoluim effect and the development of mutants hyperproducing ESBLs during therapy even if in vitro testing shows susceptibility. The aim of this study was to determine postexposure effects (PAE and PLIE) of ceftazidime combined with clavulenate against isogenic Escherichia coli strains producing low level and high level of SHV-2 and SHV-5 β-lactamases.

Methods and Materials: The experiments were performed on a set of isogenic laboratory E. coli strains producing low level and high level of SHV-2 and SHV-5 β-lactamase. Minimum inhibitory concentrations (MIC), time-kill curves, PAE and PLIE were determined as described previously. PAE and PLIE were induced with ceftazidime (32 mg/L) combined with clavulenate acid (4 mg/L). This ceftazidime concentrations correspond to the serum concentrations during therapy. Experiments were performed in triplicate and mean value and standard deviation were calculated.

Results: The MICs of ceftazidime/clavulenic acid of SHV-2 producing E. coli were 0.12 (low level) and 1 mg/L (high level) whereas SHV-5 producer showed higher MICs of 0.25 and 2 mg/L respectively. SHV-2 producer with high level enzyme production displayed shorter PAE (0.5±0.2 h) and PLIE (1.5±0.1 h) compared to low level enzyme producer (1.4±0.2 h and 2.2±0.3 h respectively). The duration of PAE and PLIE in SHV-5 producing E. coli was also significantly shorter for high level enzyme producing organism (1.6±0.1 and 1.9±0.1 h respectively) in comparison with low level enzyme producer (2.1±0.2 and 2.19±0.2 h respectively).

Conclusions: This study proved that hyperproduction of SHV-ESBLs due to the mutations in the promoter region of the blaSHV gene significantly shortened the duration of both PAE and PLIE. The fact that the amount of β-lactamase determined the length of PLIE points out that PLIE corresponds to the lag time before production of sufficient amount of β-lactamase following inhibition by clavulenate. This finding could be important for the dosing schedule of β-lactam/ inhibitor combinations.

Vaccines against emerging diseases

10.1081 – 10.192 Room: Bruckner/Mahler/Brahms – First Level

Economic analysis of pre-pandemic influenza vaccination strategies in Singapore

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Background: Pre-pandemic vaccines are currently being considered worldwide to mitigate the impact of a future pandemic. This study analyzes...
the economic outcomes of pre-pandemic vaccination compared with treatment with anti-viral agents only in the tropical globally-connected city of Singapore.

**Methods:** We used a decision-based model to perform cost-benefit and cost-effectiveness analyses for vaccination in additional to treatment-only. The Singapore population was stratified by age and risk groups and input variables were obtained from local and international sources. Sensitivity and Monte Carlo simulation analyses were performed to model uncertainty. The break-even cost was calculated which includes all vaccination-associated costs.

**Results:** The treatment-only strategy resulted in 350 to 1,122 deaths at an economic cost of US$285 to 1,310 million. The overall costs were most sensitive to the case-fatality rate, attack rate, vaccination cost, and efficacy of vaccination. At a vaccine cost of US$47, vaccination increased cost-benefit by US$19 to 53 million and reduced deaths by 22 to 69 for every 10% increase in vaccine efficacy. Treatment-only was dominated by vaccination for cost-per-life-saved when the vaccine efficacy exceeded 85%. Break-even costs for vaccination reached almost US$133 at vaccine efficacy of >90%. In addition, higher attack rates and higher case-fatality rates resulted in higher break-even vaccination costs. Under most conditions, vaccination is cost-beneficial for all high-risk groups with break-even costs of between US$67 to 467. At case-fatality rates of >1.2%, vaccination is always cost-beneficial in all Monte Carlo simulation iterations.

**Conclusion:** As the current vaccine efficacy level and time to a pandemic is unknown, the decision to stockpile is based on perception of disease severity. Policy makers considering stockpiling as insurance against worst-case scenarios will find it cost-effective. In addition, high-risk subpopulations will benefit most from vaccination, and the benefit will increase with higher proportion of the population receiving early vaccination.

### 10.183 CD40-Ligand-Dependent-CD4+ T Cell Mediated Control of the Acute Venezuelan Equine Encephalitis Virus Infection in Murine Brain

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Previous studies have demonstrated the safety and efficacy of chimeric alphaviruses in protecting against lethal encephalitis caused by Venezuelan equine encephalitis virus (VEEV). Here we demonstrate in several independent studies that CD4+ T cells are required for protection from lethal encephalitis using a gain of function approach via adoptive transfer of memory and effector T cells into cTCR-deficient recipient mice. In contrast, adoptive transfer of memory and effector CD8+ T cells as well as CD4+ T cells from donors with CD40L-deficiency does not confer protection to cTCR-deficient mice. The effector activities of CD4+ T cells in cTCR-deficient recipients likely extend beyond the capacity to provide T-dependent help to B cells, as high-dose passive (neutralizing) antibody transfer prior to infection is not protective in cTCR-deficient or wild-type mice. In this model the early infiltration of the brain by T cells correlates with protection and is detected in the presence of CD4+ T cells but not CD4+ T cells with CD40L-deficiency. CD4+ T cells also displayed a bias to Type 2 cytokines (IL-10, IL-5, IL-13) rather than Type 1 cytokines ex vivo, and had no significant impact on virus growth in the brain during the first five days of the infection. This finding suggests an important functional role for CD4+ T cells in orchestrating the inflammatory response to VEEV infection in the brain needed for survival.

### 10.184 Vaccine Development to Control the Emergence of Disease in Livestock and Humans in New Zealand Caused by Salmonella Brandenburg

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**Background:** Since 1996, an epidemic of salmonellosis caused by Salmonella Brandenburg has arisen in New Zealand. The disease has been manifest in livestock and humans as gastroenteritis (but also abortion with case fatality rates up to 50% in the former), leading to considerable economic and social cost. Hygiene and vaccination measures ‘on farm’ have proven only partially successful in preventing inter-animal and inter-farm transmission of the infective agent.

**Methods:** The establishment of infections with S Brandenburg in twin bearing, 2-year-old sheep by administration per os, following starvation, was developed in order to mimic natural infections. In the first trial, sheep were treated with either a cell wall fraction from S Brandenburg (subunit), or a commercial whole cell bacterin—both given sub-cutaneously—or an attenuated S Typhimurium preparation—by eye-drop. All were then experimentally infected (challenged) orally. In the second trial, sheep were treated with the attenuated vaccine followed by the subunit vaccine and then experimentally challenged. Vaccines were assessed for their ability to prevent infection and disease. Various immunological traits were measured including antibody titre (IgG, IgM and IgA) and gamma interferon release from whole blood cultures, in vitro.

**Results:** In both trials, there was no significant protection against mortality and abortion following vaccination. However, in trial 1, there was a significant but transient decrease in the number of ewes shedding S. Brandenburg (live attenuated, p<0.05; subunit, p<0.05; inactivated, p=0.01), and in the quantity of these bacteria in the sheep from the vaccinated groups (p<0.05) compared with controls, 6 weeks after challenge. In both trials, there was a marked serological response following both primary and secondary vaccination (IgG and IgM) and challenge (IgG, IgM and IgA) which was unrelated to protection. There was a significant negative association (p<0.05) between gamma interferon release from whole blood cultures and death and/or abortion of animals.

**Conclusion:** Two different vaccination regimes were ineffective in inducing protective immunity to disease resulting from experimental infection with S Brandenburg, even though faecal shedding of causal microorganisms was affected. Protection at the time of challenge was associated with cell mediated immune function.
Compliance for Three Doses of RotaTeq® is Similar to That for Three Doses of DTaP in US

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A pentavalent 3-dose rotavirus vaccine, RotaTeq, has been available in the US since February 2006. The recommended schedule is first dose given at age 6–12 weeks and doses 2 and 3 at 4- to 10-week intervals; dose 3 administered before 32 weeks. We assessed implementation for the first 18 months compared with that for DTaP vaccine using a US health insurance claims database. We identified infants enrolled in the health plan within one week of birth, with first RotaTeq or DTaP vaccination from January 2006 through June 30, 2007. We assessed adherence to the recommended schedules. They were followed until one year old, study end (March 2008) or disenrollment from the health plan. Median age at first dose for the 93,394 infants who had received DTaP and for the 48,006 infants who had received RotaTeq was 9 weeks; median age at doses 2 and 3 were 18 weeks and 26 weeks, respectively, for both. The relative compliance (i.e. proportion of infants who received RotaTeq on schedule divided by the proportion of infants who received DTaP on schedule) for dose 1 of RotaTeq compared with that of DTaP increased from 0.66 in Q1 2006 to 1.0 in Q2 2007. The changes for doses 2 and 3 were 1.3 to 1.0 and 1.23 to 1.08, respectively. Less than two years after launch RotaTeq uptake has increased from 0.3% in February 2006 to 48% in November 2007 and is approaching that of DTaP (62% in November 2007). The dosing patterns, including completion of schedules for RotaTeq and DTaP appear to be similar and are consistent with the recommended dosing schedules of both vaccines.

A New Vaccination Approach Against Helicobacter pylori Infection Through Application of Recombinant Bacterial Ghosts

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Background: Bacterial ghosts (BGs) are empty cell envelopes derived from gram negative bacteria by expression of cloned lysis gene E. The induced trans-membrane tunnels expel cytoplasmic contents while keeping intact the antigenic properties of the target living cell. This approach has offered new insights in developing non-living vaccines specially those targeted for oral administration.

The high prevalence of Helicobacter pylori (Hp) infection is a major health concern especially in developing countries, demanding the development of effective vaccines to prevent infection. Since BGs can mimic bacteria in structural properties, Hp ghosts expressing immunogenic proteins maybe a suitable approach.

Methods: Hp omp18 gene and an antibiotic section marker were cloned in a suitable shuttle vector. The expressions were induced in E. coli cells by IPTG and confirmed through immuno-blotting. E. coli cells were then co-transformed with two lysis and shuttle vectors. Development of BGs was performed by elevating the temperature to 42°C after primary IPTG induction.

Results: Kanar was cloned as the 2nd antibiotic marker. The specific 25kDa Omp18 protein was observed on SDS-PAGE and its immunogenicity was confirmed by immunoblotting. Co-expression of Omp18 and lysis protein was performed and generation of BGs expressing Hp Omp18 was confirmed by Western blotting.

Conclusion: The expression of r-Omp18 and BG production in E. coli has been confirmed. The expression of two cloned genes is being optimized and the constructed ghosts are going to be examined in a Helicobacter animal model.
ABSTRACTS

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10.189 Chemotherapy and Immunotherapy Along With Chemotherapy (Mycobacterium w) in Treatment of Tuberculosis Infected Mice

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Introduction: Among the TB vaccines, BCG shows various degrees of protection in different parts of the world. Hence novel vaccination strategies are needed to fight against tuberculosis. Mycobacterium w (Mw) is a saprophytic cultivable mycobacterium and shares several antigens with M.tuberculosis. It has shown good immunomodulation in leprosy patients. Hence in the present study, efficacy of Mw vaccine alone and in combination with chemotherapy was studied in mice infected with tuberculosis.

Methods: BALB/c mice were infected with M.tb H37Rv (susceptible to all first and second line drugs) and three clinical isolates taken from the repository of the Institute. The dose of 100 bacilli was used for infection via respiratory route in an aerosol chamber (inhalation exposure system, Glassco Inc.). Chemotherapy (5 days/week) was given one month after infection and the vaccinated group was given a dose of 1×107 bacilli by s/c route. Bacterial load was measured at 4 and 6 weeks after initiation of chemotherapy.

Results: Mw when given along with chemotherapy (4 weeks and 6 weeks) lead to greater reduction in the bacterial load in lungs and other organs of TB infected animals. However, the reduction was much more in terms of the number of CFU in both organs (lungs and spleen) and was statistically significant (P<0.05) in both of the organs.

Conclusion: Mw (as immuno-modulator) has the beneficial therapeutic effect as an adjunct to chemotherapy.

10.190 Detection and Sequencing of CPV-2 DNA in Fecal Samples of Puppies After Vaccination

V. Benetka, N. Affenzeller, M. Lebsch, K. Moestl. University of Veterinary Medicine, Vienna, Austria

In order to determine whether and for how long attenuated vaccine strains of CPV-2 are shed in the feces after vaccination, 6 healthy 8 weeks old dogs from two litters were separated from all other adult animals and vaccinated. Fecal samples were collected daily for a period of 2 weeks and tested by antigen ELISA, all samples tested negative.

First results of testing by real time PCR showed that viral DNA was shed from day 0 (before vaccination) until the last day of the period investigated. Samples positive in the real time PCR and the vaccine strain were then further submitted to sequence analysis and compared. From day 0 to at least day 10 two different sequences were detected in the feces of the dogs. All sequences detected on day 0 differed in three nucleotide positions from the vaccine strain and were therefore assigned to a field strain, sequences identical to the vaccine strain were detected from day 5 after vaccination on. In the following either the vaccine strain alone or both vaccine strain and field strain sequences were present in the feces.

During the investigation period all dogs remained clinically healthy and were at no point suspicious for CPV-2 infection. According to the results of the antigen ELISA no virus particles were shed. Nevertheless our results show that DNA of a field virus was detectable at the time of first vaccination showing that the puppies possibly had encountered infectious virus when still protected by maternal antibodies. Furthermore vaccine virus DNA is shed over extended periods of time. The detection of DNA had no diagnostic or prognostic value as all dogs remained healthy. These results show that positive PCR results have to be interpreted with care and only in the context of other anamnestic and clinical parameters.

10.191 Asia and Development of New-Generation Vaccines for Rabies

E. Moughdadai Khorasagani. Islamic Azad University of Shahrekord, Shahrekord, Iran (Islamic Republic of)

Rabies is an enzootic viral disease widespread throughout the world. Although it is a vaccine-preventable disease, the annual number of human deaths caused by rabies is estimated to be 32,000 in Asia. Phylogenetic analysis based on sequence data of the partial N gene of rabies viruses in Asia has shown that the viruses are divided into five genotypes, distributed in Middle East, South Asia, South East Asia, Malay, and Arctic regions. The genetic relationships among these rabies viruses agree basically with the results of previous studies. Meanwhile, new types of vaccines are being developed by applying gene manipulation techniques to rabies virus in order to overcome the disadvantages of current vaccines. This article reviews the molecular epidemiology of rabies in Asia and progress made in the development of new-generation rabies vaccines with the goal of elimination or control of rabies in Asia.

10.192 Immunogenicity and Protective Activity of Recombinant Omp28 from Brucella melitensis in Mice

P. Kaushik1, D.K. Singh1, P. Chaudhury2, G. Shukla2. 1BVC, Patna, India, 2IVRI, Bareilly, India

Recombinant Omp28 (r-Omp28) from Brucella melitensis produced in Escherichia coli was previously identified as group 3 proteins. In this study, we evaluated the immunogenicity and protective potential of r-Omp28 in mice. This r-Omp28 was injected intramuscularly in the mice with and without CpG oligodeoxynucleotides (ODN). The booster was given on 3rd week with the same antigenic preparation. Specific antibodies to purified r-Omp28 were detected in mice sera by western blotting and indirect ELISA (iELISA). In addition, isotype (IgG1 and IgG2a) specific antibodies were also measured by iELISA. Cellular immune response was measured by lymphocyte proliferation assay (MTT assay) after in vitro stimulation of spleenocytes by r-Omp28. Immunization with r-Omp28 induced a vigorous immunoglobulin G (IgG) response, with higher IgG1 than IgG2a. Whereas, immunization of mice with r-Omp28 + CpG led IgG2a dominated response, suggesting the induction of a Th helper 1 (Th1) response by CpG ODN. Spleenocyte from immunized mice showed significant proliferative response (3 week post booster), which was higher (P<0.05) in r-Omp28 + CpG than r-Omp28 immunized mice. The rOMP28 alone was found to suppress the IFN-γ production while incorporation of CpG induced IFN-γ. Finally the protection against B. abortus 544 challenge was observed highest in S19 vaccinated mice followed by rOMP28+CpG-vaccinated mice. Our results indicate that r-Omp28 is a good immunogen, capable of inducing both humoral and cellular immune response. The humoral response was biased towards Th1 type when it was co-administered with CpG ODN.

SESSION II (Parallel Session)
Roundtable: Surveillance Systems in Practice
Sunday, February 15, 2009
Room: Park Congress/Ground Level
08:30–10:30

11.001 MECIDS: Cross Border Surveillance and Response in the Middle East

A. Leventhal1, D. Cohen2. 1Ministry of Health, Jerusalem, Israel, 2Tel Aviv University, Tel Aviv, Israel
Background: The Middle East Consortium for Infectious Disease Surveillance (MECIDS) was formed in 2003 by public health leaders and professionals from both respective Ministries of Health and academia in Jordan, Palestinian Authority and Israel.

Methods: The first goal of this inter-governmental partnership was to improve detection, data sharing and control of food-borne diseases in the ME.

Results:

– During the coming years upgrading of surveillance systems has evolved due to need to bridge gaps between the existing capabilities by: harmonizing diagnostic and reporting methodologies, establishing workshops and training programs, and facilitating cross-border data sharing.

– MECIDS has proven as a platform to broaden surveillance and cross-border communication in case of a serious emerging threat like Avian Flu in late 2005. MECIDS activities included the participation of the in Ministries of Agriculture (veterinary services). MECIDS called a workshop to orchestrate future reaction in case of outbreak in term of preparedness, exchanging national plans, and exchanging information in real time.

– In March 2006, an Avian Flu epidemic actually broke out among poultry within the MECIDS participating nations. Immediate effective lines of communication were established, cross-border assistance carried out in laboratory diagnostic, supply of both Personal Protective Equipment and preventive antiviral drugs. This cooperative control measures between partners proved essential in the relatively quick mitigation of the human and economic impact of the outbreak.

– The scope of MECIDS activity has been expanded to include building of a memorandum of understanding in case of a Pandemic Flu, including series of table-top exercises focused on preparedness at the country and regional levels.

Conclusion: MECIDS has become a viable regional surveillance network that has far exceeded its set up goals and demonstrated great potential to expand its scope through inclusion of additional communicable diseases and other countries of the region as well.

11.002 CaribVET: Animal Disease Surveillance Network in the Caribbean

T. Lefrançois, P. Hendriks, N. Ehrhardt, S. Ahoussou, M. Kalloo, J. Shaw, D. Martinez, M. Trostan, CIRAD, Petit Bourg, Guadeloupe, AFSSA, Lyon, France, CARICOM Secretariat, Georgetown, Guyana, USDA-APHIS-IS, Santo Domingo, Dominican Republic, CIRAD, Montpellier, France, Veterinary services, St Michael, Barbados

The Caribbean animal health network (CaribVET) is a collaboration network among veterinary services, laboratories, research institutes, and regional/international organizations to improve animal and veterinary public health in the Caribbean. For more than 5 years, approximately 25 English, French, or Spanish speaking countries and/or territories in the Caribbean have been participating to the network. Its specific objectives are to foster exchange of information and collaboration, promote a regional approach for emergency preparedness and diseases control especially for emerging and zoonotic diseases, reinforce regional diagnostic capacities, and strengthen national epidemiological surveillance systems.

Meetings, trainings, skills building and development of regional tools for information and data exchange are the main strategies used to achieve these objectives. The steering committee of CaribVET is responsible for the regional strategy while seven working groups organize the collaboration on specific diseases (Tick and tick-borne diseases, Avian influenza, Classical swine fever, Salmonellosis, Rabies) or activities (epidemiology, quality assurance in the laboratories).

The epidemiology working group has developed criteria for the definition of priority diseases, core surveillance databases, and an evaluation questionnaire of national surveillance systems. It participates to the updating of a participatory website (www.caribvet.net), which encompasses information on surveillance systems, diagnostic laboratories, conferences, and major diseases of the region. This website also includes an online database for surveillance of specific diseases (ticks and tick-borne diseases) and a notification system for emerging events in Guadeloupe. The working group for avian influenza has developed a regionally harmonized surveillance protocol with performance indicators, a diagnostic network and a specific web page for avian influenza surveillance in the region.

CaribVET network is expected to host regional projects on animal health from different funding sources. The coordination unit of CaribVET provides cadre of scientific expertise in the area of epidemiology and vector-borne diseases and a biotechnological platform for research and diagnostic located in Guadeloupe. The interaction between surveillance and research within CaribVET allows the definition of research questions in conformity with regional strategies and facilitate the access to surveillance data and field samples for the development of research studies.

11.003 Healthmap/ProMED: Enhancing Disease Surveillance Through Collaboration

J. Brownstein, Children’s Hospital, Boston, MA, USA

One of the most important recent changes in global pandemic and emerging disease surveillance is the increasing availability and reliance on informal information sources, which may include press reports, reports from individual clinicians or field-based NGOs, web logs and other Internet outlets. Systems using informal information have been credited with reducing time to outbreak recognition, preventing governments from suppressing outbreak information, and facilitating the ability of WHO and others to respond to outbreaks and emerging diseases. Two keys systems, the Program for Monitoring Emerging Diseases (ProMED-mail) and Healthmap.org have formed a strong collaboration to enhance global public health by providing a common platform to enhance disease surveillance and to facilitate the sharing of expertise, resources and disease threat intelligence. The International Society for Infectious Diseases’ (ISID) Program for Monitoring Emerging Diseases, ProMED-mail, is one of the largest publicly available emerging disease outbreak and reporting systems in the world, with more than 45,000 subscribers in over 165 countries. HealthMap.org is an openly available public health intelligence system that brings together disparate data sources to produce a unified and comprehensive view of the current global state of infectious diseases and serves over 50,000 users a month, ranging public health officials to international travelers. During the past 2 years, ProMED and HealthMap have worked together to produce a system that automatically parses ProMED reports, recognizing disease names and geographic locations, and places them on an interactive world map. Current collaboration is focused on the potential synergy of combining ProMED’s international network of infectious disease experts with HealthMap’s automated searching and curation tools. Specific activities include building the capacity of regional networks to detect and report outbreaks, creating tools to more effectively screen electronic informal sources for possible outbreaks and quantitatively assessing the type and quality of information used to identify emerging disease outbreaks. Through this collaboration we plan to increase the timeliness access to and dissemination of reports of emerging and reemerging infectious diseases of interest to the plant, animal and human health sectors including the use of geographic localization tools.

11.004 EpiSouth: from a European Project to a Mediterranean Network for the Control of Communicable Diseases

M.G. Dentel, M. Fabiani, R. Gnesotto, G. Pultoto, F. Simon Soria, P. Barboza, M. Kojuhuvarova, R. Vorou, C. Montagna, C. Martin, Pando, F. Ait-Belghithi, N. Vladimirova, K. Mellour, S. Declich, on behalf of the Project’s Network. 1 Italian National Institute of Health (ISS), Rome, Italy, 2Padua Teaching Hospital-Azienda Ospedaliera (PTH), Padua, Italy, 3Carlos III Health Institute (ISCIII), Madrid, Spain, 4French Institute for Public Health Surveillance (InVS), Saint Maurice Centre, France, 5National Center of Infectious and Parasitic Diseases (NCIPD), Sofia, Bulgaria, 6Hellenic Center for Diseases Control and Prevention (HCDCP), Athens, Greece, 726 Countries from South Europe, North Africa, Balkans, Middle East, Italy

Background: The Mediterranean countries share common epidemiological characteristics and public health problems. In 2005, the “Year of the Mediterranean,” some Public Health Institutes (PHI) proposed a
framework of collaboration for communicable diseases surveillance and training in the Mediterranean Basin. This initiative led to EpiSouth Project, co-funded by EU Public Health Programme (DG SANCO) and by Italian MoH (EpiMed Project).

Methods: EpiSouth works through Work Packages (WP) lead by PHI. The project is coordinated by the main partner (ISS, Italy) while three WPs, “Cross-border epidemic intelligence” (InVS, France), “Vaccine Preventable Diseases and migrants” (NCIPD, Bulgaria) and “Cross-border emerging zoonoses” (HCDCP, Greece), constitute the technical pillars on which the project develops. “Networking” (PTH, Italy) and “Training” (ISCIII, Spain) are WPs dedicated to capacity building.

The Project Steering Committee guides the activities while all countries collaborate through WP Steering Teams and Focal Points.

Results: Since its starting in 2006, EpiSouth struggled to develop its Mediterranean vocation. From an initial involvement of 5 countries (Italy, Spain, France, Greece and Bulgaria) it includes now 26 countries of Southern Europe, Balkans, North Africa and Middle-East and international organizations (EU, ECDC and WHO). Several outcomes, including website with a restricted area, five electronic bulletins, two trainings for 70 epidemiologists, the assessment of Network’s building progress, the evaluation of national epidemic intelligence, epidemiological weekly bulletins, the preliminary survey on vaccine-preventable diseases and migrants and a list of priorities for emerging zoonoses in the Mediterranean, have been already accomplished and documents are available at EpiSouth website.

Lessons Learned: The project focuses on countries cross-border issues and succeeds in creating cohesion and concrete collaboration among 26 countries. It fills a geographical area with common public health problems (Balkans and Mediterranean Basin), that is not addressed as a whole neither by European Networks, as it includes also non-EU countries, nor by WHO, as it encompasses three different WHO regional offices. The methodology and approaches adopted (such as the creation of Steering Teams constituted for each Work Package), have enhanced co-ownership of participant countries, and the presence of international institutions (ECDC, EC, WHO-EURO, WHO-EMRO, WHO-HQ) has allowed sharing views and facilitated interaction which have amplified the international impact of the project.

Conclusions: EpiSouth is a unique project covering all sides of the Mediterranean. The collaboration among countries is giving a clearer picture of the context and will identify gaps in public health.

The project is in line with the EU’s external actions of Neighborhood Policy and the Euro-Mediterranean Partnership where networks are among the tools for cooperation and integration with neighboring countries.

11.005 The World Animal Health Information System: WAHIS
K. Ben Jebara, OIE, Paris, France

We assist to an unprecedented need for quality and timely animal disease information, including zoonosis, not only for those involved in the different animal sectors, but also for many other stakeholders, including the general public. Since its creation in 1924, the World Organisation for Animal Health (OIE) has played an active role in sharing disease information among countries and in the prevention and control of animal and zoonotic disease spread. Recent changes in requirements for epidemiological events and disease notification by OIE Members, as well as the recent use of information and communication technologies have strengthened the place of the OIE as the principal source of official quality animal diseases information for OIE Members and for the benefit of the international community as a whole.

The OIE World Animal Health Information System is composed of an early warning system and a monitoring system covering all aspects of animal health information. In early 2006, the OIE launched the World Animal Health Information System (WAHIS): an internet-based computer system that processes data on animal diseases. The access is dedicated only for authorised users, one official Delegate for each OIE Member and their authorised representatives, who use WAHIS to notify the OIE of relevant animal disease information.

The data and information provided by Members through WAHIS are publicly accessible on the OIE web site via the Web interface WAHID (World Animal Health Information Database). This interface offers all available data on animal diseases and zoonoses, per country, region, month and year. In addition, it compiles country animal population, exceptional epidemiological events maps, global animal diseases distribution maps or comparative disease status between two countries. The latter can help define potential health hazards linked to the trading of live animals and animal products between countries.

SESSION 12 (Parallel Session)
Oral Presentations:
Vector-Borne Diseases in Humans and Other Animals

Sunday, February 15, 2009
Room: Klimt Ballroom 2 & 3 / First Level
08:30–10:30

12.001 Mapping the Risk of Tick-Borne Encephalitis by Use of Low-Resolution Remote-Sensing
G.E. Olsson1, M. Hjertqvist1, M. Arneborn1, A. Lundkvist1, S.E. Randolph2, D.J. Rogers3, 1Swedish Institute for Infectious Disease Control, Solna, Sweden, 2University of Oxford, Oxford, United Kingdom

Tick-borne encephalitis (TBE) is caused by the TBE virus, a Flavivirus in the family Flaviviridae, and transmitted through bites of ixodes ricinus ticks. Across Europe TBE incidence has doubled since the 1980s, with the major increase occurring in the early 1990s. In Sweden, however, two step-increases in 1984 and 2000 caused an overall 4.5-fold rise, with foci appearing in new regions.

Figure 1. Predicted TBE risk areas in Sweden, based on 1,704 geo-referenced localities of human TBE (1986–2007).

Remote-sensed environmental conditions at 1,704 geo-referenced localities of human TBE (1986–2007) in Sweden, derived from the MODIS sensor on the NASA Terra satellite, were temporal Fourier processed to extract environmental signatures of seasonality, using middle infra-red, day- and night-time Land Surface Temperatures, and the Normalised Difference and Enhanced Vegetation Indices data. The TBE cases and satellite data were used within a bootstrap, non-linear discriminant analytical framework to produce risk maps of TBE in Sweden.

Overall, kappa values were highly significant, ranging between 0.990 (±0.0024) for the best ten models and 0.943 (±0.0185) for the worst ten models of the 100 bootstrap samples. The equivalent figures for Sensitivity were 99.6% (±0.52) and 98.8 (±0.85) and for Specificity were 98.5 (±0.53) and 97.7 (±0.82) respectively. Daytime Land Surface Temperature...
12.002 Threat of Malaria Outbreak Following Tsunami Disaster in Andaman and Nicobar Islands, India and Its Control
R. Kumari, S. Lal. National Institute of Communicable Diseases, Delhi, India

Background: In India, Andaman and Nicobar Islands has been highly endemic for malaria for nearly a century with perennial transmission. Anopheles sundaicus is known as a main vector for malaria, which prefers to breed in brackish water. Tsunami waves entered the inhabited coastal areas on 26th December 2004, caused heavy devastation and left large areas inundated causing exceptionally high breeding source for An. sundaicus besides making thousands of people homeless. They were living in temporary open shelters, getting more exposed to mosquito bites. The population had high parasite load. Hence, there was a threat for malaria outbreak.

Methods: An extensive survey was carried out in these areas to assess the potential breeding sites of malaria vector created by seawater flooding and for planning of intervention methods. Surveillance was strengthened and malaria situation was monitored closely and regularly. Detailed action plan for prevention of any potential epidemic outbreak and containment of malaria situation was prepared and implemented in the affected islands.

Results: There was a declining trend of malaria in the islands prior to tsunami. After tsunami, in Nicobar Islands, malaria incidence and proportion of Plasmodium falciparum cases significantly increased from January 2005 till April 2005 as compared to the corresponding period of the preceding 10 years, but it was under control during second half of the year in 2005, subsequently in 2006 and 2007.

Conclusion: In these Islands, the tsunami created favorable conditions for malaria transmission. There was a threat for malaria outbreak. However, the situation was effectively managed by strategic planning and timely preventive measures. As a result of this concerted effort, early warning systems were strengthened and enhanced in affected areas enabling early recognition and initiation of appropriate interventions. Currently, three years after the disaster, malaria situation is under control. There have been no major outbreaks of malaria.

12.003 Infection of Antigen Presenting Cells with Crimean-Congo Hemorrhagic Fever and Dugbe Nairoviruses: Effect on Key Immunomodulators
C. Peyrefitte1, M. Perret2, S. Garcia3, A. Bagnaud2, R. Rodrigues4, G. Vernet5, D. Garín6, M. Bouloy7, G. Paranhos-Baccalá4. 1CRSSA, Lyon, France, 2Emerging Pathogens Laboratory, Fondation Méièreux, Lyon, France, 3CRSSA, Grenoble, France, 4Institut Pasteur, Unité de Génétique Moléculaire des Bunyaviruses, Paris, France

Background: Crimean-Congo hemorrhagic fever virus (CCHFV) and Dugbe virus (DUGV), are two segmented single-stranded RNA of negative polarity arboviruses belonging to the genus Nairovirus of the Bunyaviridae family. All members of the Nairovirus are tick-borne viruses. Little is known about the human pathogenesis of CCHF except for the general feature of hemorrhagic fever viruses to interfere with host defence mechanisms. Some studies reported recently the interaction of CCHFV with the type I interferon and cytokine responses. To better understand the innate mechanisms in response to CCHFV infection, we compared the ability of CCHFV a highly pathogenic virus and DUGV a occasional mild human pathogenic virus to infect antigen presenting cells (APC): human macrophages (MDMs) and monocyte-derived dendritic cells (DC).

Methods: All viral infections was carried out in a BSL-4 or BSL-2 respectively for CCHFV or DUGV respectively. The susceptibility of APcs to be infected with CCHFV or DUGV was tested at three MOI 0.01, 0.1, and 1. Immunomodulators were quantified by ELISA.

Results: Both APCs were able to replicate and produce CCHFV and DUGV. The percentage of infected MDMs was higher than the one of DCs. The viruses were detected as soon as day one (D1) post-infection in both cell types. The phenotypic alterations and the cytokine secretion profile of these cells types revealed differences that could participate in the different clinical outcomes observed with this two related viruses.

Conclusion: The differences observed in CCHFV infected APCs compared to DUGV infected APCs, and in the soluble mediators pattern strongly suggest that they are key elements of the viral pathogenicity with responses observed in fatal CCHFV infections. These differences may also suggest a specific host signature in response to different viruses such as DUGV and CCHF.
Background: Dengue haemorrhagic fever (DHF) is an acute viral disease characterized by flu like symptoms, high fever, haemorrhages over the body. It is a global health problem and is caused by four serotypes of Dengue virus which are immunologically distinct and does not provide mutual cross protection. In Pakistan first confirmed epidemic appeared in Karachi during 1994 and disease remerged in 2006 again with few hundred cases in the city. Hence Lab based surveillance was started in a public tertiary care hospital having an announced isolation ward for DHF during 2004, at Lahore the capital of densely populated province Punjab, Pakistan, which is also directly connected to the epidemic area through daily based mass movement.

Methods: The blood samples of the suspected patients of dengue haemorrhagic fever were tested for the detection of IgG and IgM against the DENV 1 and DENV 2 serotypes.

Results: In the year 2006 there were found a total number of 186 cases of DHF followed by 57 patients in 2007. While in year 2008 a fulminating and massive epidemic occurred in which 903 were confirmed with IgG and IgM captured ELISA against Dengue virus 1 and Dengue virus 2 serotypes, Lab based data derived from the same aforementioned public hospital showed that among the confirmed patients 60% were male and 40% were female.

Conclusion: In the years 2006, 2007 and 2008 the secular trend revealed that in each year more than 70% dengue fever patients were reported during month of November. The occasional cases start coming from mid August and epidemic terminates in mid of January as per records of 2006-07. Hence the disease was not endemic in this area but disease trend shows that it is moving toward endemic status.

12.006 Dengue Fever Imported from West Africa to France between 2006 and 2008

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Background: Dengue is usually not considered a significant health problem in Africa because severe forms of dengue illness are rarely reported. In absence of specific dengue surveillance systems the circulation of dengue virus in this area is likely to be underestimated. We report imported dengue cases to France to contribute to document dengue virus circulation in West African countries.

Methods: In July 2006, notification of confirmed viraemic, symptomatic dengue infections imported to France became mandatory. Cases are confirmed either by polymerase chain reaction or by serology (detection of specific IgM).

Results: From July 1, 2006, to August 15, 2008, 117 imported dengue cases to metropolitan France have been reported through the mandatory notification system. Of the 117 patients, 12 came from West Africa, of whom 7 came from Cote d’Ivoire. The number and the proportion of patients returning from West African countries increased significantly in 2008 up to August 15 (7.5 months) when compared to the 18 month period from July 2006 to December 2007 (8/30 = 26.7% versus 4/87 = 4.6%; p < 0.002). Of the 12 patients from West African countries only one (8%) had minor hemorrhagic symptoms whereas 28/105 (27%) patients returning from other countries had minor (25 cases) and major (3 cases) hemorrhagic symptoms (p = 0.15). Thrombocytopenia (platelets ≤ 100,000/mll) was documented for 27% of patients returning from West Africa and for 49% of the remaining patients (p = 0.18). The only serotype identified in 2008, DENV-3, has never been detected in West Africa.

Conclusion: Despite a probably low level of completeness, the mandatory notification surveillance system in France allows monitoring the trends of imported cases. Since the beginning of 2008, dengue cases imported from West Africa, particularly from Cote d’Ivoire, are increasing. This could be due to increased dengue circulation in this area. The introduction of a new serotype could have an important epidemic potential and be associated with a higher proportion of severe forms. This could have public health implications for epidemiological surveillance and management of patients locally.

12.007 Description of Mortality Fluctuations in the Reunion Island Population During the Chikungunya Outbreak in 2005–2006

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Background: Reunion Island has been affected by an important outbreak of chikungunya disease between March 2005 and June 2006, an estimated 270,000 cases have been reported. This abstract describes the surveillance of mortality during this outbreak of chikungunya using a syndromic surveillance system for mortality monitoring.

Methods: We compared the number of death observed in 2005 and 2006 with an expected number of death computed from the 2002–2004 historical data, modified by an estimation of the evolution of population size for the period 2005–06. The number of deaths in Reunion was obtained daily from. Data were transmitted automatically and daily to the National Institute for Public Health (InVS) from a network of 13 computerized registry offices. Data were encrypted and transmitted through direct FTP in a predefined format. For each death certificate, the following information was recorded: zip code, age, sex, date of death.

Surveillance outbreak was based on active case finding during the first months (April to December 05) and on estimates of suspected cases based on a sentinel network (January to June 06).

Results: Over the year 2005, observed deaths remained within expected range of statistical variation. From January to April 2006, numbers of observed death were higher than expected (respectively +7.1 %, +34.4 % (p < 0.01), +25, 2% (p < 0.01) et +10,1 % (p < 0.01). This corresponded to a 230 excess deaths in the 13 communes participating in the study and to a 267 excess deaths when extrapolated to the entire island’s population. Excess mortality was mainly observed in the age group 75 and above. The case-fatality rate (CFR) on Reunion Island was estimated to be 1/1,000 population. From May 2006, numbers of observed death remained within expected range of statistical variation.

The outbreak reached a peak in February 06 (week 05) with 47,000 new cases. A total of 266,000 cases have been recorded during the outbreak (attack rate of 34%).

Conclusion: Our results suggest that chikungunya outbreak was possibly responsible for a large part of the excess mortality observed in Reunion during the first 4 months of 2006 and were the first description of mortality increase during such outbreak. In an other hand, no other abnormal health event affected the island at this time.

Syndromic surveillance allowed an on-going surveillance of mortality during the outbreak near real time and was useful for public health authorities.

12.008 Re-emergence of Chikungunya Fever outbreak in Southern Provinces of Thailand, 2008

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Background: Chikungunya fever is of public health concern in South and South-East Asia countries. In Thailand, Chikungunya fever outbreaks were recorded between 1976 and 1995, since its first report in 1958. The previous six outbreaks occurred in all parts of Thailand with no extended spreading. In October 2008, the Bureau of Epidemiology was notified by a local health authority for suspected Chikungunya infections which were
subsequently laboratory confirmed. The surveillance of Chikungunya fever was introduced nationwide in November 2008 with aims for early detection and prompt intervention to control the disease.

**Methods:** The information of Chikungunya fever cases was reported to the National Notifiable Disease Surveillance and analyzed by Epi Info (US CDC). The human laboratory tests for Chikungunya infections were done at the National Institute of Health, Thailand. The mosquito vector study was conducted by the Armed Forces Research Institute of Medical Sciences in Bangkok, Thailand.

**Results:** By December 12th, 2008, a total of 528 Chikungunya cases were reported to the surveillance system. All cases arose from the southern part of Thailand including Narathiwats (481 cases), Pattani (44 cases) and Yala (3 cases). The first case’s onset was in early August 2008 and most cases lived in a Thai-Malaysian border district. The monthly number of cases has dramatically increased from August to November. (2, 60, 228 and 234, respectively). The male to female ratio was 1:1.5. Majority of the cases were adults (86.7%) and the median age was 38 years (IQR=24, 50). The main occupation was agriculture (42.0%). The OPD cases accounted for 80.0%. Totally, 628 cases were reported by the lab center and 283 cases (45.1%) were laboratory confirmed by RT-PCR and/or four-fold rising of Hemagglutination Inhibition Test (HI). The highest proportion of confirmed case was from Narathiwat (90.8%). The yield of PCR and HI were 55.9% (262/469) and 36.2% (47/130), respectively. *Aedes aegypti* and *Aedes albopictus* were identified as main vectors. Chikungunya viruses were isolated from both *Aedes* species.

**Conclusion:** The re-emerging Chikungunya fever is confirmed after the 13-year absence. The outbreak tends to spread out in the adjacent provinces. No confirmed case was reported from other parts of the country. The vector control measures were limited in this violent area with high density of both species of *Aedes* mosquito circulation. The major interventions include early case detection by PCR, rapid investigation and implementation of control measures.

**12.009 Surveillance and Investigation of Chikungunya in Singapore**

L.C. Ng¹, L.K. Tan¹, C.H. Tan¹, S.S.Y. Tan¹, H.C. Hapuarachchi¹, K.Y. Poh¹, Y.L. Lai¹, S.G. Lam-Phua¹, G. Bucht¹, R.T.P. Lin¹, Y.S. Leo², B.H. Tan¹, P.L. Ooi¹, L. James³, S.P. Khoo¹. "Environmental Health Institute, Singapore, Singapore, ¹Ministry of Health, Singapore, Singapore, ²Communicable Disease Centre, Department of Infectious Diseases, Tan Tock Seng, Singapore, Singapore, Singapore, National Environment Agency, Singapore, Singapore

**Background:** After its emergence among the Indian Ocean Islands in 2005, outbreaks of Chikungunya in Asia affected India, Sri Lanka and Maldives in 2005-06. Chikungunya virus is classically transmitted by *Aedes aegypti*. An A226V mutation in the virus had enabled efficient transmission of the virus by *Aedes albopictus*. Local transmissions in Singapore were first detected in Jan 2008. Virological and entomological investigations were conducted to assist in epidemiological understanding and development of vector control strategies.

**Methods:** The surveillance was enabled through a network of general practitioners (GP) and hospitals. Diagnosis was confirmed by PCR and serology. Virological and entomological investigations were conducted through phylogenetic analysis of E1 gene of the viruses from patients and mosquitoes; and adult and larval *Aedes* mosquito surveillance in cluster areas, respectively.

**Results:** A small outbreak comprising 13 cases was detected in Jan 2008 within Little India, an urban part of Singapore. Five months after the first episode was contained, local transmissions were again detected in June 2008, in various parts of Singapore. By September 2008, a total of 231 cases were laboratory confirmed for 2008. This comprised of 123 local cases and 108 imported cases from Malaysia, India, Sri Lanka, Maldives and Indonesia. Genetic characterization of viruses revealed that the circulating virus was related to East, Central and South African genotype and that the latter local outbreak was interconnected with an outbreak in Malaysia. Results also showed that local transmissions were due to at least 3 clades of viruses with genetic signatures of viruses from India, Malaysia and Sri Lanka. *Aedes albopictus* was the predominant mosquito species found in all cluster areas investigated, except for Little India, the first outbreak area, where *Ae. aegypti* was predominant. The changing pattern of vector predominance from *Ae. aegypti* to *Ae. albopictus* coincided with a switch in circulating virus, to one with an A226V substitution in E1 gene of the virus. The entomological findings led to the revision of the vector control strategy in Singapore to include outdoor fogging and external residual spraying in Chikungunya outbreak areas to target *Ae. albopictus*.

**Conclusion:** Our results showed that Singapore, being a travel hub and a cosmopolitan city, is vulnerable to multiple importations of CHIKV virus. The aggressive A226V variant of the ECSA genotype, that has established itself in the region, is posing a challenge to Singapore.

**12.010 West Nile in Europe: A Review of the Current Situation and its Implications on Blood Donation**

H. Zeller, K. Leitmeyer, D. Coulombier. European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

West Nile virus (WNV) is a mosquito-borne flavivirus transmitted in natural cycles between wild birds and mosquitoes. Historically, WNV has been associated with asymptomatic infections and sporadic disease outbreaks in humans and horses in Africa, Europe, Asia and Australia. However, since 1996 when the first large outbreak occurred in Romania, outbreaks of severe neuroinvasive disease in humans and horses have been sporadically detected in Europe and the Mediterranean Basin. In 1999, WNV underwent a dramatic expansion of its geographic range, and was reported for the first time in the Western Hemisphere during an outbreak of human and equine encephalitis in New York City and transmission of WNV was first documented through blood transfusion in North America in 2002. Since 1999 more than 10,000 neuroinvasive cases have been reported from the USA and donated blood and organ transplants are systematically screened using a nucleic acid test (NAT).

As a consequence, different surveillance systems (e.g. serosurveillance of vertebrate species, investigation on clinical cases in humans, horses) were established in some European countries. In 2008, Italy, Romania and Hungary reported human cases of WN disease from July to the end of September, revealing a critical period for WN surveillance of 3-4 months. In Central Europe, WN viruses from different lineages had been identified in birds and mosquitoes.

West Nile NAT screening is an expensive assay. It has been evaluated in several European countries but does not seem relevant in low endemic areas or when very few cases are notified. In 2004, a directive on the quality and safety of human blood was issued deferring blood donation from individuals living in or with history of travel to an area where human cases have been reported within 28 days. The fact that the 14 encephalitis cases notified in Hungary were from 9 districts might indicate a new epidemiological pattern and/or a result of improved surveillance. Answering this question is crucial as the current directive on blood donation in the EU would prevent in a country like Hungary donation from 9 districts, which would threaten the blood supply within the country.

Therefore, it is urgent to conduct a thorough threat assessment of the epidemiological situation and revisit strategies for blood donation (e.g. definition of an infected area, time period for transmission) and to revise options for blood screening.
Background: During 2007–2010, the Global Alliance for Vaccines and Immunization (GAVI) will provide funding to deliver the live-attenuated 17D yellow fever (YF) vaccine to 48 million people in 12 African countries. Some rare but serious adverse events following immunization (AEFI) have been reported with this vaccine. The importance of YF vaccine associated AEFI is poorly understood, in part because of difficulty in conducting systematic field surveillance and collecting biological specimens in developing countries. In 2007–2008, Togo, Senegal, Mali, and Burkina Faso became the first countries to implement mass YF preventative vaccination campaigns and to establish enhanced AEFI surveillance systems as part of the YF Initiative supported by GAVI.

Methods: Active and passive surveillance for YF AEFIs was established in all 4 countries based on written protocols, case definitions to screen for suspected cases, standardized notification forms and procedures to collect and transport biological specimens. Expert Committees were established to review data and assign case status. Training on AEFI surveillance was provided and laboratory capacity expanded.

Results: Togo, Senegal, and Mali distributed 2.4, 3.1, and 5.9 million vaccine doses, respectively. Vaccination coverage in all countries was >95%. Togo reported 8 suspected cases of YF-vaccine related serious AEFI (incidence: 0.3/100,000 doses), Senegal 2 (incidence: 0.1/100,000 doses), and Mali 1 (incidence: 0.02/100,000 doses). The median delay between vaccination and symptom onset was between 1.4 (Togo) and 4.3 days (Senegal) (range 0-13 days). Unlike Mali and Senegal, 3 deaths were reported from Togo. Data for Burkina Faso are pending as the country finished its campaign December 10, 2008.

Conclusions: The rate of reported suspected YF-vaccine related serious AEFIs in the 3 African countries was not greater than those reported in US and European travellers. However, interpretation of these data is limited by lack of adequate laboratory diagnosis of suspected cases, incomplete case ascertainment, failure to obtain all appropriate biological specimens, and other problems. To improve AEFI surveillance for future campaigns planning should begin well in advance and be appropriately funded; extensive training and sensitization at all levels of the health system should occur and systematic mechanisms for collecting, and processing biological samples, including for differential diagnoses, should be implemented.

**SESSION 13 (Plenary Lecture)**

**When Germs Travel: Social, Economic, Political and Cultural Aspects of Contagious Crises Across Time**

**Sunday, February 15, 2009**

**Room: Park Congress/Ground Level 11:00–11:45**

**13.001 When Germs Travel: Social, Economic, Political and Cultural Aspects of Contagious Crises Across Time**

H. Markel. University Michigan, Ann Arbor, MI, USA

This lecture will discuss the broad historical themes of epidemics and other public health crises across time and national context from Antiquity to present. The origins of isolation and quarantine policies, the development of national and international sanitary or public health regulations, and the history of immigrant medical inspections during periods of significant human migration will be explored followed by a comparative discussion of major pandemics, including bubonic plague of the Middle Ages, cholera during the 19th century, and more recent concerns over influenza. This lecture will also demonstrate how the historical record can inform contemporary pandemic preparedness planning and public health policies in the 21st century.

**SESSION 14 (Parallel Session)**

**Global Movements of Humans, Animals and Diseases**

**Sunday, February 15, 2009**

**Room: Park Congress/Ground Level 14:30–16:00**

**14.001 Travelers as Sentinels of Emerging Diseases**

E. Barnett. Boston Medical Center, Boston, MA, USA

International travel has increased substantially in recent years, with the greatest increase in inter-Asian travel. The rapidity with which travelers can navigate the globe results in potential for disease transmission. This presentation will address transmission and potential transmission of emerging diseases as a result of global travel.

**14.002 Diseases that Travel with Animals**

N. Marano, G. Galland. Centers for Disease Control and Prevention, Atlanta, GA, USA

Animals are imported to the US for multiple reasons; for exhibitions at zoos, scientific education, research, and conservation programs, and as food and products. Individual companion animals travel with their owners across international borders. Increasingly, however, animals are being imported for a thriving commercial pet trade. In many cases the animals that are imported and traded are non-native species and/or animals not traditionally kept as pets and can represent a significant risk to human health. In 2003, monkeypox was introduced to the US when a shipment of African rodents was sold to dealers, one of whom housed the rats with prairie dogs intended for the pet trade in a distribution facility. The prairie dogs contracted monkeypox infection from the rodents and subsequently transmitted the infection to 37 people, including their owners and veterinary staff caring for the ill animals.
This presentation will address the specific risks associated with travel and transport of animals and vectors including West Nile Virus, SARS, avian influenza, rabies, viral hemorrhagic fevers, and leishmaniasis. CDC response includes surveillance, regulation, science and education. CDC’s regulations control the importation of nonhuman primates, dogs and cats, small turtles, African rodents, civets, and Asian birds. CDC partners with industry to educate the public and CDC’s ‘Healthy Pets Healthy People’ website assists physicians and veterinarians seeking to counsel their patients and clients about zoonotic disease prevention.

CDC has begun revising its animal importation regulations, soliciting public comment and feedback on animal importation to determine the need for further rulemaking. Recent initiatives such as the Farm Bill and the Non-Native Wildlife Invasion Prevention Act will also be discussed. CDC is working to develop proactive approaches to preventing the importation of animals and vectors that pose a public health risk.

### Globalization and Human Migration

**M. Cetron.** CDC, Atlanta, GA, USA

Human migration has been an interest of historians, anthropologists, demographers and other academicians for centuries, but the intersection between migration patterns and disease epidemics has been explored by a more limited circle of scholars. Significant drivers of human migration include a combination of “push factors” e.g. war, persecution, pestilence, natural disasters, as well as “pull factors” e.g. economic opportunity, family reunification, political and religious freedom. Global migration was first highlighted in 1992 by the Institute of Medicine as a major factor in the global emergence of infectious diseases. The advent of air travel in the 20th century has fundamentally altered the migration landscape. Routinely accessible jet airline travel over the last 40 years has led to an unprecedented increase in the volume and speed of global migration. According to WTO, there are now over 1 billion international arrivals annually. Modern air travel enables us to circumnavigate the globe in <36 hours compared with 365 days more commonly experienced by those traveling by ship a century ago. When transcontinental travel times become shorter than the incubation period for many infectious diseases, new paradigms for global health security are required to replace antiquated approaches. This talk will focus on the intersection of global migration and emerging disease threats through illustrative examples as well as discuss new models for EID detection, response and prevention.

### Feeling the Heat: Climate Change and Emerging Diseases

**Sunday, February 15, 2009**
**Room: Klimt Ballroom 2 & 3/First Level**
**14:30–16:00**

**15.001 Climate Change and Infectious Disease: Checking the Horse before Hitching the Cart**

**P. Reiter.** Institut Pasteur, Paris, France

Man-made climate change has become a defining moral and political issue of our age. Speculations on its potential impact often focus on infectious diseases, and on vector-borne pathogens in particular. Alarming predictions are common: in the coming decades, tens—even hundreds—of millions more cases of malaria will occur in regions where the disease is already present; transmission will extend to higher altitudes and latitudes; dengue and chikungunya will increase (are already increasing) their range and incidence in the tropics; mild winters have enabled West Nile virus to become enzootic in the United States and so on. Such predictions, sometimes supported by simple models, are persuasive because they are intuitive, but they sidestep factors that are key to transmission and epidemiology: the ecology and behaviour of humans, the ecology and behaviour of the vectors, and the immunity of the human population. A holistic view of these factors in the precise setting where transmission occurs is the only valid starting point for assessing the likely significance of future changes in climate.

**15.002 Climate and Emerging Diseases: What Kind of Information Will Help Save the Most Lives?**

**D. Campbell-Lendrum.** World Health Organization, Geneva, Switzerland

Climate is an important determinant of the transmission dynamics of many infectious diseases. The ongoing trend of increasing average temperatures and more extreme precipitation therefore raises genuine risks for infectious disease control.

However, projections of future climate changes are uncertain, and their effects will interact strongly with non-climatic determinants. These include the broadly protective effects of socioeconomic development and coverage of control interventions, and the broadly risk-enhancing effects of increased globalization and human, vector and pathogen dispersal. This presentation discusses what kinds of new information will be most useful to decision-makers aiming to help control infectious disease in a changing climate (e.g. advances in climate science, projecting future trends in non-climatic determinants of infectious disease, investigations of the effect of gradual climate changes on individual health events in the past).

The presentation argues that although these have some relevance, there is a need for a more pragmatic approach. This should include a greater focus on assembling evidence on the current costs and effectiveness of interventions to control climate-sensitive diseases, and a review of whether these investments are likely to be robust to future changes in climate and other conditions. This should serve as the basis for greater investment in surveillance and control of infectious disease both as an immediate priority, and as effective protection from any increased risks that climate change may bring.

### Vector-Borne and Zoonotic Diseases: Climate, Landscape and Transmission

**U. Kitron.** Emory University, Atlanta, GA, USA

Transmission of vector-borne and zoonotic diseases depends on climatic and environmental conditions that in turn impact the population biology of arthropod vectors and vertebrate reservoir hosts. Landscape and climate conditions and anthropogenic changes determine habitat suitability for vectors, reservoirs, maintenance of pathogen infection and occurrence of disease.

The complex impact of anthropogenic changes on various climatic, landscape, land use and demographic factors need to be understood, in order to explain and predict changes in pathogen transmission and disease occurrence. Predictions based only on the impact of one type of change (e.g., rise in temperature, deforestation) do not take into account the complex interactions among such changes and their potentially conflicting impact on components of the transmission system. Eco-epidemiology is an approach that incorporates spatial epidemiology (and other epidemiological methods) into an ecological approach, particularly landscape ecology and metapopulation biology. Examples from our eco-epidemiological research on Chagas disease in Argentina, schistosomiasis in Kenya, and Lyme disease and West Nile virus in the US, together with examples from other studies will be presented. Among the key questions that need to be considered in order to understand changes in transmission patterns of vector-borne zoonoses are: How do the key measures of risk and transmission dynamics and the impact of anthropogenic changes vary with scale? How do we integrate processes occurring at diverse spatial and temporal scales? These questions can only be addressed through solid biological, epidemiological and socio-economic understanding of the system in time and space.
SESSION 16 (Parallel Session)
Roundtable: Avian/Pandemic Influenza
Sunday, February 15, 2009
Room: Park Congress/Ground Level
16:30–18:00

16.001 Detecting Influenza Activity Using Search Engine Query Data
J. Ginsberg. Google Inc., Mountain View, CA, USA

Analysis of aggregated online search queries, submitted by millions of users around the world each day, has been demonstrated as an effective method of detecting seasonal influenza epidemics. By comparing hundreds of billions of Google web searches against 5 years of CDC influenza-like illness data, we generated models for use in influenza surveillance.

Google Flu Trends, a free service launched in November 2008, uses these models to estimate the current level of influenza activity in the United States in near real-time, with regional and state-level estimates of influenza-like illness. We present a brief overview of the automated method used to build our model, together with an analysis of the accuracy of our estimates throughout the 2007–2008 influenza season and the early weeks of the 2008–2009 season.

16.002 Intervention Strategies for Highly Pathogenic Avian Influenza Outbreaks
J. Lubroth1, J. Domenecchi2. 1FAO, Rome, Italy, 2Food and Agriculture Organization of the United Nations, Rome, Italy

Until the H5N1 HPAI crisis of Eurasia, Middle East and Africa, the occurrence of highly pathogenic avian influenza was historically confronted by the culling of infected flocks and those potentially infected (with, perhaps, the notable exception in incursions of the disease in turkey flocks in northern Italy, where pre-emptive vaccination was practiced). Thus unlike the previous outbreaks of HPAI in Western Europe (H7N1, H7N7, H5N2), North America (H5N2, H7N3), Chile (H7N3), Australia (H7N3), the H5N1 strain affected in impoverished countries or sectors requiring a different approach to intervention. With the widespread occurrence of the zoonotic H5N1 HPAI (2004-to-date) affecting all poultry rearing sectors—from highly integrated commercial systems to scavenging poultry—emphasis has been placed in awareness, capacity building for its prevention (improved biosecurity), rapid detection, analysis of market and commercial poultry practices and risk management, vaccination using quality vaccines, monitoring viral genetic and antigenic viral changes, developing regional, national or local intervention strategies (including the introduction of compensation policies, establishment of diagnostic laboratory and epidemiological unit networks, development attempts at behavioural change, improved hygiene at the market places, wild bird risk analysis and field studies) that would not unjustly further impair the livelihoods of millions of families. The international community—those responsible for animal and human health—have come together in an unprecedented manner to engage regional organisations, financial institutions, and the private sector to confront the impending threat of a wider problem of food security and pandemic potential.

16.003 Communicating Infectious Diseases Information

The journal Nature was one of the most prominent media outlets to give early sustained attention to the threat of the H5N1 avian influenza virus, and the issues needed to prepare for a pandemic. This talk will provide a science media perspective on the challenges of informing and communicating about infectious diseases and disease surveillance.

SESSION 17 (Parallel Session)
Oral Presentations:
Zoonoses and Animal Health
Sunday, February 15, 2009
Room: Klimt Ballroom 2 & 3/First Level
16:30–18:00

17.001 Zoonoses and Exotic Animal Importation
S.L. Babcock. US Department of Homeland Security, Office of Health Affairs, Washington, DC, USA

Background: The vast scope of exotic animal importation, potentially serious health ramifications, and differing regulatory authorities requires an analysis of the impact of this practice, existing US federal and state legislation, laws and regulations, private industry guidelines, as well as international treaties governing this practice.

Methods: Conduct a policy analysis that considers 1) which mechanisms are currently in place to allow an agency to regulate the importation of exotic animals 2) situations where a lack of authority may exist for an agency to regulate and 3) circumstances where there is adequate authority in place to regulate, but there may be a lack of resources to effectively enforce this authority.

Analysis: The current U.S. regulatory framework to address the issues raised by exotic animal imports is multi-faceted. While there are effective regulations in place, they are not uniform across the regulating agencies and agencies may need additional capabilities to enhance compliance with their regulations. The majority of the responsibility for imported exotic animals remains with the states. Private industry has been very successful in its efforts to minimize negative consequences of exotic animal importation through accreditation, voluntary corporate codes, and mandatory guidelines. The traditional roles and mission space of federal agencies needs to be considered in light of these emerging issues and evolve to meet the challenges.

Conclusion: There is a great need to create proactive science-based policies to prevent and minimize the risks and respond to the threats posed by exotic animal imports while facilitating the flow of legitimate trade and travel. An all-encompassing approach to include animal, ecological, and human health issues is needed to address the importation of exotic animals and should include federal, state, local, international and private partners. This overlap requires stakeholders to take novel and proactive steps towards collaboration in this area.

17.002 “Moving” Continuous Source in an Outbreak of Q Fever
C.H. Winter1,2, S.O. Brockmann1,3, C. Meier1, H. Merz1, P. Reith1, G. Pfaff1, C. Wagner-Wiening1, I. Flechotowski1. 1Baden-Württemberg State Health Office, District Government Stuttgart, Germany; 2Robert Koch-Institute, Berlin, Germany; 3Postgraduate Training for Applied Epidemiology (PAE, German FETP); 4Local Public Health Office Freudenstadt, Germany; 5Local Food and Veterinary Office Freudenstadt, Germany

Background: Q fever, a worldwide zoonosis caused by Coxiella burnetii, is common in cattle, sheep and goats. C. burnetii is excreted via urine, faeces, birth products and milk, and can survive for long periods in the environment. Humans become infected mainly by inhalation of C. burnetii-contaminated aerosols. At the end of August 2008 one Public Health Office was informed about hospitalized pneumonia patients from one village, with suspected Q fever. We performed an outbreak investigation to identify the pathogen, the source of the outbreak, and to prevent further infections.
Methods: Laboratory confirmation of Q fever was initiated and active case finding was performed via information campaign. The detected cases were interviewed about disease pattern and exposure. Human, ovine and caprine serum samples and vaginal swabs of sheep and goat herds were tested for Q fever. The distance from the residences of cases to the herding route of the Q fever positive herd was measured.

Results: Interviews of the first patients with confirmed C. burnetii infection identified a sheep herd as the likely source of infection. The herd was subsequently tested positive for C. burnetii. Active case finding yielded 41 Q fever cases from 12 residential areas of the village (nearly 1,000 inhabitants). Cases appeared between May 1st and September 18th. Thirty-five cases (83%) participated in the interviews: 54% suffered from pneumonia and 34% were hospitalized. The sheep herd passed the 12 residential areas during the weeks of grazing and moving through the region (1724 hectares). Lamming occurred before and occasionally during the outbreak. Thirty-six cases lived more than 200m away from the herding route. All cases became ill after the herd had passed their residential area. Thirty-eight percent of cases reported contact to sheep and 77% walked on grazing land where sheep had recently been kept. As public health measures, the herd was twice vaccinated against Q fever, and outdoor lambing and grazing close to residential areas was prohibited.

Conclusions: This prolonged Q fever outbreak was related to a moving continuous source. Human infections were likely to occur after sheep passed close to their residence. The reason for the appearance of cases along the long herding route remains unclear as environmental contamination through birth products only occurred at few locations. No further human infections appeared after distancing the infected herd from residential areas, indicative of effective preventive measures.

17.003 The Emergence of Simian Foamy Virus in Human Population of Gabon, Central Africa
A. Mouinga-Ondem1, M. Kazanji2, 1CIRMF, Franceville, Gabon, 2CIRMF and Pasteur Institute, Franceville, Gabon

It is now well known that all human retroviruses have a non-human primate counterpart. It has been reported that the presence of these retroviruses in humans is the result of interspecies transmission. Several authors have described the passage of a simian retrovirus (simian foamy virus (SFV)) from primates to humans. To better understand this retroviral “Zoonosis” in natural settings, we evaluated the presence of SFV in wild monkeys and in humans at high risk such as hunters and humans bitten by wild non-human primates from Gabon, central Africa.

For serological screening, a western blot test was first performed on the collected samples. For the molecular studies, genomic DNA was extracted from the peripheral blood buffy coat and a portion of the SFV provirus (465 bp of the integrase) was amplified by PCR and then sequenced. In samples collected from primates, Mandrills were found to be highly infected with SFV (71.6%). Furthermore, SFV was found in two species of monkeys (Cercopithecus solatus and Cercopithecus nictitans) which had never been found to be previously infected. In samples collected from humans, we showed clear interspecies transmission of SFV from primates to humans in hunters and in primate laboratory workers. Fourteen individuals injured by bites from mandrills, gorillas and chimpanzee had a persistent infection of SFV.

Our data demonstrate the efficient transmission of SFV to humans in natural conditions in Gabon, central Africa; however, many questions remain, such as the biodiversity of these viruses in the wild, the possibility of inter-human or intra-familial transmission, the clinical or biological consequences of the chronic infections, and the human target tissues. Our research will seek to answer these questions.

17.004 Epidemic Cowpoxvirus Infections in Germany
A. Kurth1, A. Kuczka2, C. Becker2, G. Pauli2, A. Nitsche3
1Robert Koch Institute, Berlin, Germany, 2Chemisches und Veterinäruntersuchungsamt Rhein-Ruhr-Wupper, Krefeld, Germany, 3HELIOS Klinikum Krefeld, Krefeld, Germany

Background: Still 30 years since the successful eradication of smallpox, several zoonotic orthopoxviruses are representing a danger to humans. Beside Monkeypox in Africa, Cowpox has been enzootic in cattle for periods of time in Europe. However, no cowpox virus (CPXV) infections of cattle were diagnosed in Germany over the last decades. Instead, individual cases of CPXV infections are increasingly found in cats and their usually unvaccinated owners. Both, cats and human present local exantheme on arms and legs or in the face, probably acquired as smear infections. Although generally regarded as self limiting disease, immunosuppressed patients can develop lethal systemic disease that resembles a variola virus infection. Here we report for the first time a cumulated incidence of human cowpox virus infections transmitted directly from pet rats.

Methods: Crust material, swabs and serum of 7 patients presenting with CPXV-typical skin lesions were sent to the German Consultant Laboratory for Poxviruses between March and October 2008. Specimens were subjected either to real-time PCR assays specific for Orthopoxvirus, Para-poxvirus or Molluscipoxvirus, or to serology testing by immunofluorescence staining of CPXV infected cells with the patients serum. Orthopoxvirus positive specimens were further typed by sequencing of the complete ORF of the hemagglutinin gene.

Results: All patients were PCR negative for Parapoxviruses and Molluscipoxvirus, but PCR positive for Orthopoxvirus and had a case history showing close contact to pet rats. None of the patients was vaccinated with Vaccinia virus. The virus could be isolated and identified as an identical CPXV strain in all cases. However, in comparison to recent CPXV infections found in Germany, this virus represented a new strain. In addition, all patients showed significant orthopoxvirus specific titers.

Conclusions: We could show for the first time that the same CPXV strain is detected in rat populations over a time period of six months. In this context, the increasing popularity of pet rats implicates the risk of cowpox virus transmission to humans. The screening of pet rats for poxviruses should be discussed in the future.

17.005 Public Health Aspects of the Redlands Hendra Virus Outbreak July 2008
B. McCaill1, V. Slinks1, G. Smith2, H. Field2, G. Playford3, I. Smith1, K. Heel1, N. Kung3
1Brisbane Southside Population Health Unit, Queensland Health, Brisbane, Australia, 2Queensland Health Forensic & Scientific Services, Brisbane, Australia, 3Department of Primary Industries & Fisheries, Brisbane, Australia, 4Princess Alexandra Hospital, Queensland Health, Brisbane, Australia

Background: The Hendra virus reservoir is Pteropod bats with spillover to horses possibly through contamination of horse-feed by urine/birth products. Nine previous equine outbreaks of HeV have been described, all in coastal north-eastern Australia since 1984. Principally, horses had respiratory presentations with some neurological signs. Transmission to four human cases from direct contact with bodily fluids of HeV-infected equine cases has occurred: two recovered following influenza-like illnesses (ILI) and two fatalities, one from respiratory failure following ILI and the other from encephalitis 13 months after aseptic meningitis. We describe another outbreak involving five horses and two humans.

Methods: Testing for HeV was requested on four horses at a veterinary hospital who were exhibiting neurological symptoms. Once the outbreak was confirmed in horses, the hospital was quarantined, active surveillance for human illness was commenced and a cohort study of the potential human exposures was conducted. Cases were hospitalised and treated with ribavirin. Horse and human viral and serological testing was performed as well as genetic sequencing of the virus.

Results: Initially, four horses tested positive: all were euthanased. The fifth exhibited symptoms 16 days after the imposed quarantine and was also euthanased. Of the 37 veterinary clinic staff, 20 had potential exposure to infected horses with two (attack rate 10%) contracting the disease. Both had ILI and then upon seroconversion developed encephalitis, one of whom died. Significant exposures for the human cases included nasal cavity lavage late in the incubation period of an infected horse (minimum incubation of 9 and 11 days for human cases); the fatal case also assisted autopsy of another infected horse (16 days prior to symptom onset).
Gene sequencing showed this virus was distinct from previous outbreak strains.

**Conclusion:** This outbreak provides new details on neurological presentations in horses and clinical presentations in humans. As expected, viral RNA sequencing showed several base-pair changes which may account for the different presentations. There was under-utilisation of personal protective equipment by veterinary staff prior to recognition of the outbreak. The potential human exposures in this outbreak suggest that horses may be a source of exposure late in their incubation period or that the known incubation period in humans may be longer than originally reported.

**17.006 Molecular Origin and Demographics of Genotype-IV Hepatitis E Virus in Eastern China Implicated Zoonosis and Exotic Importation**

Y.H. Lu1, Y.J. Zheng1, Q.W. Jiang1, F.D. Wang2. 1.Key Laboratory of Public Health Safety, Ministry of Education, School of Public Health, Fudan University, Shanghai, China, 2.Center for Disease Control and Prevention, Deqing County, Zhejiang, China

**Introduction:** According to the national epidemic situation (issued by Ministry of Health of People’s Republic of China), hepatitis E (HE) is the only definite. Based on the general GTR model of nucleotide substitution and time to most recent common ancestor (tMRCA) was calculated using Neighbor-Joining method. Nucleotide substitution rate and time to most recent common ancestor (tMRCA) was calculated using molecular clock theory and Bayesian Skyline Plot (BSP) based on Markov chain Monte Carlo. The host population dynamics was inferred by best-fit demographic model.

**Results:** There was great genetic similarity and little geographical difference among genotype-IV isolates in Eastern China. It was noted that some human and swine isolates were clustered with bootstrap values of almost 100% in which the direction of interspecies transmission was not definite. Based on the general GTR model of nucleotide substitution and a relaxed molecular clock, BSP gave the evolutionary rate of 2.07x10E-3 subs/site/year which corresponded to estimated date for IMRCA of 1830s. The IMRCA of human HEV was about one year longer than that of swine HEV. The effective number of infected population increased with time gradually and rose remarkably since 2000. Exponential-growth model was proved to be the best-fit model demonstrating the demographic history with epidemic growth rate of 0.16/year and doubling time of infected population of every 5 years.

**Conclusion:** It was the first time to determine that genotype-IV HEV had been spreading since 1830s in Eastern China which was consistent with the opening of local ports for trade with the outside world. The fast evolutionary rate was probably attributable to flourishing swine industry locally which maybe leads to continual passage and rapid evolution of HEV in pig herds for market. It also implied that the locally original reservoir of HEV was human and bilateral interspecies transmission occurred consequently via breeding, slaughter and selling of pigs.

**17.007 A Risk Assessment of Bluetongue Disease in Austria**

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Bluetongue Disease (BTD) is internationally recognised as a notifiable disease with great economical relevance. In the summer of 2006 this vector-borne disease was introduced into Northern Europe and has affected most European countries since. Culicoides biting midges are cyclic vectors of the bluetongue disease virus (BTV). In the recent outbreaks the main vector C. imicola was absent and palearctic Culicoides species like C. obsoletus and C. pulicaris were detected. In this study the distribution of Culicoides spp. in Austria is analysed and the Bluetongue Disease risk zones are determined. Culicoides abundance data were collected from weekly catches of 14 months at 51 trap locations. The corresponding weather data of the trapping day originated from neighbouring meteorological stations. From the total of 7.523.947 Culicoides caught 90.3 % were classified to the C. obsoletus complex. 186 meteorological stations (years 1997–2007) were analysed to detect correlation of weather and Culicoides distribution. The regression model using R (R 2.8.0, http://CRAN.R-project.org) assigned a highly significant effect of mean temperature and wind (P ≤0.001) and a borderline significant probability (P=0.0457) of relative humidity on Culicoides spp. abundance. The majority of catch (>1000 individuals) were found at temperatures above 10 °C and at relative humidity between 65 to 80 %. The point data of the significant parameters mean temperature and relative humidity were subsequently interpolated using the ESRI ArcGIS TM version 9.3 Geostatistical Analyst tool Kriging. In a raster analysis those regions providing optimal temperature and humidity conditions were seperately investigated in all four seasons. In addition data of cattle density were included to create a risk map. The results of this project provide fundamental data on the distribution of Culicoides spp. in Austria, determine limiting climatic parameters, model most favourable Culicoides habitats and identify risk areas by including possible host interaction. These high risk areas can subsequently be given special attention for precautionary monitoring measures.

**17.008 Global Warming and Epidemic Hantavirus Trends in Belgium: of Mast, Mice and Men**

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**Background:** Nephropathia epidemica (NE), an emerging rodent-borne hantavirus disease, has become the most important cause of infectious acute renal failure in Belgium, with cyclic and increasing peaks since 1993. The responsible rodent reservoir is the bank vole. We suspected that cyclic availability of its staple food, being "mast" or seeds (mainly beechnuts and acorns) of deciduous broad-leaf trees, was the ecological causal connection.
Methods: Possible temporal relationship between “mast years” and recurrent NE peaks was examined. Yearly NE numbers were matched to preceding seasonal temperature and precipitation data.

Results: Since 1993, each NE peak is preceded by a mast year, resulting in significantly higher NE case numbers during these peaks (Spearman R = -0.82; P = 0.004). Of the total of 1,678 seroconfirmed Belgian NE cases recorded in the last 12 years, almost half (828 or 49.34%) has been documented in the last 3 years 2005-2007, meaning a recent mean of 276 cases/year versus previously only 94 cases/year (P = 0.0031). NE peaks are significantly related to warmer autumns the year before (R = 0.51; P < 0.001), hotter summers two years before (R = 0.32; P < 0.001), but also to colder (R = -0.25; P < 0.01) and more moist summers (R = 0.39; P < 0.001) three years before. July was singled out as the best predictor month.

Conclusions: NE peaks in year 0 are induced by abundant mast formation in year-1, facilitating bank vole survival during winter. This survival is further promoted by higher autumn temperatures in year-1, whereas mast formation itself is primed by higher summer temperatures in year-2. Both summer and autumn temperatures have been rising to significant higher levels during last years, explaining the virtually continuous epidemic NE state since 2005. A similar phenomenon was noted in neighbouring countries, particularly in Germany This is the first report linking an emerging human infection in North-West Europe to global warming strains in a geographically confined region.

SESSION 18 (Poster Presentations II)

Antibiotic Resistance: The Future is Now

Sunday, February 15, 2009

18.001 – 18.082 Room: Klimt Ballroom I – First Level
18.083 – 18.183 Room: Bruckner/Mahler/Brahms – First Level
11:45 – 12:45

Emerging Vector-Borne Diseases in Humans and Animals

18.001 – 18.049 Room: Klimt Ballroom I – First Level

18.001 Annual Trends of Dengue for a Decade in Taiwan
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Background: Taiwan is located among the Pacific Ocean with dual climate of Tropics and Subtropics which is contributed Northern and Southern Taiwan with the distinct ecological characteristics including climate status, distribution of mosquito vectors and human population density. We examined the annual epidemiological trends as well as the impact of the imported cases on community transmission.

Methods: The number of cases aggregated over all years of data was included in the Taiwan CDC surveillance system. The impacts of imported cases on the epidemiological trends were measured using the Pearson test and the two-way ANOVA.

Results: A total of confirmed 10,351 dengue cases including 7.1% imported cases was recorded in the Taiwan CDC surveillance systems during 1998-2007. Dengue epidemics exhibited mainly clustered and reappeared in tropic Taiwan accounted for 94.0% dengue cases including 63.2% in its metropolitan. In contrast, the subtropical Taiwan showed merely sporadic cases even in its urbanized cities. There was a common seasonal fluctuation pattern, started a slight increasing in cases in June, peaked from September to November, subsided gradually in December, and ended in the next January to April. Notwithstanding, Taiwan starting an airport fever screening since 2003 till 2007 had showed to successfully identify 45.0% imported dengue cases, the introduction of asymptomatic and/or latent dengue travelers might subsequently caused epidemics in 2005-2007 which should be paid attention.

Conclusion: The recent findings may contribute information beneficially for those areas with similar ecological status to define their dengue hotspots in order to optimally introduce the control measures. Nevertheless, facing the risk of introduction of asymptomatic and/or latent dengue cases might trigger community transmissions, for those non-endemic dengue areas, airport fever screening was indeed available for quick identification of partially imported dengue cases at first time which could spare some cost for subsequent intervention.

18.002 Decision Tree Algorithm in Deciding Hospitalization for Adult Patients with Dengue Haemorrhagic Fever in Singapore
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Background: There are several predictors of dengue hemorrhagic fever (DHF) but there are no clinically-useful tools to assist clinicians in recommending hospitalization for dengue patients. This study aims to develop a simple decision tree for clinicians to decide between hospitalization and outpatient monitoring for dengue patients.

Methods: A retrospective cohort study was conducted on all laboratory-diagnosed dengue patients admitted in 2004 to Tan Tock Seng Hospital, Singapore. Demographic, clinical, laboratory and radiological data were collected, and cases classified as dengue fever (DF) or DHF. To develop the decision tree, we used the CHAID (Chi-Squared Automatic Interaction Detector) method with bi-way and multi-way splitting, and the chi-square test to implement the splitting based on the best fit. The resulting trees were pruned to achieve the highest sensitivity with the shortest tree.

Results: In 2004, 1973 probable and confirmed adult dengue patients were admitted—DF comprised 1855 (94.0%) and DHF 118 (6.0%) of the cases. Eighty-two cases developed DHF subsequently during the hospital admission. From multivariate logistic regression analysis, patients with bleeding, decrease in total protein, increase in blood urea, and decrease in lymphocyte proportion were associated with DHF. The best decision tree prediction had only 3 branches, comprising of a history of clinical bleeding, serum urea level, and finally serum total protein level. This tree had a sensitivity of 1.00, specificity of 0.46, positive predictive value (PPV) of 7.5%, and negative predictive value (NPV) of 100%. The overall accuracy of the decision tree was 48.1%.

Conclusion: The test sensitivity and specificity compared favorably with other sophisticated laboratory tests, and would have prevented 43.9% of mild DF cases from unnecessary hospitalization. This simple decision tree is therefore effective in predicting DHF in adults, and will be useful in acute clinical settings.

Figure 1. Decision tree for selection of adult DHF cases for hospital admission
Açaí is a fruit with excellent nutritional properties, very appreciated in Amazonia but also consumed in all Brazil and even in other countries. Due to high productivity in poor north Brazil regions, its commercialization is fundamental to the local economy. In 143 cases of registered Acute Chagas Disease (ACD) in Brazil between January/2006 and April/2007, more than 80% has been occurred in Amazon region and the majority is related to the ingestion of T. cruzi infected acai pulp. Meanwhile, there are no scientific data that give evidence of ACD transmitted by this way. In order to evaluate the performance of T. cruzi in acai pulp, CBA/Uni mice plasma with 1X10^5 Y strain of T. cruzi trypomastigotes were mixed to the pulp and kept at room temperature for 28 hours. The trypomastigotes vitality has been watched at each hour in the first 1 hours and then with 8; 12 and 24 hours observation. Two different methodologies were adopted to find the parasite. The first one consisted of a direct inspection of a mixture and also of a trypan blue colored mixture. The second methodology employed a forced straining process in wool to isolate the parasite. Aliquots of 5 µL from both methodologies, were observed on a common optical microscopy. The straining process allowed getting free pulp residues suspension. The adopted rule to the analysis was done according to the trypomastigotes forms movements: Very Active (VA), Active (A) Slow (S). Until 6 hours from the start of experiment, 100% of parasites has been classified as (VA), while with 28 hours, 67% of them were considered as (A) and 33%, as (S) suggesting that the acai pulp does not interfere on the vitality of T. cruzi trypomastigotes.

**Conclusions:**
Fatality due to malaria is seen even with good diagnostic facilities and treatment, especially in developing countries like India. Importance of complete autopsy and histopathological examination in identifying malaria related mortality is stressed.

**Background:**
Toscana virus (TOSV) (genus Phlebovirus) is an important agent of acute meningitis and meningocerephalitis in residents and visitors from Mediterranean countries in which the virus circulates. A significant proportion of infection results in asymptomatic or pauci-symptomatic forms. TOSV is transmitted during the summer-fall period by phlebotomine sandflies, basically represented in Catalonia by an important presence of Phlebotomus perniciosus, known vector of TOSV, and Sergentomyia minuata. Although the first case of TOSV infection reported from Spain occurred in a Swedish tourist after a visit to Catalonia, since then no more cases have been reported and no seroepidemiological studies have been carried out in this region. With this background we carried out a seroprevalence study of TOSV infection.

**Methods:**
Sera were obtained from a representative sample of Catalonian civilians. The population studied was representative stratified by gender and age for each geographic area. Eight different areas were established taking into account the localisation and the altitude. A total of 833 serum samples were submitted. Anti-TOSV IgG were detected by a commercial enzymatic immunoassay, Enzymewell Toscana virus IgG (Diesse, Italy), following the manufacturer's instructions.

**Results:**
Fifty samples showed IgG positive ratios, with an overall seroprevalence of 6%. Anti-TOSV IgG was more often detected in persons >60 years [OR] 2.79, [95% CI] 1.46-5.30, p=0.001) with a seroprevalence of 12.3% in this age group. IgG-positive persons were equally divided by gender. When different geographical areas were compared, seroprevalence rates varied from 0% in the regions of Pirineic belt and Ebre lands (higher and lower altitudes), followed by 3.5% and 3.7%, respectively, in the regions of Region Centre and Barcelona-Maresme, 7% in Lleida-Gironés1 (>1000m), 9% in Lleida-Gironés2 (<=1000m), 10.7% in Costa Brava and 19.3% in Tarragonès.

**Conclusions:**
The study found evidence of TOSV circulation in Catalonia. The higher seroprevalence rate found in the area of Tarragonès (19.3%) indicates a higher circulation, suggesting that infection cases can be misdiagnosed in this area. Despite increasing evidence of TOSV as a major cause of aseptic meningitis or meningocerephalitis during warm seasons in countries in which circulates, the epidemiology of TOSV in Catalonia is still unknown and few physicians are aware of its potential to cause central nervous system infections.

**Background:**
Malaria is one of the most common infectious diseases and an enormous public health problem. According to World Malaria report 2008, about 247 million malaria cases were present worldwide among 3.3 billion people at risk in 2006, causing nearly a million deaths.

**Methods:** This autopsy based retrospective research was conducted at KMC, Mangalore using Autopsy files of cases from September 2004 to August 2008. Histopathologically confirmed cases of malaria were included in the study. Data was analyzed using Microsoft excel and SPSS, version 11. A detailed epidemiologic profile was made.

**Results:**
Out of 2515 cases autopsied during the study period, sudden death was reported in 257 cases, out of which five cases were diagnosed as of sudden death due to malaria. The histopathology examination revealed infiltration of acute and chronic inflammatory cells with numerous parasitized RBCs containing malarial pigment in the visceral organs.

**Conclusion:**
Fatality due to malaria is seen even with good diagnostic facilities and treatment, especially in developing countries like India. Importance of complete autopsy and histopathological examination in identifying malaria related mortality is stressed.
18.007 Occurrence of Tick Bites and Serological Evidence of Exposure to Rickettsioses Among Sri Lankan Military Personnel
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Background: In Sri Lanka, rickettsial diseases were documented among military personnel during the Second World War when British troops were dramatically infected by Orientia tsutsugamushi (OT). Now, however, Rickettsia conorii and OT are reemerging in Sri Lanka but their prevalence among military personnel in active services in the Northern (NP) and Eastern (EP) provinces is not known.

Objectives: To study the frequency of tick bites and the sero-prevalence of rickettsial diseases among military personnel in active field services in Sri Lanka.

Methods: 57 army personnel admitted with war injuries to Colombo North Teaching Hospital, Ragama, were interviewed using an interviewer administered questionnaire to determine socio-demographic data and the frequency of tick bites. A 3 ml venous blood sample was taken with informed written consent and tested for common rickettsial species using Immuno Fluorescent Antibody (IFA) test for IgG against RC and OT antigens.

Results: The mean (SD) age and period of active service of the population were 25.8 (5.5) years and 6.7 (5) years respectively. Participants were from 20/25 districts in Sri Lanka. All had served in NP; 13 had also served in EP. Although all were in military uniform most of the day, they had frequently slept on scrub land. 35/57 (61.4%) had never used insect repellents while the rest used them infrequently. None were on doxycycline prophylaxis. 48/57 (84%) had experienced tick bites during field services. 50/57 (88%) had serological evidence of exposure to rickettsioses (IFA-IgG titer > 1:64): 33/50 (66%) to RC, 1/50 (2%) to OT and 14/50 (28%) had mixed titers for both (in all, titers were higher for RC). However only 24/57 (42%) had a history of febrile illness during their service period (four had malaria; the rest were undiagnosed).

Conclusions: A higher proportion of this study population showed evidence of exposure to R. conorii (transmitted by hard ticks) than to O. tsutsugamushi (transmitted by mites), suggesting a change in the pattern of rickettsial diseases when compared to the 1940s. The high frequency with which the tick bites were reported in the study population also suggests that exposure to Rickettsia disease is a neglected but preventable occupational hazard among military personnel engaged in active field services in Sri Lanka.

18.008 Chikungunya and Dengue Fevers: Differentiating Clinical and Laboratory Factors
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Background: Chikungunya fever has recently emerged in tropical Singapore. Although endemic for dengue fever since 1960s, autochthonous chikungunya transmission was only reported since January 2008. Clinical features of chikungunya mimic that of dengue. Hence, it is important to identify simple clinical and laboratory characteristics that can differentiate the two Aedes mosquito-borne infections.

Methods: We conducted a matched case-control study to identify clinical and laboratory factors associated with chikungunya fever. We included 99 patients confirmed with chikungunya infection on reverse transcription-polymerase chain reaction (RT-PCR) during the August 2008 outbreak, and hospitalised at the national infectious disease referral centre in Singapore. Ninety-nine controls matched for age, gender and ethnic group, were selected from a cohort of PCR-confirmed dengue fever patients hospitalised during the 2004 dengue outbreak.

Results: At presentation to hospital, chikungunya patients were more likely to have a rash than dengue patients (OR 3.71, 95% CI 1.61-8.56). However, dengue patients had an odds of 6.2 times (95% CI 2.80-14.61) and 4.5 times (95% CI 1.52-13.30) respectively, of presenting with vomiting and abdominal pain than chikungunya patients. A unit increase in leucocyte and platelet count (x100/L) respectively, had an odds of 1.53 times (95% CI 1.25-1.88) and 1.04 times (95% CI 1.02-1.07) of being infected with chikungunya than dengue. During hospitalisation, chikungunya patients had an odds of developing myalgia/arthritis 10 times (95% CI 3.05-32.77) that of dengue patients, and a unit increase in nadir leucocyte and platelet counts of 1.82 times (95% CI 1.33-2.47) and 1.05 times (95% CI 1.02-1.08) respectively. Nadir platelet and leucocyte counts (x100/L) were significantly higher in chikungunya patients (platelet median 168, range 102-376; leucocyte median 3.4, range 1.0-13.0) than in dengue patients (platelet median 30, range 7-206; leucocyte median 2.5, range 1.0-5.0).

Conclusion: Simple clinical and laboratory factors like rash, vomiting, abdominal pain, myalgia/arthritis, leucocyte and platelet counts can differentiate chikungunya fever from dengue.

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Dengue fever is a public health problem worldwide. Mild elevation of aminotransferases is a common feature of dengue virus infection and severe acute liver injury has been described. The aim of this study was to assess relationships between antipyretic drugs and chronic alcohol use, with the increase in serum alanine aminotransferase (ALT) level in patients hospitalized with dengue fever during the 2005-2006 dengue epidemics in French Guiana.

In this retrospective study, only patients with available ALT level and biologically confirmed dengue diagnosis were included. Clinical, biological data were collected from charts and anamnestic informations by a direct patient's interview.

In the 162 included patients (99 (62 %) adults, and 63 (38%) children), 2 analysis were performed: (i) 64 (65%) adults with ALT>2N and 35 (35%) controls and (ii) 24 (39%) children with ALT>2N and 38 (61%) controls. In each univariate analysis some factors were found to be associated to ALT elevation: (i) acetaminophen exposure and length of intake in adults, and (ii) acetaminophen overdose during the hospitalization in children. Another analysis found that alcohol consumption was significantly more frequent in adults with ALT > 10N. 5 patients had ALT>50N and PR<50%. Acetaminophen and alcohol consumption should be searched and taken into account when a patient with dengue fever is hospitalised.

18.010 Epidemiological and Phylogenetical Studies on Crimean-Congo Hemorhagic Fever (CCHF) in Iran
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Background: CCHF is a viral zoonotic disease, which causes several haemorrhages in humans with mortality up to 50%. The virus is transmitted through the bite of ixodid ticks or by contact with blood or livestock or Nosocomially.

Methods: From June 2000 till 3 Nov 2008, sera were collected from Iranian probable patients for CCHF and tested in the laboratory of
Arboviruses and Viral Hemorrhagic Fever (National Reference Lab). The sera were analyzed by ELISA (IgM & IgG) and by RT-PCR. After Purification and sequencing, we did phylogenetic studies on some RT-PCR positive samples based on S-segment.

**Results:** Between 1275 probable human cases, 520 were CCHF confirmed cases. Between them, 449 were IgM positive and 71 cases were only RT-PCR positive. Nucleotide sequencing of the S-segment revealed that the different isolates were related closely to each other with nucleotide sequence identities exceeding 98% for S-segment. Phylogenetic analysis of partial S-segment nucleotide sequences showed that the viruses clustered along with strains from Pakistan.

**Conclusion:** CCHF is the most important hemorrhagic fever in Iran and the Sistan-Baluchestan province, in vicinity of Afghanistan and Pakistan is the most infected province. As this province has a very long common border with Pakistan, this fact corroborates phylogenetic analysis that the Iranian CCHF strain is similar to Pakistani strain. It is worth mentioning that due to a successful scientific collaboration between CDC of Iran and Veterinary organization, we achieved the great performance of reducing notably the number of deaths among the confirmed cases of CCHF in recent years in Iran.

### 18.012 Epidemiological Study of Canine trypanosomosis in an Urban Focus of Ivory Coast

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**Background:** African trypanosomiasis due to *Trypanosoma congolense* is a major threat to livestock production in Sub-Saharan Africa. A cross-sectional study was undertaken to determine the prevalence of *Trypanosoma congolense* canine infection in the urban focus of Abidjan, Ivory Coast.

**Methods:** The data were collected in 2003, during a cross-sectional survey of the dogs which are more exposed to the disease because of their outdoor way of life. Blood samples from 123 dogs were collected and subjected to PCR using specific primers for *Trypanosoma congolense* "forest type." The entomological survey was carried out using 22 traps. Insects were counted, selected by sex and dissected to determine their age and detect the presence of trypanosomes.

**Results:** The prevalence obtained in the whole population studied was 30.1%. There was no association between positivity and the sex of the animals nor with their age. *Glossina palpalis palpalis* was the only captured species. They were present in high densities, mainly in the traps situated on the east side near the forest. The rate of infection in the flies was high, with a mean value of 21.7% (80/368). PCR analysis showed that the parasites identified belonged to the species *T. congolense* "forest type" and *T. vivax*. We found no *T. congolense* "savannah type" nor to *T. brucei*.

**Conclusion:** This study demonstrates the high contamination rate of dogs by *Trypanosoma congolense* in enzootic zones, and the risk they could represent if they were introduced in disease free animal populations where they could be spread by other means than the tse tse flies. It also emphasizes the need for routine quarantine including health checks and eventually chemoprophylactic treatments for dogs coming from enzootic zones.

### 18.013 Trypanosoma congolense Infection in One Dog with Surprising Histopathologic Features

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**Background:** Canine trypanosomiasis, caused by *Trypanosoma cruzi*, and known as Chagas disease, is well recognised in America where it may constitute a zoonotic problem. Canine trypanosomiasis caused by *Trypanosoma congoense* is non zoonotic and is rarely reported outside the endemic area of Africa where the main vector, the tsetse fly (*Glossina* species) is found. Under certain circumstances the disease was exported from the endemic area such as occurred in this report.

**Case Report:** Trypanosomiasis, caused by *Trypanosoma congoense* was diagnosed in one military working dog in France after having spent 3 months in Ivory Coast. The major clinicopathological findings include anaemia, leucopenia, haemorrhages, hepatic failure and hepatosplenicomegaly. Protein electrophoresis revealed hypoalbuminaemia and hypergammaglobulinaemia with polyclonal gammapathy. In spite of a symptomatic treatment the dog quickly died. Necropsy was performed. The main gross lesions were subicteric mucous membranes, focally extensive necrotic cutaneous elbow wound, subcutaneous oedema, diffuse splenomegaly, hepatomegaly with discoloration and a moderate left ventricle hypertrophy with several petechial haemorrhages. Histopathologic changes were non specific except amastigotes clusters in the heart (in endothelial cells and in some degenerative myocardial fibers) and in
the skin. PCR on a spleen fragment revealed a strong positivity for *Trypanosoma congolense* "forest type." The dog had never gone in an endemic zone of *Trypanosoma cruzi*. He had lived in France and Ivory Coast only. So *Trypanosoma cruzi* infection was not possible. The hypothesis that amastigotes were leishmanias was eliminated by the negativity of leishmaniasis serology and PCR. Furthermore leishmanias are located usually in macrophages and not in endothelial cells and myocordial cells.

**Conclusion:** At our knowledge it is the first report of *Trypanosoma congolense* infection with amastigotes nests in tissues.

**18.014 Survey of the Seroprevalence of Anaplasmosis, Lyme Disease and West Nile in Horses (France and Sub-Saharan Africa)**


**Background:** Equine anaplasmosis (EA) and Lyme disease (LD) are tick-borne diseases caused respectively by *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *Ixodes ricinus* tick is responsible for the transmission of these two agents in Europe. The infection can be asymptomatic or induce various symptoms. West Nile (WN) infection due to a *Flavivirus* and transmitted by mosquitoes is often inapparent in horses but may cause severe encephalitis. The purpose of our surveys was to assess the seroprevalence of these emerging zoonoses among horses in France and in Africa.

**Methods:** For EA and LD, sera were tested using a rapid test dot-ELISA in solid phase SNAP4Dx® (Idexx, Westbrook, Maine, USA). For WN, an ELISA test was used to detect IgG and positive samples were confirmed using an immunoblotting test. The results were compared using the chi-2 test (p<0.05).

**Results:** For EA and LD, in France, the seroprevalence was respectively 16% and 31% in the middle-west (N=144), 19% and 48% in the east (N=188), 0% and 12% in the south (N=105). The prevalence was equal to zero in Africa (N=113). The comparison between the north of France (middle-west and east groups) and the south shows a statistical difference. For WN, in France, the seroprevalence was equal to zero (N=105). In Africa, the seroprevalence was 97% in Chad (N=30), 92% in Senegal (N=25), 30% in RD Congo (N=20), 28% in Ivory Coast (N=95), 9% in Djibouti (N=11) and 3% in Gabon (N=64).

**Conclusion:** The results of these seroprevalence surveys are correlated with the distribution of the tick vector for EA and LD, and the presence of migratory birds, reservoirs for the WN virus. Horses can be considered as sentinels for the circulation of a number of arthropod-transmitted agents. Equine seroprevalence could be used to assess epidemiological changes among some emerging zoonoses and even help in anticipating them.

**18.015 Rickettsia slovaca in Dermacentor marginatus Ticks Removed from Wild Boars (Sus scrofa) in Emilia Romagna Region, Italy**

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**Background:** Various tick-borne pathogens occur in the Emilia Romagna region (Italy), where Lyme disease, transmitted by *Ixodes ricinus*, is frequently diagnosed in humans. The epidemiology of other diseases vectors by ticks is still to be defined in this area, e.g. the tick-borne lymphadenopathy TIBOLA or DEBONEL caused by *Rickettsia slovaca*. Recently, human cases of TIBOLA have been reported in Italy, but in a different region (Tuscany; Selmi et al. 2008). Abundance of wild boars and ticks of the species *Dermacentor marginatus* in the Emilia Romagna region suggests a high risk for the transmission of the *R. slovaca*. The aim of this study is to determine the prevalence of *R. slovaca* in *D. marginatus* ticks removed from hunted wild boars, in order to evaluate the risk of infection for humans.

**Methods:** Seventy-nine ticks were removed from 18 hunter-killed wild boars from four different areas of Emilia Romagna region during the period September-November 2008. Ticks were identified as *D. marginatus* using standard taxonomic keys. DNA was extracted from ticks using a commercial kit and PCR analysis was applied to determine the presence of *Rickettsia* spp. Different primers sets were employed, targeted for gltA, gyrB, D-antigen genes. Sequencing of the amplification products allowed identification of *R. slovaca* in a subset of the PCR-positive samples.

**Results:** A total of 50 ticks (50/79–63%) yielded the expected bands by PCR amplification. The sequencing of a subset of the positive samples confirmed the presence of *R. slovaca*.

**Conclusion:** Our results shows that *R. slovaca* is present in ticks of the species *D. marginatus* collected on wild boars in Emilia Romagna. Work is in progress to obtain gene sequencing from all of the positive samples, in order to generate a clearer picture of the *Rickettsia*spp circulating through *D. marginatus* ticks. The potential pathogenic role of *R. slovaca* is well documented, and the risk for transmission to humans in Emilia Romagna must be considered.

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**18.016 Occurrence and Characterization of the Tick-Borne Encephalitis Virus in the Wood Ticks *Ixodes ricinus* in North-Eastern Italy**


Tick-borne encephalitis (TBE) is the most serious tick-borne disease currently reported in humans in North-Eastern Italy, leading to long-lasting or permanent neuropsychiatric sequelae and rarely to fatal infection. From 2005 through 2008, we have monitored five provinces in North-Eastern Italy, in order to assess the presence and rates of infection of TBE virus (TBEV) in the wood tick *Ixodes ricinus*, both in previously recognised infected areas and in sites never monitored before.

Ticks were collected by dragging in fixed sites (monitored monthly), and itinerant sites, visited once or twice per year. RNA was extracted from single adults, and from pools of 5/10 nymphs and 10/20 larvae. A real time RT-PCR was applied for TBE RNA detection and positive results were confirmed by a nested RT-PCR and sequencing of the PCR products. Virus isolation by intracerebral inoculation of sucking mice was attempted from positive samples by RT-PCR. Sequence of the gene encoding the envelope glycoprotein was obtained with specific primers and compared to those available in the public database.

Overall, 5831 *Ixodes ricinus* ticks (3185 larvae, 2428 nymphs and 218 adults) collected in 209 sampling were tested for TBEV during the sampling period. Tick density in fixed sites ranged from 2.24 to 70.72 ticks/m2.

TBE virus infection in ticks was found in two provinces in nymphs collected in 2006 (expected rate of infection=0.52%–6.65%, respectively) and in a different province in adults (4/36; 11%) collected in 2007. In the last site, infection was confirmed in nymphs collected in March 2008 (expected rate of infection=0.98%) and the virus was successfully isolated after intracerebral inoculation in sucking mice. As expected, preliminary phylogenetic analysis indicate that the virus currently circulating in North-Eastern Italy belongs to the Western TBEV group, as isolates from neighbouring countries.

Three foci of TBE infection in adult ticks and nymphs in different provinces were found. Despite the fact that TBE infection is reported to be transmitted vertically in ticks, in this study larvae resulted always negative. In all these areas human infection is routinely reported. The variety of tick density and infection rates highlights the need of assessing human risk of TBE exposure on a local basis.
Genetic Variability of Dobrava Hantaviruses Carried by Apodemus Mice in Hungary and Croatia

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Background: Dobrava hantavirus belongs to the genus Hantavirus, family Bunyaviridae and is carried mainly by yellow necked (Apodemus flavicollis) and striped field (Apodemus agrarius) mice. The virus may cause severe hemorrhagic fever with renal syndrome (HFRS) in many European countries.

Objectives: The goal of this study was to detect and genetically characterize Dobrava hantaviruses occurring in the Transdanubian region of Hungary and Northern Croatia.

Methods: Rodents were trapped in five different locations during the summer and autumn seasons of 2005–2007. Small mammals were dissected and lung tissues were used for Dobrava virus detection. The viral RNA was extracted from lung suspensions with TRIzol reagent according to the manufacturer’s recommendation. Dobrava hantaviruses were detected by SYBR Green-based real-time PCR, using newly designed virus specific primers. Positive samples were selected for sequence and phylogenetic analysis.

Results: In the present experiment a total of 125 Apodemus sp. (A. agrarius n=63, A. flavicollis n=62) were tested for the presence of Dobrava hantaviruses. Three (4.8%) A. agrarius and 7 (11.3%) A. flavicollis rodents were RT-PCR positive for Dobrava hantavirus. Phylogenetic and molecular sequence analyses showed, that at least two different genotypes of Dobrava hantaviruses occur in Hungary and Croatia according to the Apodemus host species. Viruses identified in the region were most closely related to those viruses detected in Slovenia.

Conclusion: In this study, we provided comprehensive molecular data describing the occurrence of Dobrava hantaviruses in the Transdanubian region of Hungary as well as in Northern Croatia. Based on our new data from the region we concluded that extended reservoir studies would be necessary in the future.

18.018 Human Dirofilaria repens Infection: Increased Laboratory and Clinical Problem in Serbia

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Background: Dirofilaria repens is a filarial parasite autochthonous in Europe reported with increasing prevalence in the Mediterranean area and Southeast Europe. It is transmitted by mosquitoes and humans are infected by insect which fed previously on an animal with microfilariaemia. The infection usually presents as subcutaneous nodule or ocular disease. Differential diagnoses include neoplasia and other granulomatous diseases. Diagnosis requires surgical removal and parasitological/histological examination. According to earlier observations in Serbia D. repens was found in 3% of examined mosquitoes. In 2006/2007 microfilariae of D. repens were found in 7.2% of 193 privately owned dogs in the Northern part of Serbia. From 1971–2001 nine cases of human dirofilariasis were diagnosed in Serbia.

Methods: Surgically excided nodules, intact worms or fragments from different sites such as skin, eye and scrotal tissue were collected. All samples were observed macroscopically and further diagnosis were made by histology and microscopic study. For some samples PCR was performed.

Results: From 2002–2008, 19 new cases were observed which means that 67% (19/28) of all D. repens human infections reported in Serbia have been diagnosed in the previous seven years. In 58% (11/19) patients parasites were localized under the conjunctiva or in pericircular tissue, although they were considered as conjunctival granuloma or halazion.

18.019 Travellers Returning from Epidemic Regions as Sensitive Sentinels for Detection of Dengue Outbreaks

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Background: Dengue fever is the medically most important Arbovirus infection in the world. An estimated more 50 to 80 million infections occur annually. Areas of endemic occurrence all year around can be distinguished from areas with epidemic occurrence. Diagnosis in epidemic outbreaks is often delayed due to unspecific “flu-like” symptoms of dengue fever which are very common and of various causes in tropical and subtropical countries. However, early detection of virus circulation in a population is essential for rapid or increased efforts of reducing mosquito populations.

Methods: We report two instances, one in a traveller returning from the Maldives Islands, and another traveller returning from New Caledonia who to our knowledge were the first reported cases of dengue fever outbreaks in the respective countries with hundreds of cases following weeks to months after our detection. Both patients were diagnosed by real-time PCR and dengue virus was isolated. PCR amplicons could be sequenced and typing of the causative was done within few days.

Results: The sequences allowed the determination of the geographical origins of the respective dengue virus strains. The Maledives isolate showed highest homology to Indian strains. The South pacific isolate exhibited highest homology to Hawaiian dengue virus strains.

Conclusion: The results show that diagnosis of dengue fever in travellers returning from tropical and subtropical countries is important for several reasons. Beside the personal diagnosis of illness and possible diagnostic and therapeutic impact on individual medical care, the diagnosis and rapid communication of detection of dengue viruses in a starting epidemic situation can provide important information for public health systems and provide valuable data on possible ways of spread for combating the epidemic. In these instances returning travellers may prove to be sensitive sentinels for detection of dengue fever.

Detection of Flavivirus and Phlebovirus in Culicidae Mosquitoes and Phlebotomus in Catalonia (North-Eastern Spain) in 2008

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Background: Arthropod-borne viruses, i.e., arboviruses, are an important concern for human and animal health due to their emergence or resurgence in many regions around the world. These viruses are comprised of different genera including Flavivirus (e.g., West Nile (WN) and Dengue) and Phlebovirus (e.g., Toscana and Rift Valley). Several arbovirus surveillance and vector monitoring systems have been implemented in many European countries. In Catalonia (North-Eastern Spain), a program...
of surveillance for WN virus and a monitoring system for genus Phlebovirus in arthropod vectors have been operating since 2006. In this study, we present the results obtained in these programs during 2008.

**Methods:** The study was carried out from May to November during the period of highest activity of the vectors at high risk locations. The mosquitoes were trapped every two weeks using CDC and light traps. Two RT-nested-PCR followed by sequencing was used to detect Flavivirus and Phlebovirus genomes. The obtained sequences were compared with database sequences. Those pools that were positive for genus Flavivirus were tested later for WN virus specifically by real time RT-PCR. Those pools which were positive for genus Phlebovirus were cultured in Vero cells.

**Results:** A total of 5045 Culicidae mosquitoes were collected and grouped according to species, location and date in 336 pools. In 14 of these pools a band that corresponds to flavivirus amplification was amplified nevertheless none of them were positive for WN virus. The obtained sequences were different to database sequences and most of them were similar to flaviviruses of mosquitoes.

A total of 555 phlebotomines were collected and grouped in 66 pools from which 3 positive pools for phlebovirus amplification were obtained. After that, all 3 positive were isolated in one passage in Vero cells, obtaining approximately 6 log TCD50/mL. Isolated phleboviruses were related phylogenetically with Massilia virus detected in France in 2005, Arbia virus detected in Algeria in 2006 and Toscana virus.

**Conclusion:** According to the results, the detection of new flaviviruses, whose importance linked to public and animal health remains unknown, implies that there is a need for further research in this field. In the same way, in reference to the results of phleboviruses, the fact that the detected phleboviruses were easily isolated in Vero cells could be an indicator of their capacity for infecting vertebrates including humans.

**ABSTRACTS**

18.021 Dengue Infections Diagnosed at Bulovka University Hospital in Prague during 2004–2008

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**Background:** Dengue fever is a common febrile illness in travellers returning from tropical countries. In TropNetEurope database there were 1273 patients registered with acute dengue infection during 1999–2007, but there is a lack of detailed information on the number of patients and on clinical and epidemiological features of dengue fever among travellers in the countries of Central and Eastern Europe region. In the Czech Republic there were reported 46 cases of dengue virus infection in the period from January 2000 to November 2008 in the national surveillance system of infectious diseases (EPIDAT) but this number probably does not represent all cases.

**Methods:** This retrospective study collects information on travel destination, clinical and laboratory features of patients with acute dengue fever diagnosed at Bulovka University Hospital, which is a tertiary care centre specialized in tropical medicine. Acute dengue infection was confirmed by serological methods based on positivity of anti-Dengue IgM and seroconversion of anti-Dengue IgG antibodies.

**Results:** Medical records of 14 patients (6 women and 8 men; aged from 17 to 56) with confirmed acute dengue fever were investigated in the period from December 2004 to November 2008. 10 patients were hospitalized, but only one (21-year-old woman) had a severe clinical course and acquired ICU stay for 22 days. 10 patients were hospitalized and 4 patients were observed as out-patients. All cases were imported from the region of South-East Asia, except for 3 cases that were acquired on Indian subcontinent or Sri Lanka. Fever was a predominant symptom in all patients, lasting from 2 to 11 days. Rash and cephalea were both observed in 8 cases, followed by arthralgia in 6 and myalgia in 5 cases. Laboratory findings were leukocytopenia (range 0.9–5.6; median 3.0 x10^9/l), thrombocytopenia (range 43–298; median 125 x10^9/l) and moderate elevation of transaminases: AST (range 0.43-8.89; median 1.27 µkat/l), ALT (range 0.6–6.17; median 1.49 µkat/l), CRP ranged from 0.7 to 26 (median 4 mg/l).

**Conclusion:** Infections caused by dengue virus range among the most emerging in tropical countries. Due to increasing risk of import of these infections to non-endemic countries, it is necessary to improve the diagnostic approach to febrile patients returning from tropical countries.
West Nile Virus Surveillance in Sicily (Italy)

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Background: West Nile Disease (WND) is considered an emerging zoonosis caused by a Flavivirus. In Italy WND first appeared in Tuscany in 1998. On October 2008, a new case of disease, including horses and one women, was registered in Emilia-Romagna. In 2002, Italian Public Health Department established a surveillance programme of the disease. The control regards geographic areas at the risk of introduction of WN virus. The programme is developed from spring to autumn, by serological surveillance of horses and “sentry” poultries, entomological surveillance and study of mortality in wild birds. In Sicily, the control of WND is developed in a natural reserve, including some coastal ponds, in the south east of the island. In this study, the A.A. have showed the activity of surveillance of WND in Sicily since 2004.

Methods: For serological survey 20 poultries were tested once a month and the horses were checked twice, in spring and in autumn, to verify seroconversions. For adult mosquitoes catching, CDC traps and electric and manual aspirator were used. Also, mosquitoes larvae were collected. The CDC traps were located in proximity of poultries. The larvae were caught at marsh and puddle. Sera and blood from poultries and horses, tissues (kidney, hearth and brain) of dead wild birds and mosquitoes were performed by National Reference Center (CESME).

Results: Since 2004 to 2008, were tested 302 samples of poultries and 530 samples from horses by serological test. WN virus was investigated by PCR in blood samples of 349 horses and 248 poultries and in 376 wild birds. In 2007, one horse was IgG seropositive but not viremic; in 2008, the IgG seropositive horses were two. In 2008, others Flaviviridae were found in 26 tissues of wild birds samples. The most of the mosquitoes caught belong to Ochlerotatus detritus, Ochlerotatus zammitii and Ochlerotatus caspius. Small presence of Anopheles maculipennis, Culiseta sp and Culex sp was detected.

Conclusion: The recent case of WND in the north of Italy, the seropositive found in horses in Sicily and the caught of mosquitoes involved in the WN virus transmission, show that the disease can be considered an emerging issues of public health. Moreover, the surveillance system for WNV can be used for the control of others Flaviviridae responsible of encephalomyelitis in human and animals. The obtained data are relevant epidemiological findings since they demonstrate the presence of the host, the virus and the competent vector in a full-risk area.

Chikungunya Virus (CHIKV) Infection: Analytical Performance of A Real-time PCR Assay Suitable for Blood Screening

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Background: Chikungunya virus (CHIKV), an alphavirus belonging to the Togaviridae family, is transmitted by human by several species of mosquitoes, with Aedes Aegypti and A. Albopictus being the two main vectors. The virus is endemic in Africa, India, South-East Asia and recently in southern-Europe and is responsible for an acute infection of abrupt onset characterized by high fever, asthenia, headache, rash, myalgia and a painful polyarthalgia. Occurrence of CHIKV asymptomatic infections, whose epidemiological consistency is still to be assessed, leaves hypothesis of spread of infection by blood transfusion or by tissue or organ transplant and highlights the need for highly sensitive CHIKV-specific tests. Objectives of this study is to develop and analyze the analytical performance of a real-time PCR assay suitable for blood screening and the opportunity to operate on mini-pools.

Methods: The analytical sensitivity of the assay was validated with a panel of blood donor plasma samples spiked with 10-fold serial dilutions of CHIKV, previously quantified by TCID50 assay. 10 replicates for each viral concentration were analyzed on single units and on diluted samples consistent with mini-pool (MP) of 3 and 5 units. Following RNA extraction, all samples were amplified by real-time PCR. For each virus concentration, the detection rate (n.positives/n.total) and the limit of detection (LOD95%) was evaluated.

Results: Analytical sensitivity (LOD95%) of the real-time PCR assay, when performed on undiluted samples, is 36 TCID50/mL with an amplification rate 100%for virus concentrations >50 TCID50/mL. For MP of Sunitis: LOD95%: 230 TCID50/mL; amplification rate 100%: >200 TCID50/mL for MP of 5 units: LOD95%: 340 TCID50/mL; amplification rate 100%: >2000 TCID50/mL.
Conclusions: The results of this study document a good analytical sen-
sitivity of the assay, when applied on undiluted samples and, thus suit-
able for blood screening during epidemics. The analytical sensitivity was
substantially lower when the assay was performed on mini-pool suggest-
ing that pooling might be used only for surveillance.

Assessing the UK Public Health Risks from Vector- and Rodent-Borne Zoonoses

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Background: Arthropod-transmitted infections continue to cause large-scale outbreaks of human disease. Climate change, increased global
tourism and changing agricultural practices may also impact upon poten-
tial vectors and the dynamics of infections. Understanding the ecology of
vector-borne and wildlife zoonoses, and the influence of environmental
factors upon them, can help predict the incidence of vector- and rodent-
borne human disease in the UK and help target surveillance and mitiga-
tion and develop appropriate public health strategies to prevent or reduce
the incidence of human infection.

Methods: A variety of approaches may be used to investigate driving
forces governing vector-borne disease (VBD). Nationwide data from vol-
untary recording schemes, supplemented and refined by targeted field
studies, can identify vector distributions and identify what factors influence
infectiousness and abundance. Geographical Information Systems (GIS) and math-
ematical modelling can use environmental and ecological data to inter-
pret disease cycles and aid the assessment of risk to the UK.

Results: MEZE have used these various approaches to: develop GIS-
based climate models that simulate extrinsic incubation of pathogens in
vectors (e.g. Dirofilaria, Malaria); predict and model establishment potent-
tial and seasonal activities of exotic disease vectors in the UK (e.g. A.
albopictus); develop high resolution risk mapping of disease vectors within
tick endemic risk areas and incorporate the influence of climate and tree
mast on the rodent host of Puemula (PUUV) hantavirus.

Conclusion: Wildlife and vector-borne zoonoses occur in nature and
therefore by taking an ecological approach to risk assessment and trying
to understand the dynamics of enzootic cycles, our ability to understand
the occurrence of human zoonotic diseases and their outbreaks is greatly enhanced.

Rising Seroprevalence of Antibodies against Vector- and Rodent-Borne Infections in Forestry Workers in Germany

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Background: In South-Germany, forestry workers have an occupational risk of acquiring vector- and rodent-borne infections, such as Puemula virus (PUUV) infection, borreliosis, tularemia and tick-borne encephali-
tis (TBE). The objectives of our study were to measure the antibody sero-
prevalence of forestry workers against vector- and rodent-borne infections, compare the data with the general population and to examine
whether their risk of infection has increased in the last decade.

Methods: We conducted a seroprevalence study with forestry workers from four administrative districts of Baden-Württemberg in 2007 and 2008. The workers were interviewed, e.g. about vaccination and exposure, and serum samples were taken. Antibodies against PUUV, Borreli a burgdor-
feri, Francisella tularesis and TBE-Virus were measured. The calculated seroprevalences were then compared with seroprevalence data from the
general population (literature) and with seroprevalences of forestry work-
ers from the same districts in 1997–1999, which were obtained using the
same tests and conducted at the same laboratory.

Results: 221 forestry workers participated (mean age 44 years, 98% males). Sixty-nine percent reported more than ten tick bites. The measured
seroprevalence for PUUV was 15%, for B. burgdorferi 41%, for Francisella
tularesis 5% and for TBE-Virus 29% (in non-vaccinated participants). TBE-Virus vaccination coverage was 73%. The detected seroprevalences were 5-to-8-times higher against PUUV and 20-times higher against Fran-
cisella tularesis as in the general population. Compared to 1997–1999 the current seroprevalences against PUUV were 6-times higher, against B. burgdorferi 1.3-times higher and against TBE-Virus 5-times higher.

Conclusion: Our data confirm that forestry workers are at higher risk
for vector- and rodent-borne infections than the general population. Espe-
cially the high seroprevalence of tularemia was unexpected but is well in
line with recent observations of a re-emergence of this zoonosis in Ger-
many. Our investigations further demonstrate that the risk of vector- and
rodent-borne infections increased over the last decade for forestry workers
in Baden-Württemberg. This increase in a highly exposed population
shows the growing importance of these infections in endemic regions.
Therefore, forestry workers and other risk groups should be informed about
their risk and preventive measures such as prevention of tick bites, TBE-
Virus vaccination and respiratory protection during high risk activities

Surveillance of Dengue Hemorrhagic During First Epidemic in Punjab Pakistan

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Background: An estimated 2.5 billion people are at risk from dengue infection globally, dengue viruses are one of the most important arthro-
pod-borne viruses transmitted to humans, whether measured in terms of
the number of infections or deaths. Dengue haemorrhagic fever (DHF) is
an acute viral disease caused by four serotypes of Dengue virus which are
immunologically distinct and does not provide mutual cross protection. In
Pakistan first confirmed epidemic appeared in Karachi during 1994 and
disease remerge in 2006 again with few hundred cases in the same city. But
only few cases and 1 death in Lahore only while no death in the year 2007
with only 57 cases. But in 2008 from mid of October a sudden rise in DHF
cases happened hence a province level DHF surveillance was conducted.

Methods: Prospective disease surveillance was done in all the Public,
private district hospitals, diagnostic laboratories, and data regarding DHF
positive cases was collected from all 35 districts of Punjab Pakistan.

Results: From mid of August 2008, since the notification of first DHF
positive case in Lahore there were found that 47.75% (n=16) districts have
positive cases ranging from 1 to 1149 in number and 54.28% (n=19) have
not any positive case. A total of 2497 patients were found positive out of
which 35.88% (n=896) taken admission in hospitals while 64.11% (n=1601)
did not have admission in the hospitals. Only 1.88% (n=17) case
fatality rate out of admitted patients were observed. Highest number of
cases were found in Lahore district 91.83% (n=2293) while lowest in 0.20%
(n=5) in Multan district. Case fatality rate of Rawalpindi district was
32.35% (n=11) out of positive cases as compare to Lahore with 0.73%
(n=6).

Conclusion: During the years 2008 DHF epidemic in Punjab, the high
positivity rate of DHF in Lahore having a geographical resemblance with
other areas of Punjab where a very few who cases were reported may have
involve an act of bioterrorism.Moreover there is need of improvement in
the early disease warning system (EDWS) and surveillance system.

Entomological Survey of Aedes albopictus (Diptera: Culicidae) in Agrigento Province, Sicily

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Background: Ae. albopictus is a competent vector of many viruses dis-
eases including Dengue fever, Eastern equine encephalitis virus and
Chikungunya virus. In 2007 an outbreak of Chikungunya occurred in Emilia Romagna region, for this reason an Entomological Survey was activated by the Health Prevention Offices of many Italian provinces.

Methods: during 2007 and 2008 was carried out an entomological survey to evaluate the distribution and the abundance of Ae. albopictus, in details: from September to October 2007 (4 weeks) and from June to September 2008 (13 weeks). The survey was carried out by ovitraps, placed on the territory depending on people density. The ovitrap is made by a Black plastic flower pots. A strip of Masonite® is suspended vertically in the middle of the pots to provide a suitable surface for oviposition. Pots were filled with 350 ml of water. Every week, pots were rinsed and refilled and strips were changed and checked for egg presence. Eggs were counted by observing the strips under a dissecting microscope.

To evaluate the distribution and the abundance of the species in the study area, two parameters were considered: the number of positive ovitraps/total ovitraps operating, and the average number of eggs in the total ovitraps operating. Positive strip were subdivided in classes in according with the eggs number C0=0 eggs; C1= 1–10 eggs; CII= 11–50; CIII=51–100; CIV=101–300; CV=301–600; CV=601–<=700.

Results: On 2007 at 35 towns observed only 7 resulted positive, 121 ovitraps was placed and 17 resulted positive for the presence of Ae.albopictus. Only 4 ovitraps belongs to the classes CIV the most ovitraps belonged to CII and CIII classes.

On 2008 at 39 towns observed only 20 resulted positive, 170 ovitraps was placed and 69 resulted positive for the presence of Ae.albopictus. Only 5 ovitraps belonged to the classes CV and CIV, 18 ovitraps belonged to CIV class but the most ovitraps belonged to CII and CIII classes.

Conclusion: These results show a spotted distribution of Ae. albopictus at Agrigento province. However, comparing the same period in the two years studied, a biggest diffusion of mosquitoes was noted during 2008 rather than 2007 and the number of eggs is higher on 2008 than 2007. These results as a whole suggest that Ae.albopictus rather than 2007 and thenumber of eggs is higher on 2008 than 2007. However, comparing the same period in the two years studied, a biggest diffusion of mosquitoes was not ed during 2008 at Agrigento province. Howver, comparing the same period in the two years studied, a biggest diffusion of mosquitoes was not ed during 2008.
A Fatal Mediterranean Spotted Fever Case in Greece

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Background: Mediterranean Spotted Fever (MSF) is a tick-borne disease caused by Rickettsia conorii, which currently includes R. conorii strain Malish 7, and R. conorii subspecies caspia, canorii, indica, and israelensis. MSF is usually a mild disease with mortality rate 1-3%. During the recent years, however, severe MSF cases have been reported. We present a fatal MSF case in Greece, resembling severe hemorrhagic fever. The case was observed in the same region (Rhodope prefecture) where the first Greek Crimean-Congo Hemorrhagic Fever (CCHF) case was confirmed, with 45 days interval.

Case Report: In August 2008, a 62-year-old previously healthy woman was admitted to University Hospital of Alexandroupolis, because of a 1-week febrile syndrome, accompanied by stiff neck, stupor, and acute renal insufficiency; she presented hematomas in mucus membranes and in sites of injection. Main laboratory findings were thrombocytopenia (50,000/mm3), elevated aPTT and extremely high levels of serum transaminases. She didn’t report tick bite; she was working in the field. Twenty-six hours after her transfer to ICU (9th day from the onset of the disease), she died with multiple organ failure. As CCHF was included in the differential diagnosis, preventive measures for probable nosocomial or family transmission were taken.

Results: A serum sample taken from the patient upon admission was tested by serologic and molecular methods for Crimean-Congo Hemorrhagic Fever (initially), hemorrhagic fever with renal syndrome, leptospirosis, and rickettsiosis. Serology was negative, but PCRs for rickettsia (17-kDa antigen, OmpA) were found positive. Sequencing of the PCR products revealed that the causative strain was R. conorii, with 100% identity with R. conorii strain Malish.

Conclusion: Two fatal cases with similar epidemiological characteristics, clinical symptoms and laboratory findings were observed in summer 2008, in Rhodope prefecture, Greece: one caused by CCHF virus and a second caused by Rickettsia conorii. As MSF is becoming a severe disease, it has to be included in differential diagnosis of febrile syndromes with thrombocytopenia.

Uptregulation of a Novel eIF5A in C6/36 Cells is Associated with Cell Survival During Dengue 2 Viral Infection

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Dengue (Den) viruses generally induces apoptosis in mammalian cells, but frequently result in persistent infection in mosquito cells. Through PCR-select cDNA subtraction, eukaryotic translation initiation factor 5A (eIF5A) was identified to be upregulated in C6/36 cells with Den-2 virus infection. eIF5A is the only known protein in nature that contains hypusine, an unusual amino acid derived from the modification of lysine by spermidine. The full length of eIF5A derived from Aedes albopictus consisted of 1488 bp of nucleotides with a 41.39% G+C content, and possessed a higher similarity and shorter evolutionary distance with insects than with other organisms. It has been reported that eIF5A plays a role in cell proliferation, cell viability, and cell cycle progression. In this study, cell death increase and cell cycle transition from the G1 to the G2 phase occurred when eIF5A activity was inhibited in C6/36 cells. Synthesis of viral RNA and protein was not evidently affected in infected C6/36 cells in the absence of eIF5A activity. It is postulated that eIF5A plays a role in preventing the death of mosquito cells in response to Den-2 viral infection, eventually supporting continued viral growth of persistent infection in mosquito cells. In conclusion, eIF5A is not responsible for regulating replication of the Den-2 virus in C6/36 cells. However, it regulates the G1/S/G2 transition and allows virus-infected C6/36 cells to survive, helping mosquitoes be efficient vectors of viral transmission in nature.

Introduction, Establishment and Present Status of Aedes albopictus in Albania

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Background: In 1979, Adhami firstly identified as Ae. albopictus, adult mosquitoes collected around a large pile of used tires in Albania. Following the first finding after several years other entomological surveys were carried out to identify the spread and establishment of Aedes albopictus in the country.

Methods: The past and the present distribution of Aedes albopictus in Albania is analyzed through previous published papers and entomological surveys carried out in 2001 and 2006. Hundred specimens of larvae and adults have been collected in several districts by using the standard equipment: for larvae oviposition traps and Fay-Prince traps for adult surveillance.

Results: Ae. albopictus has been recorded in 6 towns during 1979, being present also in a dump for discarded tires, 2.5 km from the nearest human settlements. The most northern-infested town was Shkodero. The species was particularly common in the gardens of suburban home tires. During summer 2001 an entomological survey was carried out in some sites where the species was previously reported. The presence of Ae. albopictus was confirmed this time inside buildings in Tirana as well as outside in the pine beach of Durreses. The species was also found in households surrounded by water containers and gardens in the south of Albania. In 2006 another entomological survey was undertaken confirming that Ae. albopictus is spread and established in 20 out 36 districts of Albania. In the Northern Albania, near the border with Montenegro, the density was very high.

Conclusions: It could be concluded that now this species is common not only in the used tires but it is spread inside of the buildings, houses and in the gardens. The species is very well established all over the country and especially in the costal plain. The widespread of Ae.albopictus in Albanian is a serious concern for public health.

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E. Velo, E. Rogozi, S. Bino. Institute of Public Health, Tirana, Albania

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Conclusions: It could be concluded that now this species is common not only in the used tires but it is spread inside of the buildings, houses and in the gardens. The species is very well established all over the country and especially in the costal plain. The widespread of *Ae. albopictus* in Albanian is a serious concern for public health.

**18.037** Surveillance for Dengue Fever in Eastern Kolkata, West Bengal, India: Preliminary Results

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**Background:** Dengue is reported from most states in India and a significant proportion of nationally reported cases come from West Bengal. However, most reported cases lack laboratory confirmation and the degree of underreporting is not known, making it difficult to estimate true disease incidence.

**Methods:** To determine the incidence and burden attributable to dengue, and the epidemiologic, clinical and virologic characteristics of severe dengue, health care facility based, enhanced sentinel surveillance for febrile illness has been established among a population of 22,199 persons living in an urban slum area of Kolkata, starting August 2008. Among all identified febrile persons, a sample of 2,000 will be enrolled for collection of paired acute and convalescent serum samples for laboratory testing following informed consent. IgM anti-DENV was determined by microplate ELISA (IgM ELISA kit for dengue, National Institute of Virology, Pune and/or Dengue IgM capture ELISA, PANBIO Diagnostics) and DENV detection and molecular typing were done by RT-PCR.

**Results:** During the first 17 weeks of the study, a total of 1333 persons were identified with a febrile illness. Of them, 1306 with less than 7 days of onset of fever were enrolled in the follow-up study: 1058 (79.7%) outpatients and 270 (20.3%) in-patients. 37.1% of enrolled persons were less than 15 years of age. 1286 serum specimens were tested for dengue and 157 (12.2%) were positive by IgM anti-DENV by either test kit. Molecular typing showed that all four types of DENV (DENV1-28, DENV2-27, DENV3-03, and DENV4-16) were present. RTPCR tests were performed, but no data presented here.

**Conclusion:** Available data suggests that dengue is a major cause of febrile illness in Kolkata. Data from this study will be important in decision making for future trials of candidate dengue vaccines in a country with the potential for dengue vaccine manufacturing capacity.

**18.038** Dengue in Thailand and Cambodia: An Assessment of the Degree of Underestimated Disease Burden based on Reported Cases


**Background:** Disease burden data are needed to guide decision making for public health interventions. Although dengue is a reportable disease in Thailand and Cambodia, the degree that reported incidence underestimates true disease burden is not known. We utilized dengue incidence calculated from laboratory confirmed outpatient and hospitalized cases in prospective cohort studies to estimate the magnitude of dengue under detection and reporting, and establish more accurate disease burden estimates for these countries.

**Methods:** Cohort studies were conducted by members of a dengue field site consortium over at least 2 dengue seasons between 2004 and 2007 among children aged less than 20 years. Age-specific multiplication factors were computed by comparing data from three cohort studies to national surveillance data in the same province and year. These multiplication factors were used to estimate age-group specific disease burden at the country-level from reported surveillance data.

**Results:** In Thailand, 14,627 person-years of prospective data were obtained in two provinces and 16,661 person-years were obtained from one province in Cambodia. Average annual incidence of laboratory-confirmed dengue was 2.3% and 2.5% in Thailand, and 3.3% in Cambodia. Calculated multiplication factors varied by age-group and year (range 0.4-29). Applying average age-group specific multiplication factors to country-level surveillance data indicated that in Thailand a median 405,773 (range 343,676-576,371) dengue cases occurred annually during 2003-2007 and a median 113,382 (range 82,453-383,525) cases occurred in Cambodia. Average underestimation of total and inpatient dengue cases was 8.7 and 2.6-fold in Thailand, and 9.6 and 1.4-fold in Cambodia, respectively. During the high-incidence year 2007, more than 143,000 patients in Thailand and more than 58,000 patients in Cambodia were hospitalized due to dengue.

**Conclusion:** Calculating multiplication factors by comparing prospective cohort study data to locally-reported national surveillance data is one approach to more accurately assess disease burden. These data indicate that although dengue is regularly reported in many countries, it significantly underestimates true burden of this disease.

**18.039** Monitoring of *Aedes albopictus* by Ovitraps in Marche Region (Italy): 2008 Seasonal Data Report


After the autochthonous Chikungunya virus (ChkV) outbreak spread in Emilia Romagna region in summer 2007, Regional Public Health Department and Istituto Zooprofilattico Sperimentale dell’Umbria e delle Marche have improved a surveillance scheme for monitoring the tiger mosquito *Aedes albopictus* seasonal activity and for early detection of human cases of ChkV and Dengue.

No humans cases are detected in 2008 but an heavy infestation is recorded weekly from June to October 2008 by the 330 o-ov-traps installed in 31 municipalities. Complete data of monitoring is presented and preliminary results are discussed; main values in o-ov-position are founded in the urbanised areas with up to 400 inhabitants/km2 (Kruskal-Wallis H =4,1671; P=0,0412), whereas in the municipalities up to 200 meters in altitude the means of eggs for traps recovered decreased significantly (Kruskal-Wallis: 91,0021; P: 0.0000).

These results could be used for a risk-based surveillance scheme for vector-born diseases.

**18.040** Interaction of Tick-Borne Encephalitis Virus Strains with Plasmacytoid Dendritic Cells and T-cells

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**Background:** Tick-borne encephalitis virus (TBEV) is the most prevalent arbovirus in Europe and in Germany. Clinical symptoms range from mild to severe neurological disorders. The pathogenesis of TBEV is still not completely understood and mechanisms of virus host response interaction remains to be discovered. Plasmacytoid dendritic cells (pDCs) are a key component of the innate antiviral response and play an important role in bridging innate and adaptive immunity, we analyzed the interaction of TBEV with human pDCs and T-cells using the TBEV-strains Absettarov, Neudorfer and Hypr71 as well as Langat virus.

**Methods:** pDC and T cells were infected with different TBEV strains. Cell surface marker expression was characterized by FACs analysis and cytokine expression profiles were measured using CBA and ELISA.

**Results:** None of the strains was able to enhance the expression of mat
urination markers and co-stimulatory markers in pDCs as monitored by FACS analysis, likewise expression of antigen-presenting molecules MHCI and MHCI owed unchanged. On the other hand, interferon-

ELISAs and cytometric bead arrays revealed that TBEV induced a strong interferon-alpha (IFN-alpha) and proinflammatory cytokine response, although pDCs did not support viral replication. However, virus replication was not required, since UV-inactivated virus preparations were also effective in initiating the production of antiviral and proinflammatory cytokines. Interestingly, more virulent strains (e.g. Bypr71) induced a stronger response compared to attenuated strains (e.g. Absettarov). In contrast to pDCs, T-cells supported the replication of all investigated TBEV strains. Furthermore replication-competent TBEV was more effective in the upregulation of CD69 expression (i.e. T-cell activation) compared to UV-inactivated virus.

Conclusion: Our data suggest that pDCs mount an adequate innate antiviral and proinflammatory response to TBEV infection and that this response does not require TBEV replication in these cells. T-cells however are productively infected and might therefore play a crucial role in the dissemination of the virus in infected individuals. Further experiments will be carried out to investigate whether TBEV-infected T-cells are (i) functionally impaired and (ii) might be involved in the spread of TBEV across the blood-brain-barrier.

18.041 Evaluation of Relationship Between the Hyaluronic Acid, Svcam-1, Sicam-1 and Vegf-A Serum Levels and Severity of Illness in CCHF

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Background: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne viral hemorrhagic disease. Pathogenesis of the disease has not been well described yet. A common pathogenic feature of CCHF virus is its capability to damage endothelium. Hyaluronic acid (HA) is an extracellular matrix polysaccharide, which is metabolized by liver sinusoidal endothelial cells. Increased HA levels indicate liver sinusoidal endothelial damage. sICAM-1, sVCAM-1 and VEGF-A play a role in the inflammatory process, vascular damage and plasma leakage. The aim of this study was to evaluate the relationship between levels of sICAM-1, sVCAM-1 and VEGF-A serum levels and severity of illness in CCHF.

Methods: Between 2007 April and 2008 September, 65 patients which were confirmed by RT-PCR and serological tests for CCHF, included in the current study. Plasma samples were collected from 61 patients in acute phase and 43 patients in convalescent phase. The disease course was defined as follows; first seven days after onset of the disease was acute and the after tenth day of the illness was convalescent phase. Patients were divided into two groups according to Swanepoel criteria on admission day. HA, sICAM-1, SVCA-1, VEGF-A levels in serum samples were analyzed by ELISA. Comparisons between groups were examined by independent-samples t-test.

Results: Thirty five (54%) of the patients were male while 30 (46%) of them were female. Eight (12%) of the patients died. According to Swanepoel criteria, 42 (65%) of the patients were severe and 23 (35%) of them were mild. Acute phase of HA, sICAM-1, sVCAM-1, VEGF-A scores were compared for survivors and non-survivors. There were statistically significant differences in HA, sICAM-1, sVCAM-1, VEGF-A scores compared for survivors and non-survivors. There were no significant differences in sVCAM-1 scores for survivors and non-survivors. Acute phase of HA, sICAM-1, sVCAM-1, VEGF-A scores were compared for severe and mild cases according to Swanepoel criteria. There were statistically significant differences in HA, sICAM-1, sVCAM-1, VEGF-A scores while there was no significant difference in sVCAM-1 scores for severe and mild cases. There were no significant differences between male and female for conducted analyses.

Conclusion: HA, sICAM-1, VEGF-A levels were found significantly increased in severe cases compared to mild cases. It was thought that these results may be used as an indicator of disease severity.

18.042 The 2008 West Nile Disease Outbreak in Italy: Entomological Investigation and Virus Detection in Mosquitoes

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Background: In 1998 West Nile Disease (WND) has been reported for the first time in Italy. The outbreak occurred in central Italy affecting 14 race horses. Cx. pipienns and Cx. impudicus were the most abundant potential vectors collected in this area. After ten years, in August 2008, a West Nile virus (WNV) epidemic is occurring in the north east of Italy involving humans, birds and horses. Following the first case, in order to identify the species of mosquitoes involved in the virus transmission, an exhaustive entomological survey has been implemented in the infected stables. This study reports the preliminary results of the survey.

Methods: The mosquitoes collections were performed between 12th September and 16th October 2008, in affected farms of Ferrara and Bologna provinces. The insects were collected by CO2-baited CDC miniature light-traps, bird-baited traps, CDC gravid traps and mouth aspirator. Each specimen was identified at specific level by morphological observation and 152 pools of female mosquitoes were examined by both PCR and virus isolation.

Results: A total of 1725 mosquitoes were caught in 92 collections. The most abundant species resulted Ochlerotatus caspius (1263, 73,2%), followed by Culex pipiens (429, 24,8%). WNV RNA was detected in 3 pools of Culex pipiens and 4 pools of Ochlerotatus caspius: 1 pool of Culex piperiens was collected in the province of Bologna, the rest of the positive pools were collected in the province of Ferrara.

Conclusion: The dominance of Culex piperiens and Ochlerotatus caspius nearby the affected animals might suggest an important role played by these species in transmitting the infection from viremic birds to horses. Although detecting RNA virus is not always equal to transmission capability, the fact that in several pools of these species WNV was detected, still strongly support their vector role assumption in this WND outbreak.

18.043 A Critical Reappraisal of the A226V Mutation in Chikungunya Outbreaks: Possible Role in Increased Pathogenesis?


Background: Chikungunya virus (CHIKV) is a mosquito-transmitted alphavirus diffused in Africa, Asia, Indian Ocean Islands, India, responsible for several imported case in Southern Europe, giving rise, in 2007, to the first autochthonous European outbreak in the Emilia-Romagna Region of Italy. Several mutations of E1 glycoprotein are considered as molecular signatures of the Italian Ocean outbreak, particularly the A226V mutation. We have previously analysed 7 CHIKV isolates, 5 imported to Italy and 2 coming from the Italian outbreak, with respect to the presence of A226 mutation. All imported and autochthonous strains showed the A226V mutation with the exception of the isolate imported from India in 2008, suggesting that the acquisition and fixation of the A226V mutation may be a common pathway of CHIKV outbreak explosion, in a parallel interplay with the mosquito vector dynamics. Since this mutation has been associated with enhanced replication and fitness in A. albopictus vector, we investigated the possible involvement of A226 mutation in enhanced infection capability in primate cells.

Methods: Vero E6 and A. albopictus mosquito cells (C6/36) were infected with two CHIKV isolates, one carrying the A226V mutation and one with wild type aminoacid, using single replication cycle conditions, i.e. Multiplicity of Infection (MOI) of 10. After 3, 6, 24 and 48 hours of infection, progeny virus was harvested and measured by both quantitative real time RT-PCR and viral infectivity assay.
**Abstracts**

**Results:** Under single replication cycle conditions the virus yield was about 10 times higher in mosquito cells as compared to primate cells. No significant differences were observed between the two isolates either in terms of replication kinetic or in virus yield, on both Vero E6 and C6/36 cells.

**Conclusion:** The results suggest that the A226V mutation does not influence replication ability in both host species, when using single replication cycle conditions. Other factors, such as increased ability to spread in cell culture, under conditions more similar to the natural infection, i.e. at low MOI, as well as decreased sensitivity to innate immune mechanisms, could account for the explosive diffusion of mutated strains in the establishment of novel human outbreaks.

**18.044** Trends in Rift Valley Fever Outbreaks in Kenya

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Rift Valley Fever is a viral disease transmitted to humans and livestock by mosquitoes of Aedes spp. The outbreak is closely associated with abnormal high prolonged rainfall and floods in Kenya. The first outbreak was positively reported as a specific viral disease in 1931. Epidemic outbreaks of the disease have continued to be reported after a cycle of every 10 to 15 years. Recent trends tend to show that the inter-epidemic period is becoming shorter with instances where the outbreaks have been reported after (two)2 to (five)5 years. It is also emerging that in some areas such as highlands, the disease could be becoming endemic. Climatic factors such as precipitation and temperatures are thought to play a significant role in this new trend.

**18.045** In Vitro Effects of Antimicrobial Drugs on Motility of *Brugia malayi* Microfilariae and *Wolbachia* Susceptibility Determined by Quantitative Polymerase Chain Reaction

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**Background:** Lymphatic filariasis, a mosquito-borne disease, affects approximately 120 million people in the tropical and subtropical regions. In Thailand, this disease is mainly caused by *Wuchereria bancrofti* and *Brugia malayi*. Fertile adult female worms, residing in lymphatic vessels, can release an abundance of offspring microfilariae into host circulatory system. Microfilaria stage is the important transmission key. Transmission-blocking agents, such as ivermectin, diethylcarbamazine, albendazole, as well as antimicrobial drugs (e.g. doxycycline and rifampicin) have been used to reduce microfilaria density in human patients and animal reservoir hosts. The antimicrobial drugs have effects on *Wolbachia*, a mutualistic endosymbiont, responsible for the filarial nematode embryonic and larval growth and development, adult female fertility, and survival. However, the minimum effective concentration (MEC) and minimum inhibitory concentration (MIC) of the antimicrobial drugs are needed to study.

**Methods:** The MECs of the antimicrobial drugs (e.g. doxycycline, ciprofloxacin, rifampicin) on *B. malayi* microfilaria motility were evaluated. The MICs were determined by the *Wolbachia*/nematode ratio after antibiotic exposure using the quantitative polymerase chain reaction (qPCR) technique.

**Results:** The MEC results demonstrated that doxycycline at the concentration of 256 milligram/litre (mg/L) inhibited the microfilaria motility at 12 hour after antibiotic exposure. Ciprofloxacin and rifampicin were less effective, with the MECs of 512 mg/mL at 94 hr and 111 hr, respectively. Using qPCR to detect the *Wolbachia*/nematode ratio at 12 hr after antibiotic exposure, we showed that doxycycline and rifampicin were the most effective antimicrobial drugs with MIC of 0.25 ml/L. Ciprofloxacin was less effective with MIC of 1 mg/L.

**Conclusion:** This study showed that doxycycline was the most effective antibiotic for the treatment of *B. malayi* microfilariae. The *Wolbachia* nematode ratio could be used to evaluate the anti-*Wolbachia* agents for in vitro study and follow-up lymphatic filariasis patients after treatment.

**18.046** Development of a Double Recognizing Direct Immunoassay for Detection of BTV’s VP7 Specific Antibodies in Sera from Different Animal Species

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**Background:** Current situation of BT in the EU has considerably changed in recent times with incursions in an area of the Community where outbreaks have never been reported before and which was not considered at risk of bluetongue. Due to the necessity of detecting BTV presence at very early states, INGENASA has developed a precocious, sensitive and highly specific ELISA able to detect antibodies specific of VP7 of all 24 serotypes in sheep, goat, cattle and camels.

**Methods:** The assay (1), based on the double recognising ELISA technique, uses BT VP7 recombinant protein both as HRPO-conjugate and as antigen.

**Results:** Differences were used for evaluation: Reference sera specific of the 24 BTV serotypes from different Reference Laboratories; 758 sera from BTV free herds; 288 sera characterized as positive by other techniques; sera from BTV serotype 8 experimentally infected cattle and sheep; sera from BTV serotype 4 experimentally infected cattle and sheep; 254 sera from vaccinated and non vaccinated alpacas and llamas.

**Conclusion:** All Reference Sera Specific of the 24 BTV serotypes show to be positive. 757 of the 758 sera studied from free herds were negative. 100% of confirmed positive sera studied were positive. Sera from experimentally infected animals showed to be positive at day 5-6 p.i. for sheep and at day 7 p.i. for cattle. New world camelid’s samples showed a correspondence of 99.6% with the expected results.

**Discussion and Conclusion:** The present situation in the EU requires the use of early detection tools in order to control the outbreak as soon as possible, avoiding its spread. Results obtained have indicated that, due to its characteristic of sensitivity and specificity (near 100%) and its precocity to detect antibodies specific of BT in sheep, goat and cattle (days 5-7 p.i.), the assay is very useful for these proposes. Moreover, it has been demonstrated that, it can be used for diagnostic in new world camelids.

**Acknowledgments:** We appreciate the support given by Dra. Concepción Gómez Tejedor (Director of Spanish Reference Laboratory, Laboratorio Central de Veterinaria, Algete, Madrid, Spain).

(1) INGEZIM® BTV DR

**18.047** Crimean—Congo Hemorrhagic Fever in Greece

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**Background:** In June 2008 the first case of Crimean-Congo hemorrhagic fever (CCHF) was recorded in the Prefecture of Rhodopi, Northern Greece. The case concerned a woman with a history of tick bite 5 days before the onset of symptoms. Travel abroad, contact with an animal or an ill person within the previous 2 weeks was not reported. The patient developed hemorrhagic manifestations and succumbed. The diagnosis was confirmed by reverse transcriptase-nested PCR and quantitative real time PCR. Sequencing revealed that the strain was phylogenetically similar to the CCHF strains circulating in other Balkan countries, Turkey, and South-West Russia.

**Methods:** Description of public-health interventions implemented. The possibility of CCHF becoming endemic in Greece is discussed.

**Results:** An ad hoc committee was established for the development of case definitions for CCHF notification and contact-tracing. Guidelines for isolation and management of CCHF cases were sent to hospitals in
Northern Greece. Information regarding prevention of tick bites and their proper removal was disseminated. Available data from the 1908's indicate 1–6% serologic evidence of previous CCHF infection among Greek farmers and shepherds. Given the paucity of clinical CCHF cases during the past three decades in Greece, it appears that the these rates most probably reflect previous infection with the AP-92 strain that was isolated from Rhipicephalus bursa ticks from goats in Northern Greece, and differs from the pathogenic Balkan CCHF strains.

**Discussion:** The possibility of CCHF becoming endemic in Greece under conditions favoring suitable infection levels among animal hosts is possible. However, it appears that the current CCHF case was an isolated event, and transmission was succeeded through a tick infected from a small herbivore that may move over long distances. CCHF should be considered in a patient with a history of tick bite and a compatible syndrome in areas neighboring endemic areas.

18.048 Characterization of Leishmania Isolates from Patients with Cutaneous Leishmaniasis (CL) in New Foci, North Baraan, Isfahan 86-87 by PCR Technique

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Leishmaniasis is a zoonosis with a wide range of clinical aspects. Depending on the species of the infective parasite and immune response of host, including three main pathological forms: cutaneous, mucocutaneous and visceral. Iran is one of the endemic centers of this disease as well. There are many severely infected locations of different forms of this disease all over the country. Planning of any preventive program in order to eradicate this disease, control and treatment of patients in any region necessitates precise determination of features of involved parasites. In this study, PCR technique was used to identify species of Leishmania in barean region of isfahan. Smears were prepared by scraping doubted lesion of 50 patients. A pair primer was designed from L. major DNA kinetoplast: Primer 1: 5’_TCGCAGAACGCCCCTACC Primer 2: 5’_AGGGGGTGTGGTGTTGAATAGGC Next step standard PCR was carried out using classic protocol (72). Samples were cultured in NNN and RPMI 1640 media. Total DNA was extracted from RPMI 1640 cultured samples. Negative and positive control and clinical samples was applied for PCR in the same condition. Out of 50 smears L. major found in 46 cases and in 2 cases L. tropica and in 1 case L. major and L. tropica was reported. Statistical analysis were carried out using SPSS soft ware and chi-square test and showed that L. major specie is more prevalence (p-value<0.001)

18.049 New Phleboviruses from Different Antigenic Complexes Circulate in Sandflies in Algeria

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Trapping campaigns of sandflies (Psychodidae, Phlebotominae) were organized in Algeria. Sandflies are trapped alive and identified through morphologic keys. Pools were constituted of a maximum of 30 individuals sorted by species and gender. Due to difficult access to liquid nitrogen or dry ice, pools were placed in guanidinium isothiocyanate from which total RNA was purified using the standard protocol. In Algeria, 314 sandflies (17 pools) were trapped in Algiers, and 471 sandflies (23 pools) were trapped in Tizi Ouzou (Fort National) in the Kabylbian province of Algeria. PCR screening was performed with a combination of assays targeting the L and S genes. Positive PCR products were cloned and sequenced. Resulting sequences were aligned with homologous sequences extracted from the Genbank, and used to analyse genetic and phylogenetic relationships. In Algeria, a total of 4 pools were found positive for phleboviruses RNA. Sequence analysis revealed that 2 distinct species were detected: Sandfly fever Sicilian virus, Sandfly fever Naples virus. Here we report for the first time, sequence data of phleboviruses in northern Africa. This study indicates that there may be much more phleboviruses circulating that initially believed. Further studies should be designed to investigate deeper the genetic diversity of this genus in the regions surrounding the Mediterranean.

Figure 1. Phylogenetic tree based on nucleotide sequences of phleboviruses from different antigenic complexes.

**Models of Disease Surveillance, Detection and Reporting**

18.050 – 18.082 Room: Klimt Ballroom 1 – First Level

18.050 Pakistan Public Health Information Network

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PakPHIN is designed to integrate both the silos of programs that typically develop over time as various donors provide segregated resources to attack specific health issues and the data being collected from different facilities. PakPHIN is capable of supporting the creation, collection and analysis of data from all possible conditions, diagnostic tests (lab as well as radiology), procedures and medications. Using PakPHIN, health authorities and care providers can address communicable diseases as well as chronic and acute care disease management and even accidents and injuries.

PakPHIN consists of a series of modules that are deployed at the Federal, Provincial, District and Local levels. At the Federal level authorities use Code Servers and control what data the system will allow to be collected by selecting a set of codes from a library of all possible codes and thus defining the master code set.

Finally at the District and Local levels Authorities use the Disease Reporting Module that is part of the Medical Record Server to collect disease data and Care Providers (Clinics, Labs and Hospitals) use the eLog-Book to manage clinical data as part of their daily business and export reportable disease data to the appropriate Report Server.

The application provides reporting at all levels, for instance the basic health unit is able to run reports specific to that unit, the district hospital is able to analyze data from all hospitals in that district and Provincial and Federal authorities can run Province wide and country wide reports. A particular strength of this system is the ability to collect laboratory data, integrate it with Patient data and then analyze this data to generate alerts and recommendations for both the Public Health Personnel and for the Care Provider using our Clinical Decision Support system.
Health Professionals have been using the tuberculin skin test (TST) for more than 90 years to identify people with TB infections. This test requires intra-dermal injection of purified protein derivative (PPD) derived from the TB bacteria followed by observation of the injection site by a trained skin test reader between 48 and 72 hours after injection. The two visits are not feasible and as a result, many workers who have skin tests are advised on how to “self-read” the results—a practice without any proven reliability or validity. Reading a TST response relies upon interpretation and discrepancies can occur.

In 1989, the Centers for Disease Control and Prevention announced the goal of eliminating tuberculosis (TB) from the United States by the year 2010. In 2001 the QuantiFERON-GOLD test (QFT-G) [manufactured by Cellestis Limited, Carnegie, Victoria, Australia] was approved by the U.S. Food and Drug Administration (FDA) as an aid in detecting latent Mycobacterium tuberculosis infection and the current QFT-G test (using ESAT-6 and CFP-10) was approved in May 2005. In addition, the U.S. Centers for Disease Control recommends the QFT-G may be used in all circumstances where the TST is currently used. This test uses two specific highly antigenic proteins derived from the TB bacteria (as opposed to the hundreds of antigens in PPD preparations) to stimulate white cells in people infected with TB to produce interferon gamma (IFN-γ). This test requires only a single clinic visit, does not have the same problems with false positive and negative tests, eliminating the limitations of the TST. This paper reports on the experience with TB screening in a population of more than 4600 screenings of business travelers, offshore workers and workers in remote locations and compares the process, procedure, and documentation of results between the workers screened using TST or QFT-G test.

Comparison of TB QuantiFERON—GOLD (QFT-G) and Tuberculin Skin Test (TST) Screening Data in a Worker Population Surveillance Program

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Background: The purpose of nationwide web-based surveillance reporting and management system is to monitor the emerging diseases and to detect outbreaks rapidly by integrating various infectious disease surveillance networks. In July 1999 when Taiwan Centers for Disease Control (Taiwan CDC) reorganized, the National Diseases Surveillance Systems began with notifiable diseases surveillance to detect epidemics. The nationwide web-based surveillance reporting and management system was accomplished in July 2001, which enabled easier and more complete to transfer the reporting information. Medical and public health professionals can join the epidemic surveillance network and real-time alert system, the system can therefore considerably increase public enthusiasm and commit more people to focus on epidemic control. Any related and essential measures of epidemic control can be immediately implemented to avoid serious infection transmission. In this study, we used TB/HIV co-infection cases survival probability as indicator to monitor the impact of implementing nationwide web-based surveillance reporting and management system.

Methods: The main source was based on nationwide web-based surveillance reporting and management system data bank from Taiwan CDC. Kaplan-Meier Method from SAS software was used to evaluate and compare the impact on the survival probability of TB/HIV co-infection cases after onset of one year.

Impact of Nationwide Web-Based Surveillance Reporting and Management System on TB/HIV Co-Infection Cases for 5 years in Taiwan

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Results: The mission of nationwide web-based surveillance reporting and management system is to construct diversified disease surveillance systems, collect and monitor data for disease trend analyses and provide the analysis and assessment for imported and indigenous diseases regularly. The study shows that the survival probability of the period of pre-web based surveillance system (2002–2006) is 0.93; and survival probability of the period of post-web based surveillance system (1993–2000) is 0.75. There was a significant difference in survival probability of TB/HIV co-infection cases between pre- web based surveillance system era and post- web based surveillance system era, P<0.0001.

Conclusions: In order to efficiently observe any alleged epidemic cases, thoroughly control infections, stop transmission, and prevent emerging diseases such as TB/HIV co-infection and avian flu, we need to continue bringing in cutting-edge surveillance system and technology for better epidemic control, integrating all information of epidemic control, improving our professional abilities, and agilely handling the disease prevention as in a general mobilization.
Tuberculosis in HIV Positive Persons in Singapore
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Background: HIV is a significant risk factor for progression to active TB in people with latent TB infection. The annual risk of developing TB in HIV infected individuals ranges from 5-10%. The incidence of HIV/AIDS in Singapore has been on the rise from 0.6 per 100,000 population in 1990 to 9.1 per 100,000 population in 2005. The incidence of TB was 49.9 per 100,000 in 1990 and 37.9 per 100,000 population in 2005.

Methods: We carried out a retrospective study matching TB cases that were diagnosed between 1990 and 2005 from the Singapore Tuberculosis Elimination Programme Registry, with the National HIV registry. A total of 210 TB cases among Singapore residents who had prior diagnosis of HIV were analysed in terms of socio-demographic factors, baseline CD4 counts, mode of HIV transmission, time of TB diagnosis from HIV diagnosis, as well as site of TB disease.

Results: Of the 210 cases, 96 % were males, 88% were Chinese, and 76% were heterosexual. About two-third were aged 30 to 49 years old at HIV diagnosis, and more than 50% were blue collar workers. 74% had a baseline CD4 count of less than 200 per mm3 at first diagnosis of HIV, and 41% were notified for active TB within 1 month of HIV diagnosis. Majority of the TB cases (57%) were extrapulmonary.

Conclusion: As only registry data is utilised in this study, it is likely that TB cases which were notified within 1 month of HIV diagnosis were already present at HIV diagnosis. The time lag is likely due to time required to confirm diagnosis of TB, either clinically or based on positive culture result, prior to notification. Public health intervention would potentially be effective in prevention of TB in 60% of HIV cases who subsequently develop TB one month after HIV diagnosis.

Cryptosporidiosis: A Fatal Parasitosis with High Prevalence in Leopard Geckos (Eublepharis macularius)
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Background: Cryptosporidiosis in reptiles is a latent infection with a long incubation period, eventually leading to acute to chronic symptoms and finally to the death of the infected animal. Since there still is no effective therapy available and cryptosporidia are very easily transmitted, a single subclinically infected animal poses a serious threat to any reptilian collection. The prevalence of this protozoal disease in leopard geckos (Eublepharis macularius) seems to be exceptionally high, but no definite data are available at present.

Methods: A polymerase chain reaction (PCR) assay with consecutive sequencing of the 18S ribosomal ribonucleic acid (RNA)-gene of the genus Cryptosporidium has been developed and adapted for use on tissue and faecal samples. A total of 123 faecal samples from leopard geckos were examined with this method.

Results: Seventeen faecal samples from leopard geckos (13.8%) gave a positive PCR result for cryptosporidia. The sequence of the amplification product was consistent with sequences from Cryptosporidium saurophilum in 16 cases and from Cryptosporidium serpentis in one case.

Conclusion: Infection with cryptosporidia has been described in close to 80 reptilian species and cryptosporidiosis is recognized as fatal infectious disease of snakes and lizards. In leopard geckos it has been reported to commonly result from an intestinal infection with C. saurophilum but its prevalence in captive held lizards is unknown. The examination of faecal samples from 123 leopard geckos resulted in a prevalence of 13.8% of reptilian-pathogenic Cryptosporidium species. The amount of oocyst-shedding varies considerably and small numbers of oocysts might lead to false negative results, so that the actual prevalence might be even higher. Personal observations regarding reptilian necropsies support the high prevalence of this eventually fatal disease especially in captive leopard geckos. Trade and distribution of subclinically infected geckos should be avoided to limit the further spread of this dangerous parasite.

Disease Monitoring in Wild Bird Populations
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Over the past few decades there have been a number of research projects developed to study the behaviour and ecology of migratory and other wild birds. The information gathered from these projects has provided valuable base line data on the health and population dynamics of a range of species and now serves as a useful set of bench marks for studies on the impact of climate change, and land development, on migratory and other wild bird species. With the emerging interest in ecosystem health, and the recognition that an understanding of complex problems requires a multi disciplinary approach, it is now more common for ornithologists, ecologists and other disease specialists to work together to investigate health problems in wild birds. One immediate benefit arising from this is the effective exchange of information on the health of wild populations and the subsequent expansion in the range of available literature on the subject of disease surveillance in wild bird species. This poster illustrates how a project designed to monitor the condition of migratory birds arriving at study sites in Europe (Osca, Hungary and Lago di Lesina, Southern Italy) from Africa, successfully brought together a multi disciplinary team to gather base line parasite data as part of a health monitoring programme. The quantitative and observational ecological data from the long running "Old World Song Birds project" was used to help interpret the base line health data gathered on over 30 passerine species caught using mist nets for the purpose of banding. This work demonstrated that employing the skills professional ornithology groups and ecologists significantly improves the value of health data gathered. To ensure the future success of surveillance initiatives for wild bird populations it is important to support long term studies and to ensure that the teams responsible for developing and delivering these studies are truly multi disciplinary.

Modelling Detection Strategies for an Influenza Pandemic: A Retrospective Application of Today’s Surveillance Capabilities to Yesterday’s Epidemic
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Background: Diagnostic capabilities for the surveillance of influenza viruses has improved dramatically in the last two decades, but it is uncertain how such technologies might improve our chances of detecting an influenza pandemic. We use a mathematical model to estimate whether current technologies might have detected the 1957 pandemic in Singapore ahead of clinical recognition of the outbreak.

Methods: We fitted a deterministic Susceptible-Exposed-Infectious-Recovered model to data on Influenza-Like-Illness consultations observed during the 1957 pandemic in Singapore using maximum-likelihood methods. We then superimposed a simulated epidemic of influenza on the background incidence of about 1000 acute respiratory infections (ARIs) per day, and estimated the probability that at least one sample taken from all ARIs is positive for the pandemic virus.

Results: We obtained a reproductive number of between 1.9 to 2.2, depending on assumptions on whether the epidemic course was altered by interventions (Figure 1). The best fitting model suggested that, if the epidemic had been started by a single infection, the infection might have been introduced some weeks prior to when the epidemic was noticed around 1 May 1957. If rapid diagnostic testing had been available during the 1957 pandemic, we estimate that the cumulative chance of detecting
the pandemic virus earlier than 1 May would have been more than 90%, even when testing only 1 specimen per day (sampled from ARI patients). If we had tested up to 20 specimens per day, we would have had >50% chance of detecting the epidemic 2 weeks earlier than in 1957.

Conclusion: Current laboratory capabilities for rapid diagnosis of influenza virus strains would likely have detected the 1957 influenza epidemic in Singapore caused by the pandemic viral strain earlier than the point when the outbreak was clinically recognised. Further work is needed to determine the best mix of pandemic surveillance strategies.

Figure 1. Modelled and observed ILI, assuming that the reproductive number R is constant throughout the epidemic (Model 1: R0 = 1.9), and assuming that the reproductive number changes 7 days after epidemic detection (Model 1: R0 = 2.2). Model 2 gave a better fit to the data, and was used in all subsequent analysis. Model 2 suggests that, if the epidemic was started by a single infection, this infectious case may have been introduced up to 6 weeks before the epidemic was noticed on 1st May 1957.

Figure 2. Cumulative chance of detecting an epidemic caused by a pandemic influenza virus as a function of the number of samples for virological testing taken per day, assuming that the epidemic course approximated the modelled distribution of cases as for 1957. Daily virological testing, even of a single specimen daily, would have >90% chance of detecting a 1957-like epidemic before the time-point corresponding to when the 1957 epidemic was actually reported. If we tested up to 20 specimens per day, we would have had >50% chance of detecting the epidemic 2 weeks earlier than in 1957.

**Abstracts**

**18.057 Global Travel Health Services by ExxonMobil Medicine and Occupational Health Clinics in the United States**

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Global travel health preparation is comprised of a complex variety of tasks. Over the past decade, large corporations have experienced significant growth in business travel due to globalization of markets. In the United States during 2007, we had 10,668 international travelers making 29,201 trips. A retrospective review of services provided to international business travelers by our 18 Medicine and Occupational Health Clinics revealed types of clinical encounters, immunizations provided, and preparation activities for the business traveler. The review covering a 24-month period, October 2006 to September 2008, explored country specific examinations, clinic visits, and types of immunizations provided. Country specific examinations for expatriate and rotator assignments during the 24-month time period accounted for 933 of the total clinic visits. Travel preparation visits comprised 11,272 of the encounters. Our clinics served employees with 7,263 immunization encounters with 12,848 vaccine doses administered. We utilized a country specific travel information service to remain current on developing diseases and immunization requirements, and immunized employees against vaccine preventable diseases. Data reviewed for the 24-month period provided the opportunity for clinics to manage inventory projections, staffing needs, and explore cost saving measures, as well as review traveler preparation procedures. Our previous studies revealed employee utilization of travel preparation services needed updating and changes were implemented in the clinics to improve delivery of risk prevention information. Corporations successful in globalization activities prepare employees for assignments with detailed review to reveal health risks, reduction of risk from vaccine preventable diseases through active immunization programs, and reduced travel related illness and injury through preparation. Our Emergency Medical Response System (EMERS) and active tuberculosis surveillance system further support our business travelers.

**18.058 Isolation and Genotypic Characterization of Listeria monocytogenes from Poultry Meat**

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Prevalence of *Listeria monocytogenes* in the broiler meat was studied in the Nagpur municipal corporation area. A total of 200 meat samples were collected from broilers slaughtered with in the Municipal corporation, Nagpur, India. The meat samples were processed for isolation of *Listeria* according to the method of US Department of Agriculture (USDA). The isolated *Listeria monocytogenes* were screened for the presence of pathogenic genes viz Phosphatidylinositol Phospholipase C gene (plcA), Actin gene (actA), Haemolysingene (hlyA), and p60 gene (iapA) in them by PCR. Standardization of PCR was done by using standard strain of *Listeria monocytogenes* 4b (MTCC 1143). The amplified product was screened by gel electrophoresis. *Listeria monocytogenes* was isolated from the 21(10.50%) meat samples and three isolates harboured all four pathogenic genes while nine, three and one harboured three, two and only one pathogenic genes respectively. It is concluded that broiler meat from market harbours pathogenic *Listeria monocytogenes* and for accurate identification of pathogenic species of *Listeria monocytogenes* detection of multiple virulence associated genes reliable and useful.

**18.059 A Prototype AI BioPortal: Application to Data from the Danish 2006 HPAI H5N1 Wild Bird Epidemic**

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Background: Most current disease information systems have severe built-in limitations to their use as general global surveillance and reporting tools. To overcome some of these limitations CADMS has since 2004 partnered with several national and international groups in the development
of a BioPortal system for use in global foot-and-mouth disease surveillance (FMDBioP) which became operational in January 2006. The BioPortal was also used in several local projects worldwide, including providing support for visualization and transmission of information.

Methods: The official Danish wild bird surveillance data file from 2006 includes 45 identified cases of HPAI H5N1 infection among more than 6,700 records of tested samples, of which more than 5,500 were faecal samples obtained from migrating wild birds during resting (active surveillance data) and around 1,200 were dead wild birds collected and tested (passive surveillance). In total 22 HPAI H5N1 viruses were isolated, of which 8 have been sequenced, while 23 cases were diagnosed by RT-PCR. All records have been uploaded on the most recent version of the BioPortal system to create a prototype for the AIBioP at a national scale. The AIBioP procedures include various spatial-temporal visualization tools (Fig. 1) along with displays of different phylogenetic tree-structures (Fig. 2).

Results: Preliminary spatial-temporal analyses have identified several clusters of higher incidence in the Danish 2006 data, and different observations have indicated that a burn-out of the regional epidemic of HPAI H5N1 infection among migrating wild birds in and around the Baltic Sea may have taken place in May 2006.

Conclusion: A prototype of the AIBioP was developed, using the official wild bird surveillance data collected in Denmark in 2006. The general public may access this prototype at http://fmdbiportal.ucdavis.edu logging in with the username “Denmark” and the password “avianflu”. The addition of several surveillance items from 120 countries have been captured and delivered to 350 individuals from 50 countries that have subscribed to the FMD news system.

A BioPortal System For Global Animal Disease Surveillance

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Background: Real-time decision-making is limited by the absence of informatics tools able to manage disparate streams of information, display results in space and time, identify space-time clusters of disease, facilitate the phylogenetic analysis of samples, and communicate and share results in near real time and at a global scale. Here we describe some of the attributes of a new information system, referred to as the BioPortal, which is currently operational for global foot-and-mouth disease (FMD) surveillance (https://fmdbiportal.ucdavis.edu)

Methods: The FMD BioPortal was developed as a multiagency effort aimed at creating a web-based system that would make available in near real-time FMD-related data. The FMD BioPortal has the capability to integrate several sources of data and analytical tools that can interact in real-time from various locations and at different levels of security and user restrictions. A primary objective was to be able to apply to the data basic search and analytic tools, including graphic and tabular presentation of the data and cluster analysis, to be able to download selected records, and to provide access to aligned FMD virus sequence data and to tools for real-time development and comparison of phylogenetic trees of virus isolates.

Results: Since becoming operational in January 2007, 370 users from 46 countries or international organizations have subscribed to the FMD BioPortal. Databases currently available through the FMD BioPortal include FMD serotype data for samples submitted to the World Reference Laboratory, Pirbright, since 1957; the OIE WAHID database, GenBank, and for users with the required permits, selected national and international databases. The FMD news, which is a real-time web search service aimed at identifying, capturing, and delivering FMDBioPortal-related news items, is also available via the FMD BioPortal. Since its inception in October 2004, 6,736 news items from 120 countries have been captured and delivered to 350 individuals from 50 countries that have subscribed to the FMD news system.

Conclusion: Use of the FMD BioPortal will enhance the ability of countries to prepare for and respond to FMD and other animal disease epidemics. Future initiatives will involve development of BioPortal prototypes for animal diseases other than FMD, including, for example, avian influenza and Rift Valley fever. Interested research groups and agencies are invited to participate in this multiagency effort.

Simultaneous Detection of Rift Valley Fever, Bluetongue, Rinderpest and Peste Des Petits Ruminants Viruses by Multiplex PCR Using DPO System


Background: Nucleic acid detection techniques such polymerase chain reaction (PCR) provide the potential for rapid and sensitive detection of the most serious viral infections, such as Rift Valley fever virus (RVFV), Blue-tongue virus (BTV), Rinderpest virus (RVP) and Peste des petits ruminants virus (PPRV). RVFV, BTV, RVP, and PPRV showing mucosal lesions in infected animals are necessary to be differentiated from suspected cases. We investigated to develop and validate single-tube multiplex PCR (mPCR) on RVFV, BTV, RVP, and PPRV.

Methods: RPV (Kabete-O, LATC), PPRV (Nigeria 75/1), BTV serotype 1 to 24, RVFV (Smithburn strain and inactivated Zim 688/78 strain), Ibaraki, Aino, Akabane, Bovine ephemeral fever, and Chuanzun viruses (Korean Isolates) were used in this study. Total 19 primers designed to improve the specificity comprise a tripartite structure with a polydeoxyribo- nosine [poly(dI)] linker between the 3’ and 5’ target core sequence using a dual priming oligonucleotide (DPO) system. These sets of primers were derived from conserved regions of F gene of the RVFV and of the PPRV, VP3 gene of BTV, and NS5s gene of s segment of RVFV, respectively.

Results: The single-tube mPCR method developed in this study readily identified viruses by specifically discriminating the size of their amplified
Can Surveillance of Mortality in Wildlife Help to Detect Emerging Diseases?

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Background: Since 1986 France, as a country, is covered by a wildlife mortality monitoring system, called SAGIR. A network of technicians from the federations of hunting and the National Hunting and Wildlife Agency (ONCFS), collect carcasses found by hunters and the general public, and submit them for investigation by veterinary laboratories, in each “départe-
men.” The collected data are analyzed and results reported annually. More than 50.000 cases of mortality in wildlife were recorded between 1986 and 2007, representing 244 species of terrestrial mammals and birds, and were attributed to 220 different causes of mortality. Our aim is to use the data collected by the network to study the feasibility of early detection of unusual health events we call “anomalies.”

Methods: We first analyzed the functioning of the network according to the guidelines of the Centers of Disease Control and Prevention of Atlanta [1,2], in order to evaluate its ability for the early detection of health events affecting wildlife. Syndromes of interest were then defined by a statistical typing of the lesions observed on the carcasses, using factorial analy-
sis in order to identify the information which best explains the observed variability, followed by clustering techniques. We then will carry out trend analyses on selected historical data, to establish the “background noise” of occurrence of these syndromes, characterized by multiple features (time, space, species; altitude...). Existing abnormality detection methods will then be adapted. Variation of occurrence of a syndrome should be detected by the methods as an abnormality and will lead to an alarm signal.

Results: The poster will describe the mortality monitoring system and the data collected; it will then illustrate some of its characteristics regarding detection of unusual health events. Then, it will detail the syndrome definition, and finally present the abnormality detection methods chosen.

Conclusion: The study poses questions about the relevance of the use of mortality surveillance data for a broader purpose and gives arguments in favor of the use of this type of data for the detection of emerging diseases.


Automatic Early Warning System of Infectious Diseases in China

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Background: China established a Notifiable Infectious Diseases Direct Net-Reporting System in China, which is possible for early warning of infectious diseases outbreaks through Notifiable Infectious Diseases Direct Network. The system has a good sensitivity to detect abnormal incident or spatial cluster, and helps early detect infectious disease outbreak.

Paediatric Dengue Surveillance in Colombo, Sri Lanka

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Dengue has been endemic in Sri Lanka since 1989. The District of Colombo suffers the highest burden, with around 25% of the national
Results:

A census of households in Municipal Council Ward 33, Colombo, was conducted in October 2008. From this population, 800 randomly-selected children aged <12 years have been recruited to be followed up for fever events over one year. All children with ≤ 7 days of fever are referred to dedicated healthcare centres for further investigation. Acute and convalescent blood samples are collected from all fever episodes for detection of dengue viral genome by RT-PCR and dengue antibody levels by IgM and IgG ELISA. At initial recruitment, fingerprick blood samples from all participants were collected onto filter paper discs to determine baseline dengue sero-prevalence. A repeat fingerprick sample will be collected at 12 months to determine the annual seroconversion rate. This study is one of the dengue surveillance sites supported by the Pediatric Dengue Vaccine Initiative to evaluate the burden of dengue and for future trials of candidate dengue vaccines.

18.067 Influenza Associated Excess Mortality in Slovenia, 2000–2006

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Background: Influenza epidemics occur every winter in northern hemisphere with varying timing and magnitude. Influenza is more severe compared to other acute respiratory viral infections. During an influenza epidemic, morbidity, hospitalization rate and mortality rate rise. The aim of our study was to assess the impact of influenza on all cause mortality during six influenza seasons.

Methods: Weekly numbers of death (violent deaths were excluded) from year 2000 to year 2006 were obtained from National Mortality electronic dataset (Institute of Public Health). Expected weekly number of death was calculated. For each week expected number of death corresponded to the mean of three weeks (current week, previous week and next week) for past three years. Upper and lower confidence intervals were calculated. Virological surveillance data were collected from National Influenza Surveillance Scheme: weeks with positive detections of influenza virus defined the influenza epidemic period. For influenza epidemic period crude excess mortality was computed by subtracting expected from observed mortality.

Results: Influenza activity had occurred annually from December to March. Number of weeks with influenza virus detections lasted from 10 to 19 weeks per season. In 2000/01 and 2001/02, virus influenza A H1N1 and influenza B predominated, respectively. 2002/03 and 2003/04 were almost exclusively influenza A H3N2 seasons. In 2004/05, half of flu virus detections were influenza B, 40% A H3N2 and 10% A H1N1. In the last season 2005/06, influenza A H3N2 was found in 75% of patients, and influenza B in 25%. In flu seasons from 2000 to 2006 excess mortality per 100.000 was: -21.75, -13.05, 29.75, 15.55, 25.46 and 3.9. Conclusion: Seasons in which influenza A H3N2 virus predominated exhibited higher excess mortality then those in which virus influenza B circulated. Influenza AH1N1 had the lowest impact on mortality.

18.068 Determination of the Invasiveness Properties of Iranian Shigella flexneri Wild Type and the Mutants in Hela Cell Culture by Electron Microscopy

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Objectives: Invasive bacteria such as shigella have the ability to penetrate mammalian epithelial cells both in vivo and in vitro. Morona et al, produced a S. flexneri mutant and showed invasiveness of mutants decreased compared to wild types. So, the aim of this study was determination and comparison the invasiveness properties of the S. flexneri wild types (icsA+ strains) v.s S. flexneri mutants (icsA defected) in HeLa cell culture by electron microscopy.

Methods: Shigella flexneri strains were isolated by standard bacterial methods from fecal specimens of children attending to the 3 children’s hospitals of Tehran between January 2003 to December 2003. DNA isolation, was done by sodium perchlorate 4M method. Existence of icsA gene was determined by PCR. Mutation formation was done by allelic exchange and using pCACTUS-icsA: ampr. HeLa cell culture were prepared using MEM medium and Fetal calf serum and infected with both S. flexneri wild types and mutants strains. Continuously, electron microscopy investigation was done after fixation, using resin spur and staining gold sections with saturated Uranyl acetate and Lead citrate.

Results: From 100 S. flexneri strains recovered from stool specimens, 46% were icsA+ by PCR. All transformed bacteria were selected by temperature sensitivity and sucrase levan production and resistance to ampicillin. Further PCR on mutants showed no PCR product detection. According to pictures, invasive shigella strains penetrated in HeLa cells compare to mutants which may adhered but not entered. Further more,
pseudopod structures used to facilitate bacterial cell-to-cell spread were readily identified by electron microscopy in wild types. Fig-1(a-c).

**Conclusion:** In this study, all clinical isolated S. flexneri wild types which were IcsA+, showed similar internalization, cell existence and cell disruption. In addition, pseudopod filaments were confirmed with electron microscopy. In contrast, most of mutants IcsA- could not enter to HeLa cells. These bacteria might produce necrosis later, but not detected after 3 h incubation.

**18.069 Three-Year Surveillance of Community-Acquired and Health Care-Associated MRSA Infections in Uppsala County, Sweden**

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**Background:** To calculate the cost of minimizing the risk for unintentional spread of community-acquired (CA) or health care associated (HCA) meticillin-resistant Staphylococcus aureus (MRSA) by prospective identification of patients with MRSA infections.

**Methods:** Prospective CA-MRSA and HCA-MRSA infection surveillance at hospitals and health care settings in Uppsala County was initiated on November 1, 2003 by systematic screening for MRSA carriage, regardless of symptoms, of all patients that had been treated in hospitals outside Sweden during the last six months or in hospitals in the Stockholm area where an outbreak of MRSA had started in 2002. Moreover, MRSA was isolated from all patients with skin and soft tissue infections attending primary care clinics or other outpatient settings. All isolates were genotyped by pulse-field gel electrophoresis.

**Results:** During the 3 years of the study, 17,634 isolates were collected from 7967 patients attending 2 hospitals and 33 primary care clinics, 2 outpatient settings and 2 private care providers. 82 cases of MRSA were identified (27.3 cases per year). 41 isolates were from patients with clinical symptoms of infection whereas 41 patients had no clinical infection but were carriers of MRSA in the nose, throat or perineum. Eight of 82 patients (10%) had HCA-MRSA after being hospitalized in the Stockholm area. Three of the eight isolates belonged to the outbreak strain in Stockholm. There was no spread of CA-MRSA or HCA-MRSA in the hospitals during the study period. The total cost for bacterial isolation of MRSA was 2.4 million SEK per year (equivalent to 380,000 USD/year), which amounts to 87,805 SEK (13,870 USD) per identified patient with MRSA.

**Conclusion:** Strict surveillance of MRSA from both infectious and colonized cases may minimize the risk for hospital outbreaks, increase patient safety and be cost-effective.

**18.070 A Tool for the Assessment of European Network for Highly Infectious Diseases Project**

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**Background:** EuroNHID is an EC-funded network of experts in the management of Highly Infectious Diseases (HIDs), involving 15 countries (Austria, Bulgaria, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Luxembourg, Malta, Poland, Slovenia, Spain, United Kingdom). The main objective of EuroNHID is to conduct a survey for the assessment of current capabilities in isolation, infection control and healthcare worker (HCW) management in the referral hospitals designed to deal with HIDs. With this aim, specific checklists have been developed. The checklists’ structure and some preliminary results of the survey are presented.

**Methods:** The checklists have been developed with a “networking strategy”: topics had been attributed to a participant with specific expertise, who sent drafts to the other participants, for comments and amendments. Final agreement had been reached during the first EuroNHID meeting (Rome, April 10-11, 2008). The checklists will be used to conduct the survey by each participant (or their representatives) in her/his centre and in other selected centres in the country, if any, together with Project Coordinator.

**Results:** The checklists addressed the following issues:
- Hospital Resources: infrastructure and technical issues, personnel availability, diagnostic procedures, infection control in emergency department
- Hospital Policies: administrative aspects, availability and management of Personal Protective Equipments, hand hygine, prevention and management of needle-stick injuries, transportation of patients, routine hygiene and disinfection, waste management, post-mortem procedures, surge capacity procedures
- HCWs Safety: safety of healthcare personnel (organizational, administrative and medical aspects, including vaccination and post-exposure policies), education and training of HCWs.

Complete checklists are available on EUNID web-site (www.eunid.eu), after registration, under “Documents.”

The survey of referral hospitals is currently ongoing. Results from 4 centres in Italy, France, Germany and United Kingdom will be presented in details.

**Conclusion:** The checklists would represent standard and shared tools for the assessment of referral hospitals for HIDs. The results will represent a valuable outcome both for surveyed centres, for revising and implementing their infection control procedures, and for European authorities, providing an “on-the-field,” qualitative evaluation of European hospitals’ capabilities in dealing with HIDs.

**18.071 Forensic Luminol: A Tool for Detecting Traces of Blood after Isolation of a Patient with Highly Infectious Diseases**

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**Background:** The use of forensic Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) was proposed as a suitable method for identifying invisible blood contamination of the Health-Care Settings environment. Luminol, in presence of haemoglobin, produces a bright blue chemiluminescence. The method is very sensitive although other substances cause false-positive signals. To further assess the effectiveness of our infection control procedures, we used the Luminol test in a unit where a patient with suspected Viral Haemorrhagic Fever was cared for.

**Methods:** In 2006, a patient was referred to the National Institute for Infectious Diseases “L. Spallanzani,” the Italian referral centre for highly infectious diseases, because of a suspected Crimea-Congo fever. The patient was admitted in a never used, negative pressure, intensive-care room, provided with anteroom. Strict, full isolation procedures were instituted (contact, droplet and respiratory precautions, and appropriate Personal Protective Equipment (PPE)). After two days of intensive care, including haemodialysis, the patient died with multi-organ failure due to Herpes. The test was conducted by a special unit of Carabinieri, a corp of Italian Army, experienced in crime scene investigation, one month after the patient’s death and before any cleaning, by spaying a mixture of Luminol, Acid Sodium Bicarbonate and Sodium Perborate.

**Results:** According to the Carabinieri report, in the isolation room a positive signal likely to be due to blood was observed over frequently touched surfaces (bed edges, monitor buttons, cables and pipes of auto respirator, haemodialysis machine). Not unexpected, chemiluminescence derived from 2 basinfuils where double gloved hands were immersed in 1:100 dilution of household NaOCl before external glove removing, and from the portable table where basinfuils were located, the NaOCl bottle used and some areas of the floor close to the table, suggesting dropping. In the anteroom, signals were discovered on the external surface of the box containing gloves, in the cupboard containing PPE, and on the floor. No signals were detected outside the isolation room.

**Conclusion:** Using Luminol to detect invisible environmental contamination allows to call attention on possible leaks of infection control procedures. In particular, we do not use anymore basinfuils containing NaOCl for disinfection of external gloves, and recommend to remove shoe cover before leaving the patient’s room. Detailed recommendations for PPE removal have been implemented.

**ABSTRACTS**

International Meeting on Emerging Diseases and Surveillance 2009
Influenza-Associated to Hospitalizations and Deaths in Spain

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Background: Influenza is responsible for a high burden of hospitalisation and deaths. The burden of disease attributed to influenza is difficult to determine, as respiratory syncytial virus (RSV) activity of overlap with influenza activity. Besides rate difference method, more complex statistical techniques are required to account for other variable with seasonal pattern. The objective of this study is to estimate the excess hospitalizations and deaths attributed to influenza in Spain, using age-specific generalized linear models (GLM), for the period 1997–2005.

Methods: We used hospitalisation data from Minimum Data Set for Hospital Discharge (CMBD) for the period 1997 to 2005, and mortality data from National Statistics Institute (INE) for the period 1999 to 2005. We revised the hospitalization and mortality for influenza and pneumonia, respiratory and circulatory diseases, as well as all-causes deaths, by age groups. Data supplied by CMBD were categorised using the International Classification of Diseases 9th Revision (ICD 9) and INE data with ICD 10. Virological data was obtained from the Spanish Influenza Sentinel Surveillance System and the Microbiological Information System. We used a GLM procedure, assuming Poisson-distributed errors with the response variable (hospitalisations or deaths) assumed to be linear and additive in the covariates.

Results: Globally, the excess mortality attributed to influenza was 1.31 per 100,000 for influenza and pneumonia and 13.45 for all-cause. Highest mortality rates were seen in adults older than 74 years, with an excess mortality attributed to influenza of 15 per 100,000 and 142 per 100,000 for influenza and pneumonia and all-cause, respectively. The excess of influenza/pneumonia hospitalizations attributed to influenza were 6.5, 41, and 5.3 per 100,000 for children under 1 year, adults older than 74 years, and all ages, respectively.

Conclusion: The estimated morbidity excess was particularly high in children under 1 year and in adults older than 64 years, for hospitalizations, and only in the last group for mortality. Applying the GLM models allows a more accurately estimation of the burden of the disease attributed to influenza, taken in account the differential impact of influenza and RSV circulation during the influenza activity.

Environmental Surveillance of Viruses with Enteric Habitat in Parma

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Introduction: During 1988, WHO aimed to eradicate Poliovirus before the year 2000. Despite the total number of new cases was actually reduced of 99% and three WHO regions were thereafter declares as “Polio Free,” during 2008 four countries still resulted endemic for wild type polioviruses (Nigeria, India, Pakistan and Afghanistan). Also for “polio free” countries, a rigorous surveillance is then mandatory, both at clinical (identification of paralytic diseases) and environmental level.

Aims: This survey aimed to identify circulating polioviruses (vaccinal, wild type, rotavirus) and other enteroviruses in wastewater from the Parma county.

Methods and Materials: Between February 1st 2005 and November 18th 2008, 208 samples were collected with a 2 week periodicity from the two wastewater treatment plants (Eastern and Western Plant) active in Parma county. The samples were analyzed through pharysac separation (WHO, Guidelines for environmental surveillance of poliovirus circulation). The sludge was then seeded on two continuous cell lines, in order to isolate enteroviruses (RD-cells) and polioviruses (L20B). All samples were then evaluated with RT-PCR with primers specific for enteroviruses and polioviruses. Cells lines positive isolates were then further analyzed through RT-PCR and microneutralization with specific anti-sera (Coxackievirus, Poliovirus).

Results: Positive results for enterovirus were identified in 110 of 206 samples (53.4%). A difference among the isolates from Eastern (50/103, 48.5%) and Western wastewater treatment plant (60/103, 58.3%) was identified, but resulting not statistically significant (Fisher’s test P = .4792). Ninety five isolates (86.4%) were positive for Coxackieviruses: serotypes B4 (25/95, 26.3%) and B5 (24/95, 25.3%) were the more frequently identified, followed by serotypes B3 (18/95, 18.9%) and B2 (17/95, 17.5%); 11 samples were then positive for Group B Coxackievirus (11.6%); but a further typization was not possible. Finally, a single specimen resulted positive for type 3 vaccinal poliovirus.

Discussion: Our survey confirms the absence of environmental circulation of wild-type poliovirus. During the post-eradication phase of poliovirus surveillance, the availability of reliable methodologies appears as a point of critical interest for Public Health and the identification of vaccinal poliovirus 3 confirms the sensibility and the specificity of the methodic here we present.

Surveillance Systems for Infectious Diseases Among Healthcare Workers: A Systematic Review for the REACT Project

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Previous EU-coordinated responses to infectious diseases have been challenging—largely as a result of differing levels of public health preparedness and response across Europe. REACT (Response to Emerging infectious disease: Assessment and development of Core capacities and Tools) is an EC-funded multi-agency project aiming to establish and define tools, best practices and core capacities to facilitate EU-coordinated responses to future emerging infections. This includes creating a Europe-wide surveillance system (SS) which is able to detect emerging infections among sentinel populations of healthcare workers (HCWs). To help achieve this objective, a review of existing SSs for infectious diseases among HCWs and an assessment of their utility in serving as a template for the Europe-wide SS were carried out.

Systematic review of peer-reviewed literature using seven databases (including MEDLINE and Embase). Searches were restricted to articles published in 1997-2008 and written in English and French.

We identified 891 potentially relevant articles—73 met the inclusion criteria. The review generated descriptions of 79 different SSs worldwide, including 25 from Europe. Most descriptions were incomplete, with roughly 80% mentioning fewer than 5 of the criteria (e.g. specificity, timeliness and acceptability) used by the CDC to evaluate SSs. The majority (49) were designed for occupational exposure to HIV, hepatitis C, hepatitis B or tuberculosis—all of which have long incubation and symptom-free periods. Less than one third (21) of the identified SSs were designed for highly infectious and transmissible infectious diseases such as SARS, influenza, rotavirus and pertussis.

Descriptions of SSs for HCWs in peer-reviewed journals are rare and often, incomplete. Few of the identified SSs will function as a template for a Europe-wide SS for detecting emerging infections among HCWs; however, the information obtained in this review will serve as an aid in developing concepts for the Europe-wide SS.

This project received funding from the European Union (DG-SANCO).
migrations due to business and tourism, socio-economic status, climate change and a unique ecological make-up can pose major threats in terms of disease exchange. Existing epidemiological systems miss key components, lack “dynamic” ability and were not designed to meet demands for inter-island cooperation.

A preliminary study was conducted from Martinique to test strategies, models and hypotheses for integrated surveillance in the Caribbean. Dengue fever and influenza-like illnesses were chosen for experimentation based on prevalence, modes of transmission and complexity. The approach made use of a “network of networks” linking computerized or on-line sites (data collecting points). Extraction and storage of various quantitative or qualitative data were both semi-automated and request-based. Data extraction was performed using commercially available query tools. The architecture included dedicated databases and a web house for data collection, sharing and speedy treatment.

Information was downloaded into a data warehouse, built using MS SQL SERVER (with Integration Services for ETL operations) and based on a dimensional model for query performance. The warehouse provided a unified and integrated view of all collected data and a scalable analytical engine handling large sets for multiple users; it enabled analysis through knowledge discovery techniques and selected calculation algorithms (classification, statistics of inference…). Data-mining customized algorithms were used for predictive functions. Internet was utilized for rapid transmission and timely access to raw or processed information.

The pilot system demonstrated early warning capacity based on inference from collected data (an upcoming outbreak was correctly postulated). It also proved cost-effective and fared better than the existing ones when applied to retrospective analyses. This study supports usefulness of our system for efficient EID surveillance in archipelago settings.

### 18.077 Highly Mobile Pathogens and Their Early Detection- Possibilities and Constraints of Avian Influenza Surveillance in Wild Birds

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**Background:** The monitoring of wild birds, and especially migratory birds, for pathogens is difficult. When cases of avian influenza are detected, the assessment of the true number of affected, positive animals is often difficult if not impossible. This results in limitations in calculating prevalence estimates. When the large number of different bird species is taken into account, it becomes obvious that a profound analysis of these data is a challenge for biometricians and veterinary epidemiologists.

**Methods:** For the analysis of surveillance data, we propose an evaluation model for estimating confidence intervals for (i) prevalence calculations in outbreak situations or (ii) the absence of disease in certain time intervals for certain regional units in Germany. For this point estimates the upper confidence limit (UCL) where estimated. By the introduction of a scheme of species weighting the surveillance system can be evaluated in terms of effectiveness of the birds sampled under the respective conditions.

**Results:** During outbreak situations in wild birds (e.g.: at the Baltic Sea) prevalence estimations are produced but due to reporting bias and missing data this should be interpreted very carefully. For most places and most times the UCL did not fall below 10%. Only intensive monitoring action between November 2006 and June 2007 at the Baltic Sea led to reliable confidence limits over a long period. Between the outbreaks of wild swans in Nuremberg and in domestic ducks in Middle Franconia the UCL varies around 25%. Different UCLs for different species selection scenarios can be compared and show how optimizing the sampling can lead to higher confidence in surveillance system regarding virus prevalence and virus transmission activity.

**Conclusion:** This study may prove useful for the detection of time intervals, geographical units and population subgroups where monitoring is insufficient to detect a pathogen with low prevalence. Limited resources did not allow installing a reliable surveillance system for whole Germany and the whole variety of species over a long period. In the future, it may be possible to develop a risk-based approach and to target specific animals with a better chance of detecting the virus. Altogether this supports the risk analysis for introduction of Influenza-A viruses into poultry and the food-chain in Germany.

### 18.078 A Computerized Decision Support System for Screening Precipitation Errors in Treatment for Pulmonary Tuberculosis

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**Background:** Influenza-like illness data is collected via an Influenza Sentinel Provider Surveillance Network at the state (Iowa, USA) level. Because participation is voluntary, locations of the sentinel providers may not reflect optimal geographic placement. The purpose of this study is to use a maximal coverage model (MCM) to determine the “best” locations for sentinel providers in Iowa.

**Methods:** We calculate the number of people within 20 miles of the Iowa Department of Public Health’s (IDPH) existing 20 sentinel locations, as well as the incremental benefit of selecting additional sentinel locations from the 117 available candidate sites (different primary care clinic locations) using the MCM. We compare with using the MCM to select from 1 to 117 candidate sites de novo, again maximizing the number of people within 20 miles of a selected site. A web-based MCM calculator (http://vinci.cs.uiowa.edu/~gcfairch/) was developed in order to help the IDPH select new candidate sites providing the greatest population coverage.

**Results:** The 20 existing IDPH sentinel locations cover 39% of the population. This same population coverage is achieved with just 8 sites chosen using the MCM; in comparison, using the MCM to select 20 sites de novo covers 66% of the population. The first location selected covers 15% of the population; the first two alone cover 23.5%. Additional locations provide more coverage but with diminishing marginal returns.

**Conclusions:** Given scarce public-health resources, MCMs can help surveillance efforts by prioritizing the recruitment of sentinel locations.

Figure 1. Sample map generated showing the 20 existing sites and 10 newly calculated sites. All sites have a radius of coverage of 20 miles. A potential site located in ZCTA 50703, highlighted in yellow, covers 158,571 people.
Background: High prevalence of prescription errors in treatment of pulmonary tuberculosis (TB) in Taiwan has been reported in previous studies. Due to the complexity of treatment guideline for TB, frontline health workers for TB control often find difficulties in determining the correctness of anti-TB regimens. The aim of this study was to build a guideline-based computerized decision support system (DSS) for screening prescription errors in anti-TB treatments.

Methods: To facilitate the screening work for prescription errors, we’ve created a web-based DSS implementing the paper-based “Taiwan Guidelines for TB Diagnosis & Treatment, 3rd edition.” By means of knowledge engineering, standardized prescription guidelines were transformed into computerized decision table and if-then rules. An ease-to-use user interface was designed for input of required parameters. With a single click on the analysis function, users are allowed to read the analytic results by the DSS immediately. An alert module for sending severe prescription errors to relevant medical officers was also embedded.

Results: From preliminary testing for 62 regimens with 200 tablets of drug prescribed, this system had detected errors in 48% of drug combinations, 21% in dosages and 6% in frequencies. Sensitivity and specificity were, respectively, 0.93 and 0.59 in combination check, 0.95 and 0.91 in dosage check, and 1.00 and 0.98 in frequency check.

Conclusion: With the characteristics of high sensitivity of this DSS, frontline health workers for TB control may find it useful as a screening tool for anti-TB prescription errors. The low specificity in drug combination will be improved after adding in rules used in special considerations.

Methods: For non-standardized prescriptions, this system may have the potential for increasing treatment success rate and improving patient safety.

18.079  **Homeless Youth and Their Exposure to Hepatitis B and C in Tehran, Iran**

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Summary: Nowadays the issue of street children is one of the most important issues in societies from industrial to developing cities. There are approximately one hundred million children spending their life in the streets. With regard to the last description of the International Organization for street children, many of statistical results and numbers are reported less than the actual figures. Many countries are suffering from an individual relation the issue of street children despite possessing common properties, and by controlling this phenomenon each country will get a specific picture.

Methods and Materials: 203 street children who were picked up from different places of Tehran and settled at a welfare center which provides shelter for street children were chosen for this study. These children were clinically examined by a pediatrician and requested to answer the questionnaire asking about their gender; age; birth place; educational status; the origin of the family; sleeping place; occupation; income; social security of parents; number of siblings; reasons for being in streets; period of living in the streets; street friends; means of earning money; substance use. Smoking levels was classified as heavy (10 and more per day), medium (1–9 per day), and light (0–5 per day) by controlling this phenomenon each country will get a specific picture.

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Smoking levels was classified as heavy (10 and more per day), medium (1–9 per day), and light (0–5 per day).

Methods and Materials: In order to isolate specific RNA aptamers binding to the N protein selectively with a high affinity, RNA aptamers of 40 nucleotides in length were screened from a randomized RNA pool by the Systematic Evolution of Ligands by Exponential enrichment (SELEX) procedure. Binding affinity of the selected aptamers to the N protein was assessed by surface plasmon resonance and electrophoretic mobility shift assays. Aptamer immobilized on streptavidin-coated wells was used to capture N protein, which was then detected by anti-N antibody.

Results: After 9 cycles of selection, by sequence analysis and RNA secondary structure predictions along with analysis of binding affinity to the N protein, we selected two aptamers, aptamer 4 and 8, showing a high binding affinity with a binding constant of 3.3 nM and 0.8 nM, respectively, for further characterization. Electrophoretic mobility shift assays and RNA competition experiments revealed that the selected aptamers recognize the target with a high specificity and affinity, allowing us to use them to capture the N proteins prior to their detection with N protein-specific antibody. N proteins were easily detected by modified ELISA using the selected aptamers as capture molecules. Furthermore, RNA aptamers were arrayed using Dip-pen nanolithography for on-chip detection of N protein.

Conclusion: We have established a sensitive aptamer-based chemiluminescence immunosorbent assay for detection of SARS-CoV nucleocapsid protein.

18.080  **Development of a SARS Coronavirus Diagnosis Tool Using RNA Aptamers and Nanochip Fabrication**

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Background: Severe acute respiratory syndrome coronavirus (SARS-CoV) is the ethiological agent of a new emerged disease, SARS. The possibility of reemergence of SARS-CoV has been posing a threat to us because of its high infectivity and rapid epidemic spread. Rapid and accurate diagnostic tools for the early detection of SARS are required to cope with the future outbreaks rapidly. SARS-CoV nucleocapsid N protein can be detected at the early stage of viral infection before generation of antibodies as it is the most stable and abundant viral protein, and is thus a good target for early detection of SARS-CoV.

Methods: In order to isolate specific RNA aptamers binding to the N protein selectively with a high affinity, RNA aptamers of 40 nucleotides in length were screened from a randomized RNA pool by the Systematic Evolution of Ligands by Exponential enrichment (SELEX) procedure. Binding affinity of the selected aptamers to the N protein was assessed by surface plasmon resonance and electrophoretic mobility shift assays. Aptamer immobilized on streptavidin-coated wells was used to capture N protein, which was then detected by anti-N antibody.

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Conclusion: We have established a sensitive aptamer-based chemiluminescence immunosorbent assay for detection of SARS-CoV nucleocapsid protein.
Programme in the pilot area (Anambra state). Segregation of TB case finding is not practiced in the programme and contribution of PPM to case finding needed for routine programme monitoring and evaluation is often lacking. We looked at all the tuberculosis treatment registers in Local Government Areas where the PPM DOTS facilities are located segregating TB case finding according to facilities noting contribution from PPM sites. Results: In the 2-year period reviewed, 2005 and 2006, percentage contribution by PPM to all forms of TB cases detected was 10.03 and 12.11 respectively. This represented 110 out of 1097 cases (all forms) detected in 2005 and 141 out of 1164 cases (all forms) detected in 2006. Also 12.29% and 11.40% of new smear positive cases representing 84 cases out of 744 and 83 out of 728 SM+ cases detected in 2005 and 2006 respectively.

Conclusion: The PPM pilot project though implemented within 13 private health care facilities (compared to over 80 DOTS centers within the public sector in the state) contributed about 10% and about 12% of all cases detected in the state in 2005 and 2006 respectively. Scaling up of PPM DOTS services currently going on in the programme will no doubt contribute in increasing TB case detection by the programme.

18.082 Hepatitis A in Belgium: Reemerging Disease in a Susceptible Population
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Background: A prevalence study performed in 2003 using saliva test indicated a 20.2% (95% CI 19.43-21.08) hepatitis A prevalence rate in the general population. In Belgium, vaccination against hepatitis A is only recommended for travelers to endemic areas and for targeted risk groups. Will HAV infections emerge as an outbreak related threat affecting mostly some risk groups?

Methods: Descriptive analysis is based on data coming from outbreak investigation done by health inspectorate of the Communities and on database of the laboratory sentinel surveillance network.

Results: Current analysis of epidemiological trends in hepatitis A incidence from 1994 to September 2008 aims to demonstrate the impact of the increasing herd susceptibility.

Even if data from sentinel laboratory network give an underestimation of the real incidence, they allow to observe a steady decrease over time from 7/100.000 inhabitants in 1994 to 2.46 cases per 100.000 inhabitants in 2008. The age distribution of hepatitis A cases showed a highest incidence rates in younger age groups, namely 0-10 year old (7.70 per 100.000, pvalue<0.000). If most affected age groups are kept under 14 years old, a smaller peak is observed among the 21-40 years old which could be explained by travel related transmission. At country level, geographical distribution showed an overall decrease with marked peaks corresponding to outbreaks occurring in main cities. From 2006, outbreaks emerged in schools of urban underprivileged areas. Some characteristics of HAV infection; asymptomatic forms and long incubation period, make difficult to estimate attack rate and effective reproductive number in outbreaks while these information are required to discuss changes in vaccine policy. A better description of HAV outbreaks could be easily achieved by using systematically saliva tests.

Conclusion: Since Belgium faces an epidemiological transition period in HAV endemicity, in order to evaluate the necessity to implement further control measures such as universal HAV vaccination, missing epidemiological data have to be gathered. These data could be easily collected in prevalence study and outbreak investigation thanks to the use of saliva test.

Surveillance Using Informal Sources

18.083 Survey of TBEV Infected Areas by Sheep Flock Sentinels: Preliminary Results
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Background: Tick-borne encephalitis (TBE) is a human neurologic disorder caused by a Flavivirus (TBEV) which replicates predominantly in ticks. More than 100 species of wild and domestic animals may be infected and support between ticks virus transmission. Areas of TBEV infected ticks (i.e. foci) develop slowly and are identified mainly by autochthonous human cases or infected ticks. The paper reports results of a survey aimed to explore whether sheep grazing flocks are suitable sentinels of TBEV foci

Methods: Preliminary studies were performed to assess sheep flock immunity under natural TBEV exposure. These data were used to design a 2-years cross sectional survey in North Eastern Italian Alps. The municipality area represented the epidemiologic unit and the presence of TBEV within the municipality was estimated by sheep TBEV specific humoral immunity. Only flocks grazing within the municipality borders were enrolled and sampled by convenience out of all flocks sampled for the annual brucellosis surveillance. Sheep aged ≥ 24 months were sampled according to flock size and expected within flock prevalence of 20%. Sheep immunity was assessed using a commercial competitive ELISA (Test Line Clinical Diagnostics). The flock was considered TBEV exposed if at least one sampled sheep resulted positive and the municipality was considered exposed when at least one flock resulted exposed. To analyse the ability of sheep flocks to highlight TBEV infected areas (i.e. the system’s sensitivity) exposed municipalities were compared with known TBEV foci

Results: The survey involved 311 flocks and 1550 sheep. The study area comprised 627 municipalities and 143 were explored through at least one flock (range 1-19); 32 municipalities enclosed at least one TBEV exposed flock (range 1-4). Preliminary results indicate that in the study area four out of five TBEV foci were also identified by grazing flock and within the confirmed foci up to five municipalities resulted exposed.

Conclusion: Compared to wild animals, sheep flocks have the advantage to allow a better standardization of the system but may lack of sensitivity in areas with low flock density or grazing.

18.084 Efficacy of Pentavalent Rotavirus Vaccine, RotaTeq® Against Hospitalizations and Emergency Department Visits Up to Three Years: The Finnish Extension Study
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Six 12-week-old healthy infants were randomized to receive 3 oral doses of RotaTeq or placebo in the Rotavirus Efficacy and Safety Trial (REST). To determine if RotaTeq remains efficacious beyond 2 years after vaccination, ~21,000 infants in the Finnish safety cohort of REST were followed for healthcare encounters (HCEs), defined as rotavirus gastroenteritis (RVGE)-associated hospitalizations and/or emergency department (ED) visits, in the Finnish Extension Study (FES) for up to 3.1 years. The infants were contacted every 12 weeks to determine whether they had any RVGE-related HCEs. RVGE was defined as forceful vomiting and/or ≥3 watery or looser-than-normal stools within a 24-hour period and detection of rotavirus antigen by ELISA, plaque assay, and PCR assays (P and G types). Infants with RGE, who received 3 vaccine doses, were included in the analysis; follow-up started 14 days after dose 3. The maximum follow-up time in FES was 3.1 years (1126 days). Overall, RotaTeq reduced the
rate of RGE-associated HCE, regardless of rotavirus serotype, by 94% (95% CI: 91-96), demonstrating an overall reduction of HCE similar to that of REST 95% (95% CI: 92-97). See table for further details. RotaTeq significantly reduced RGE-associated hospitalizations and ED visits for up to 3.1 years of follow-up. The results of FES are consistent with the results of REST and confirm that the efficacy of RotaTeq remains consistent beyond two years, and up to 3.1 years.

18.085 National Melioidosis Surveillance Under Limitation

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Background: Southeast Asia is the Melioidosis endemic area, with the greatest concentration of cases reported in Thailand. This infectious disease caused by the gram negative bacteria Burkholderia pseudomallei which can found in contaminated water and soil and are spread to humans and animals through direct contact. Thailand has set up the Melioidosis as national notifiable disease; the annual cases and distribution of the cases have been archived to Bureau of Epidemiology.

Methods: The study were conducted on analyzing epidemiologic characteristics of national archived data from 2003 to 2007 and comparing The 2007 data with five year Median.

Results: Numbers of cases and morbidity rates have been increased during the past five year from 2003 to 2007: 0.57, 0.64, 0.88, 1.04 and 1.46 per 100,000 persons respectively. Comparing 2007 data with five year Median demonstrated the abnormal event and the cases occurred throughout the year but associated with rainy season. During the period, ages of these patients were from all groups of ages. Most cases were adult with age more than 35-year-old. The highest morbidity rates were in the northeastern part.

Conclusion: The burden of Melioidosis is an area of great public health interest, since it is a significance and costly public health problem. Although it is a reportable disease in the countries, but what is being report is not clear. Moreover, the researches on epidemiology are limited and etiologies of disease have not been evaluated. There are a number of laboratory methods, microbiology and serology to identify. However, each method has its own advantages and limited. The laboratory confirmation in provincial hospital is limited to blood culture. Most community hospitals do not have this kind of culture. Special technique confirmations are mostly in university, research and national. So that modern methods are often not in place in hospital laboratories, leading to underestimates of frequency of B. pseudomallei and lack of data on bacterial etiology. The number of patients infected reported is also considered a minimum. Clinicians will order microbiologic cultures only when the microbiology laboratory is available and serious infections are suspected. Most of the patients are not investigated by microbiologic procedures but are given empiric therapy instead. All positive cultures are considered to be infected cases since there has been no observation of colonization in humans.

18.086 Mobilizing Volunteers to Control Avian Influenza in Traditional Markets

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Background: Indonesia has the highest number of human fatalities resulting from highly pathogenic avian influenza (HPAI) infection. The Indonesia Community-Based Avian Influenza Control (CBAIC) project strengthens Government of Indonesia coordination, planning, and pandemic preparedness, through intensifying avian influenza (H5N1) control and prevention efforts by training and deploying 20,000 community volunteers in selected high-risk districts. Information generated by these volunteers has shifted CBAIC’s focus from backyard poultry producers to the commercial sectors of the Indonesian poultry industry. An underlying assumption is that CBAIC volunteers in these high-risk areas are capable of transferring the surveillance and reporting skills acquired in traditional production systems to commercial poultry marketing systems.

Methods: CBAIC has evolved from an emergency response mechanism into a program of selected field interventions. CBAIC provides essential training on HPAI detection, reporting and bio-security while the Indonesian Red Cross and Muhammadyiah, a faith-based NGO, provide local field support. Volunteers are trained to recognize HPAI in poultry, promote basic poultry bio-security measures and identify signs of human HPAI infection. In high risk communities, volunteers receive additional training on monitoring markets where live birds are slaughtered and sold. A geographic information system records the location and cell phone coordinates of all 20,000 volunteers.

Results: CBAIC volunteers are linked in a participatory disease surveillance and reporting system, allowing for near real-time reporting of suspected outbreaks in villages, markets and commercial entities. With limited additional resources, large volunteer resources can be effectively mobilized, trained and redirected to address new animal and human health threats.

Conclusion: A community-based volunteer contingent can reduce the risk of HPAI H5N1 virus transmission in Indonesia’s traditional markets, particularly if combined with focused private sector-led biosecurity measures for commercial poultry production and marketing.

Figure 1. HPAI Control Resources and major road crossings are mapped around a suspected HPAI outbreak in Indonesia.

18.087 Investigation of Epidemiological Trends Using Individual Lots of Intravenous Immunoglobulin (IVIG)


Background: Individual lots of IVIG, produced from pools of plasma obtained from thousands of healthy donors, reflect the serostatus of a segment of the donor population and therefore allow (1) to investigate epidemiological differences and trends in the respective donor population, and (2) to identify individual IVIG lots with functionally different anti-viral efficacy.

Figure 1. Rate reductions in rotavirus-Associated HCEs (Combined hospitalizations and ED visits), in REST and REST+FES, regardless of rotavirus serotype, by year.
Methods: Virus neutralization titers of different lots of IVIG prepared from either EU or US plasma were determined for (A) West Nile Virus (WNV), a flavivirus that has recently emerged in the US, (B) several Echovirus (EV) serotypes (9, 11, 13, 30) that continue to cause potentially severe neurological infections in people with immune deficiency (PID) and (C) Hepatitis A Virus (HAV), where vaccination increasingly replaces infection.

Results: (A) Analyzing IVIG lots manufactured from US plasma between 1998 and 2008, we demonstrate the occurrence of WNV neutralizing antibody titers in IVIG lots since 2003, and a steady yearly increase in mean WNV neutralizing antibody titers since. By now, approx. 30 % of all lots produced from US plasma contain WNV antibody titers that can be expected to protect against infection [1].

(B) Echovirus neutralizing titers were significantly different for the different serotypes investigated, depending on the geography of plasma sourcing, as well as donor demographics. (C) HAV antibody titers were different between IVIG lots derived from US versus EU plasma by ELISA, yet they were equivalent when tested by a functional neutralization assay.

Conclusions:

(A) We demonstrate the WNV seroconversion of a formerly naïve population by increasing mean WNV neutralizing antibody titers in IVIG lots manufactured from US plasma within the last ten years.

(B) Echovirus neutralization titers varied significantly, for different serotypes as well as different geographies and plasma donor communities.

(C) The changing nature of HAV exposure, i.e. infection earlier versus increasingly vaccination now, is reflected in the differences observed for antibody levels determined by ELISA versus neutralization assay, i.e. determination of antibody presence versus virus neutralization.

The understanding of changes in virus epidemiology and, as a consequence, the specific neutralization capacity of individual IVIG lots might be of potential clinical interest, especially for PID, who critically depend on the presence of protective levels of antibodies in IVIG.


18.088 International Epidemic Intelligence (EI) at the Institut de Veille Sanitaire (InVS)


Background: With 4% of its population living in overseas territories and almost 6% of its mainland population being foreign nationals France is also a tropical country. Various supranational institutions have developed Epidemic Intelligence (EI) activities. However, none of them fulfill specific French needs hence in 2002, InVS implemented a specific EI unit.

Methods: InVS EI is an event-based monitoring composed of 5 steps: detection, selection, validation, analysis and communication. The detection process is based on Internet. Informal sources are collected through specific EI devices (e.g. Gphin, Medisys, ProMed). Official sources are collected on governmental or supranational organisations websites. Specific criteria have been developed to select events according to the risk they might represent, the area of occurrence and the nature of the agents involved. Once the selected events are validated through official sources and a network of reliable contacts. Only validated events are characterised and analysed. Different communication supports are targeted to specific publics.

Results: From August 2005 to November 2008, 166 weekly epidemiological bulletins were published reporting 430 events of which 34% occurred in Africa, 28% in Asia, 5% in the Pacific and 19% in the Americas, 14% in Europe and the Middle-East. In addition, 73 thematic notes on 32 health topics were distributed.

This information is used to implement preventive control measures e.g. surveillance of imported cases. EI allowed to anticipate health threats before the first imported cases were identified in France (e.g. Rift Valley fever in Mayotte) and is used since 2003 to readjust the case definition for human A(H5N1) influenza surveillance.

18.089 Wildlife Rehabilitation Centers and Disease Surveillance

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Background: In the recent past emergence of diseases has frequently been related to wildlife and the wildlife-livestock-human interface. Some species of wild animals are known to be the reservoir for certain disease agents (e.g. waterfowl and avian influenza viruses). On the other hand, endangered wild animals are put at the brink of extinction by diseases introduced by man, livestock and/or companion animals. As a result of the recognition of the importance of wildlife for the ecology of some disease agents surveillance programs include recently wildlife (e.g. IA or WNV surveillance). Sampling of wild animals is not always easy as it implies capture of the individuals to be sampled in sufficient numbers which is both time consuming and costly. Wild animals, especially birds are admitted numbers to rehabilitation centers worldwide, representing a (biased) sample of the fauna of a given region. The admitted individuals are victims of accidents with manmade structures, and exceptionally diseased animals. Screening of the collected individuals can reveal interesting information.

Methods: Wild animals admitted to a rehabilitation center in central Spain were sampled routinely upon admission between 1997 and 2007. Plasma samples were stored at -20 °C and cloacal swabs were cultured for Salmonella. Plasma samples were analyzed retrospectively for the presence of antibodies against avian influenza virus.

Results: Mean prevalence of Salmonella was 3.26%, but varied from 0.78% to 4.63% between years. No increase in prevalence was observed throughout the ten year period. Serore prevalence of antibodies against influenza viruses reflected the dynamics encountered in active surveillance studies, with a peak in prevalence during the winter migration and just after the breeding period.

Conclusions: Although patients of a rehabilitation center represent a biased sample, important information can be obtained on disease occurrence if routine sampling is carried out upon admission of these birds.

18.090 Rapid Assessment of Health Emergency Communication after Hand, Foot, and Mouth Disease Outbreak in China

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Background: In April of 2008, an outbreak of HFMD occurred in Fuyang prefecture, Anhui province in China, and after that several other HFMD outbreaks were reported, which caused the widespread concerns both in the public and the media. As a key component of outbreak control, health emergency communication in China was a new study field and needed to conduct more field research and assessment.

Objective: To find out the public people awareness, their major channel of access to information and their primary concerns after the outbreak of the hand, foot, and mouth disease (HFMD), so as to provide scientific and evidence basis for more effective health emergency communication on infectious diseases outbreak in the future.

Methods: We used questionnaire survey and surveyed to 600 persons from three areas: Bozhou prefecture in Anhui province, Pudong New Area
Phase 4 announcement, a 3% probability of 425 or more human cases of influenza from the market as of December 10 include a 3% probability of a WHO Flu Home. As sampling of predictions arising in various countries on 6 continents were traded on the market. Real-time occurrence.

These prices can be interpreted as the consensus probabilities of event occurrence.

Results: As of December 4, 2008, 261 ProMED-mail subscribers from over 40 countries on 6 continents were trading on the market. Real-time prices are displayed on our web site, http://fluprediction.uiowa.edu/fluprediction.uiowa.edu/Market_AvianInfluenza.html. A sampling of predictions arising from the market as of December 10 include a 3% probability of a WHO Phase 4 announcement, a 3% probability of 425 or more human cases of H5N1 worldwide and a 8% probability of a new human case in Africa, all by December 31, 2008.

Conclusion: The probabilities generated by the market can help public health officials plan for the future and coordinate resources. While prediction markets will not replace existing avian influenza surveillance systems, we propose their use as a supplement to aggregate expert opinions quickly based on existing information.

**18.089 Use of a Prediction Market to Forecast H5N1 Influenza**

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Introduction: Indications are that the pandemic potential of H5N1 influenza is increasing, but the likelihood of that event and the timing and path of spread are all unknown. Information about H5N1 is disparate, geographically dispersed and often subjective, limiting the usefulness of traditional methods in the collection and interpretation of these data. Prediction markets have been successfully used to forecast future events with similar uncertainties in other fields. We adapt this new method to provide estimates of the likelihood of H5N1 influenza related events.

Methods: Participants are given educational grants of approximately $100 with which to trade financial contracts whose future values depend on the outcome of selected avian influenza watershed events. These events are based on policy, numbers and locations of human and animal H5N1 cases. For example, one contract will be worth $1.00 if Phase 4 of the WHO-defined Pandemic Alert Period is declared by 1/1/2009. After 1/1/09, it will cease trading and be replaced by a similar contract with a 7/1/09 target. Traders buy and sell contracts with one another at prices that depend on their beliefs about the likelihood of the underlying event. These prices can be interpreted as the consensus probabilities of event occurrence.

Results: As of December 4, 2008, 261 ProMED-mail subscribers from over 40 countries on 6 continents were trading on the market. Real-time prices are displayed on our web site, http://fluprediction.uiowa.edu/Market_AvianInfluenza.html. A sampling of predictions arising from the market as of December 10 include a 3% probability of a WHO Phase 4 announcement, a 3% probability of 425 or more human cases of H5N1 worldwide and a 8% probability of a new human case in Africa, all by December 31, 2008.

Conclusion: The probabilities generated by the market can help public health officials plan for the future and coordinate resources. While prediction markets will not replace existing avian influenza surveillance systems, we propose their use as a supplement to aggregate expert opinions quickly based on existing information.

**18.092 Contribution of the Nigeria National HIV/AIDS and STIs Behavioral Surveillance Survey (BSS) to the Reduction of HIV/AIDS Prevalence in Nigeria—Results from Review of the Research**

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Introduction: Nigeria, with an estimated population of 140 million people has one of the highest burdens of Human Immunodeficiency Virus (HIV) and the Acquired Immune Deficiency Syndrome (AIDS) in the world with prevalence rate of 4.4% (NARHS, 2005). From the first case officially announced in 1986, the Nation has witnessed rapid increase in the spread of HIV and AIDS resulting in rise in the national Sero-Prevalence rates based on sentinel screening of antenatal care attendees, from 1.8% in 1991 to 3.8% in 1994, 5.4 in 1999 and 5.8% in 2001. Thereafter, progressive decline has been witnessed in two subsequent national sero-prevalence surveys, with a reported rate of 5.0% in 2003 and 4.4% n 2005. Despite this encouraging trend, the HIV/AIDS remains a leading health and development in Nigeria.

The National HIV/AIDS and STIs Control Programme (NASCIP) of the federal Ministry of Health adopted Behavioural Surveillance Survey in the general population and among high risk group to identify the major risk behaviours that fuel the epidemic for effective policy formulation, programme planning and implementation.

Method: The BSS was carried out through a highly collaborative process overseen by National AIDS and STIs Control Programme of the Federal Ministry of Health, Society for Family Health, Family Health International and the Harvard AIDS Prevention Initiative in Nigeria. The data were quantitative in nature and collected through interview administered questionnaire. Four broad categories of high risk group were targeted: youths (15–24), female sex workers (both brothel and non brothel), male transport workers (long and short distance drivers and commercial motorcycle riders), uniformed service personnel (police and armed forces). Random sampling approach was used in the selection of participants.

Results: In the 2-year period reviewed, 2003 and 2005, there was a significant fall in the prevalence of HIV/AIDS in Nigeria—from 5.0% in 2003 to 4.4% in 2005 and was contributed mainly by behaviour change programmes that were evidence based from the previous BSS and IBBS surveys done in Nigeria and the result from NARHS (National AIDS and Reproductive Health Survey, 2001–2005).

Conclusion: The BSS though done in 23 out of 36 states of the country was representative and has contributed to the overall trend in the fall of the prevalence of HIV/AIDS in Nigeria. This formed the basis for and sustained advocacy for more Behaviour Change programmes in the multisectoral repsonse against HIV/AIDS in Nigeria being coordinated by NACA(National Agency or the Control of AIDs).

**Syndromic Surveillance**

**18.093 Modelling Strategies for Detecting Pandemic Influenza Based on Syndromic Surveillance of Confined Populations: Insights from a Stochastic Metapopulation Model**

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Background: Many reports of influenza pandemics describe outbreaks in confined subpopulations (CSPs). We investigate the sensitivity and time-sensitivity of a syndromic surveillance system based on investigating clusters of influenza-like-illness (ILI) in CSPs using a metapopulation framework to model the interaction of CSPs with the general population (GP).
Methods: The epidemic of influenza in the GP was based on the 1957 epidemic of pandemic influenza in Singapore (R0 about 2.2), and modelled deterministically, while stochastic transitions between Susceptible-Exposed-Infectious-Recovered states were assumed for CSPs. We computed the chance that outbreak investigations in one or more CSPs would be initiated before 1 May 1957, when the 1957 epidemic was noticed in Singapore, using the mean result from 1,000 stochastic realisations. CSPs were modelled after military camps, with 20 CSPs of 2000 individuals each, with 2/7 of their contacts being with the GP and the remainder being within the same CSP.

Results: Outbreaks in some CSPs would likely precede the GP epidemic (Figure 1a). For our default parameters, we found a >95% chance of observing outbreaks of >=10 symptomatic influenza cases in one or more CSPs before the time-point when the 1957 epidemic was noticed (Figure 1b). Moderate levels of within CSP mixing maximized the chance that outbreaks in CSPs would be noticed early (Figure 2a). The chances of observing an outbreak beyond the threshold for investigation increased dramatically on assuming that transmission within CSPs was relatively more intense than within the GP (Figure 2b).

Conclusion: Syndromic surveillance systems based in CSPs such as military populations may have an important role in detecting local transmission of an influenza pandemic virus. Surveillance signals may arise in one or more CSPs early in the course of an influenza epidemic. This is even more so if we assume higher transmission intensities within CSPs.

18.094 Alberta Veterinary Surveillance Network (AVSN: Comprehensive Real-Time Surveillance for Emerging Diseases of Livestock)


Background: Many emerging diseases of farmed livestock have been detected through adhoc efforts of farmers, veterinarians or pathologists. Emerging disease detection often begins with a diagnostic or pathological dilemma, in which a veterinarian or pathologist is faced with an unusual clinical or pathological disease presentation or pattern. Elucidation of the etiology requires a champion, someone who advocates for the resources necessary for a comprehensive investigation.

Methods: The goal of the AVSN is to organize, support and formalize a process for the timely detection and response to emerging diseases of livestock within the Province of Alberta, Canada. The AVSN collects, collates and analyzes real-time animal health data from many sources to identify signals and patterns in endemic diseases that may represent the emergence of new diseases. Near real-time data are collected from veterinarians who report to the AVSN data bases via a restricted access website. Free text clinical and postmortem diagnoses from submission forms for samples entering the laboratory for BSE testing are text mined and classified to facilitate further analysis. Farmers, veterinarians, public health workers, slaughter plant inspectors and members of the public can report unusual animal disease events to the AVSN. Event detection algorithms such as exponentially weighted moving averages, CuSums, SatScan and hidden Markov models have been developed in R statistical computing software (www.r-project.org) and deployed on a data mining platform (Knime: Constanz Information Miner, Michael Berthold, University of Constanz, Germany). The AVSN has an investigative unit capable of rapidly deploying surveillance veterinarians, epidemiologists, pathologists, toxicologists and other well resourced disease investigators when intensive disease investigations are needed.

Results: The AVSN has identified, investigated and resolved many unusual disease events that have arisen within Alberta’s livestock population. All of the disease events that have been identified and investigated are the result of unusual endemic disease presentations or patterns.

Conclusion: The AVSN has identified and responded to changing endemic diseases and has the potential to identify and respond to important emerging diseases of livestock in the province of Alberta.

18.095 Syndromic Surveillance, A New Concept of Surveillance

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Background: In August 2003, many countries in Europe, including France, were hit by an exceptional and unexpected heat wave. Surveillance systems dedicated to identify pathologies were inefficient for such an unexpected phenomena.

After the crisis, InVS and the Ministry of Health decided to create a non-specific surveillance system, to be implemented in emergency departments, emergency medical associations and mortality recording structures.

Method: Data is collected from medical files and mortality records and automatically sent via a file transfer protocol every evening to InVS. Syndromic groups and age groups are followed up daily. Each morning, temporal curves of these syndromic and age groups are analysed, firstly with automatic statistics tests, and secondly by an epidemiologist, if a statistical signal is found. The results of the 3 systems are eventually compared and, if necessary, clinicians are called for further clarification and investigation.

Results: In hospitals, 120 emergency departments are already part of the network and an extension to include more hospitals is planned.

In the community, 48 of the 60 emergency medical associations are part of the network and send daily data to InVS. Over 1000 cities send their mortality data. This represents approximately 70% of the daily deaths in France. The system is used to detect and follow up seasonal outbreaks as flu, bronchiolitis, gastro entritis... It is also useful in allowing estimates of the impact of environmental events (heat wave, polluted food or other product...) or to monitor the consequences of health events (chikungunya epidemics). Some examples will be illustrated.

Conclusion: Such a system of surveillance can detect unexpected events, only if they have a large impact on health. Its detection ability for events with lower impact is not yet verified. However, this requires networking between public health services and physicians, and thus events with lower impact are consequently notified to InVS.

It can also help authorities in measuring the impact of unexpected environmental events such as industrial accidents or natural disasters. However, it cannot replace other dedicated systems nor can it answer all the questions authorities ask. The great facility and access to the data must not diminish the use of scientific and epidemiological rigor when analysing the data.

In France, although the system is not fully operational it has already been used and has shown that it can work. The next step in its development is to expand it to the regions. Regional units of InVS will soon be in charge of the local analysis and animation of the network of the participating services.

18.096 Sensitivity and Specificity of Illness Symptoms versus Rapid Tests for Detecting Influenza in a University Setting

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Background: During an influenza outbreak, both symptom assessment and rapid diagnostic tests may facilitate the identification of influenza cases among sick individuals. We sought to evaluate the sensitivity and specificity of illness symptoms and two rapid tests in a university setting.

Methods: Students from five selected residence halls provided nasal and throat swab samples at collection sites in the halls or at the student health clinic. Samples were collected from students who presented acutely with symptoms of cough, plus fever/feverishness, chills, or body aches. At sample collection, participants were also asked to report the first day on which they experienced any symptoms. Three specimens were taken from each subject: one nasal swab for each rapid test (QuickVue Influenza A+B Test (Quidel; San Diego, CA, USA) and 3MTM Rapid Detection Flu A+B Test (3M Health Care; St. Paul, MN, USA)) and a throat swab for polymerase chain reaction (PCR). Logistic regression with backward selection was used to generate adjusted odds ratios (aOR) for the association between illness symptoms (cough, fever/feverishness, chills, body aches, headaches, sore throat, congestion) and days since onset of symptoms, in relation to PCR influenza positivity. Varying dichotomies of symptoms onset were assessed, and 0-3 vs 4-7 days were chosen based on statistical significance of regression results. The sensitivity and specificity of illness symptoms, symptom onset and both rapid tests were determined in reference to PCR results.

Results: Cough (aOR= 5.62, 95% CI = 2.74–11.55), feverishness (aOR= 2.45, 95% CI = 1.25–4.78) and onset of symptoms within 3 days vs 4–7 days (aOR= 4.86, 95% CI = 2.21–10.63) remained significantly associated with a positive PCR sample after adjustment. The sensitivity and specificity for cough plus feverishness within 3 days of onset were 0.58 (95% CI = 0.46–0.69) and 0.83 (95% CI = 0.77–0.87), respectively. The QuickVue and 3M tests had similar sensitivities: 0.19 (95% CI = 0.11–0.30) and 0.18 (95% CI = 0.10–0.28), respectively and specificities: 0.96 (95% CI = 0.97–1.00) and 0.96 (95% CI = 0.92–0.98), respectively.

Conclusion: Cough plus feverishness within 3 days of symptom onset was more sensitive than either of the rapid tests. There were no differences between the QuickVue and 3M tests, which showed low sensitivity but high specificity. Clinical evaluation of symptoms may be a more reliable method than rapid tests for monitoring disease incidence during influenza outbreaks in a university community setting.

18.097 The HealthySocial Project: Monitoring Public Health and Promoting Positive Health Behaviors Through Social Networks

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The HealthySocial project explores different approaches to using social networking tools for monitoring public health and promoting positive health behaviors. HealthySocial applications are already in use by thousands of people in over 90 countries around the world. Unlike many other applications in the emerging space of health-related social applications, HealthySocial applications motivate participation by explicitly leveraging the interactive social features of a social network in order to improve health. This includes empowering friends to reinforce each other’s positive health behaviors as well as leveraging existing social networks to spread positive health habits.

Figure 1. The “Get Well Soon” interactive social network application represents a new type of informal data source for public health situational awareness.
We will describe three sample applications: the first is an interactive social application aimed at encouraging influenza vaccination and includes information resources on vaccination. The second application is aimed at encouraging blood donation, including an online donation journal and information resources about blood donation. The third application allows friends to report their symptoms to one another and send each other images and gifts. This application also provides resources with anonymous recent total aggregate counts of different symptom categories in their networks. This represents a new type of informal data source for public health situational awareness.

Many previous attempts at public-sourced syndromic surveillance have suffered from a critical lack of user motivation. Our approach is different in that users are motivated by the immediate and personal incentive of friend-to-friend communication, while also contributing to and benefiting from aggregate syndromic statistics.

Securing the privacy and security of all user information is especially important within social networks where users are accustomed to freely sharing information with others. We will discuss the broader implications of these and other issues for the emerging field of health-related social applications.

**18.098**

**Outbreak of Mycobacterium abscessus Type I Infections Following Esthetic Surgery at Private Clinics in Vila Velha City (Brazil)**

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During the last years several outbreaks of infections caused by rapidly growing mycobacteria following esthetic surgeries were described in literature. The authors reported 15 cases of M. abscessus type I infections in women with age between 20 and 49 years old (mean=35.8) in private clinics at Vila Velha city (Brazil): nine of mastitis, five mastitis and abdominal wall infection and one limited to gluteus region. These cases followed different surgical procedures performed in only one moment by one surgeon (10) and another (5) at this clinics between Feb 12th and July 29th 2008: liposuction and lipo-graft (1), lipo-aspiration and -graft, implant of breast prosthesis and abdominoplasty (12) and lipo-aspiration and -graft, implant of breast prosthesis, abdominoplasty and colostectomy (2). Involvement of both breasts was observed in nine cases and only one in five. The most common clinical complaints included hyperemia, edema, spontaneous drainage of fluid and nodules. AFB were observed in 6/15 (40%). Strains of M. abscessus type I (identified by PCR restriction analysis) were isolated of 14/15 cases (93.4%). Based on the results of minimal inhibitory concentrations (broth microdilution), clarithromycin 500 mg/12/12 h per os and amicacin 1 g/day intravenously were taken. In one case, amicacin was, later on, changed for levofloxacin. Among the nine cases with abscesses and spontaneous drainage of fluid before beginning of antimicrobials, these lesions closed in less than one month in two cases, between one and three months in four and after three months in another. One patient began antimicrobial therapy two days before and another 45 days ago. The side effects included diarrhea (4 [one case of C. difficile infection]), amarum taste (6), alopecia (1), nausea (4) and somnolence (1). After introduction of antimicrobials, surgical drainage of abscess were performed in two cases. Investigation of infection source is in course.

**Regional Disease Surveillance**

18.099 – 18.138 Room: Bruckner/Mahler/Brahms – First Level

**Prevalence of Human Herpes Virus Type 1 (HHV-1) Infection Among the Chronic Fatigue Syndrome Patients (CFS) in Northeast of Iran**

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**Background:** CFS is a disorder of unknown etiology that probably has an infectious basis. Many studies have been reported elevated serum antiviral antibody titers in patients suffering from CFS; so a viral component in the pathogenesis of this disease has been suggested. Among the variety of viruses evaluated to date, including enteroviruses, retroviruses, and human herpesviruses (HHVs), there is controversial report about HHV-1 and HHV-2 CSF-association. So our goal was to seek out any relationship between CFS and HHV-1 infection.

**Methods and Materials:** After a precise medical examination, 48 patients were found to fulfill the Centers for Disease Control (CDC) classification for CFS and 35 individuals were selected as control group. IgG and IgM antibodies to HHV-1 were measured in serum of all samples obtained from patients and control group using ELIZA method.

**Results:** Both patient (93.8%) and control (97.1%) groups had positive IgG antibody titer and negative IgM antibody titer (100%). There were not any significant differences (P>0.05) between CFS patient’s IgG antibodies (115.8 ± 38.58) versus control (105.78 ± 35.68). Also any significant differences (P>0.05) was not revealed between patient’s IgM antibodies (0.42 ± 0.13) and control (0.46 ± 0.13).

**Conclusion:** Our data didn’t show any relationship between CFS and HHV-1 viral infection and this was in parallel with a few studies with the same conclusion. CFS is unlikely to be caused or maintained by a single agent. So several strains of HHVs and other viruses could simultaneously infect the body and therefore more studies needs to be done in this field for clarifying the viral infections as etiology of CFS.

**18.100**

**Onychomycosis in Northern Iran**

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**Background:** Onychomycosis is common in our community. The most complaint of these patients is worry from visual form and it’s frequent relapses. Because of high prevalence in wet climate such as studied region and lack of researches in this subject, we decided to study this disease and determine the presentation factors.

**Methods:** Recent study done on patients refer to Tonekabon hospital clinic of dermatology in 2007. Diagnosis of disease is based on clinical manifestation and in next level (if suspected in diagnosis based on culture).

**Results:** From 210 patients suspected to onychomycosis due to candidia 75 patients (35/7%) were positive culture. Most of them were men (64%) and most of the patient were in 61–0 years old (41/1%). The disease had high prevalence in farmers (52%) and house wives (24%) and who had very contact to water (80%) and history of nail trauma (68%). The most common clinical presentation that reported was DLSO (Distal lateral subungual onychomycosis) (100%). There wasn’t any defined relations between sex and clinical view of onychomycosis.

**Conclusion:** Because of onychomycosis is due to candidia is a relapsing disease and control of causing factors have a high role in presentation of it’s relapses with more studies on causing factors we can prevent this disease significantly.
18.101 Tinea Pedis and Onychomycosis in Type 2 Diabetes Mellitus Patients

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Background: Foot disorders is the main reason for mortality, disability and morbidity in diabetic patients. Tinea pedis and onychomycosis are seriously and futher in this patients. So, because of high incidence of diabetes, we decided to search incidence of tinea pedis and onychomycosis in these patients and use its results to improve their quality of life.

Methods: This search is obtructive and descriptive and was done between diabetic patients who come to Shahid–Rajai hospital and non diabetics who come to dermatology clinic of hospital in the northern Iran.

Results: In this search, 120 cases considered who suspected to tinea pedis and onychomycosis, that 66 cases were male (%55) and 54 cases were female) 45 (%. between 120 cases, 22 cases had Tinea pedis and onychomycosis that 14 cases were diabetic (63.3%) and 8 cases were non diabetic. The most clinical type of tinea pedis were (toe cleft) 9 cases (29 %). The most clinical type of onychomycosis were DLSO (Distal Lat- eral sub ungula onychomycosis) 19 cases (82.6 %). The most pathogenic fungi was Trichophyton rubrum 9 cases (40.9 %). Between 14 cases of diabetic patients who had tinea pedis and onychomycosis 9 cases had ground disease (neuropathy and vascular disorders) 40.9 % between 14 cases of diabetic patients, 6 cases had blood sugar higher than 300 (10 %) that 5 of them had tinea pedis and onychomycosis. (8.3%). Between 14 cases of diabetic patients who had tinea pedis and onychomycosis 11 cases had diabetes more than five years.

Conclusion: In this search diabetic patients more than non diabetics had Tinea pedis and onychomycosis, and high blood sugar is a risk factor to get fungi. In diabetic patients there is direct connection between ground disease and Tinea pedis.

18.102 The Prevalence of M. tuberculosis Infection and Disease in HIV Positive Individuals in Shiraz, Southern Iran

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Backgrounds: To determine the HIV-seropositive individuals with mycobacterial infection referring to Center for Counseling and Behavioral Modification in Shiraz, Southern Iran.

Methods: From January 2004 to December 2006, 459 HIV-positive indi-viduals who referred to Center for Counseling and Behavioral Changes in Shiraz, southern Iran were enrolled. HIV antibody tests were ELISA and western blot tests and for M. tuberculosis infection were PPD skin test, chest x-ray, Ziel-Neelsen technique, culture in Lowenstein-Jensen medium, CD4+ T cell count and pathological examination.

Results: 28.5% of HIV-positive individuals had positive PPD skin test among them, 89.3% showed a latent tuberculosis infection and 10.7% active tuberculosis. Mean induration of PPD skin test in patients with latent tuberculosis was 11.13 mm. 7.9% of HIV positive patients had active tuberculosis including pulmonary (75.8%) and extrapulmonary types (24.2%). Among extrapulmonary cases, 62.5% had TB lymphadenitis, 25% pericarditis and 12.5%, TB pleuritis. 40% of patients with pulmonary tuberculosis and 50% with extrapulmonary TB had positive PPD skin test. Mean CD4+ T cell counts of patients with latent tuberculosis infec-tion, pulmonary, and extrapulmonary tuberculosis were 287, 189, and 138 respectively. Mean CD4+ T cell count in HIV-positive individuals with neg-ative PPD test was 283.

Conclusion: As tuberculosis is a common opportunistic infection in HIV-positive patients in Iran and with a higher prevalence of extrapulmonary type and the complex clinical presentation of disease, HIV-positive patients should be regularly screened for tuberculosis. Early recognition of latent tuberculosis infection and adequate chemoprophylaxis seem essential too.

18.103 Epidemiology of listeriosis in Austria

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Background: In Austria, a total of 150 human cases of listeriosis (case definition based on isolation of Listeria monocytogenes from normally sterile material) were reported from 1997 to 2007.

Methods: In Austria, listeriosis is a notifiable disease and isolates are sent to the National Reference Centre at AGES. Data from 1997 and 2007 were evaluated to show the epidemiological trends within Austria during that time.

Results: A total of 14 cases (9.3%) were pregnancy-associated (when both mother and child are ill, this is considered a single case). The mean age of pregnancy-associated cases was 29.3 (median: 26.5; range 24–36 years). Among the non-pregnancy-associated cases (n=136), 75 included men (55.2 %) and 61 included women (44.9 %). Patients from non-pregnancy-associated cases had a mean age of 64.3 years (median: 66.2; range 1–93 years). The average incidence of listeriosis in Austria from 1997–2007 was 0.168 cases per 100,000 population. The majority of listeriosis cases (90.7 %) caused systemic infection, while only 9.3 % of cases caused local infection. The case fatality of non-pregnancy-associated cases from 1997 to 2007 was 28.7% (39/136). Among 14 pregnancy-associated cases reported between 1997 and 2007, 5 (35.7%) resulted in death (miscarriage x3, stillbirth x1, and death in a newborn within 15 days of birth). Serotyping results for the 150 isolates revealed serovar (SV) 4b: 54 %, SV 1/2a: 31.3 %, SV 1/2b: 10 %, SV 1/2c: 2.7 %, 4d: 1.3 %, and SV 3a: 0.7 %. Predisposing risk factors could be determined for 131 of the 150 cases: age ≥65 years (n=73), pregnancy (n=14) and 44 cases of car-cinoma, blood malignancies, autoimmune diseases, and status post solid organ transplants (7 patients showed more than one underlying illness).

Conclusion: During the study period, the incidence of listeriosis dou-bled, despite a drastic reduction in the frequency of pregnancy associated cases. As in other countries, the reason for this increase is unclear.

18.104 Spatial Variation in Drug Therapy and Treatment Outcome for Tuberculosis in Scotland

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Background: Tuberculosis (TB) surveillance in Scotland through the Enhanced Surveillance of Mycobacterial Infection (ESMI) scheme provides information on the demographics of TB cases and their treatment and outcomes. We aimed to investigate spatial and demographic factors associated with receiving different treatment regimens, and with successful treatment outcomes.

Methods: Anonymised ESMI data from 2000–2006 for patients aged over 15 years were geo-referenced based on home postcode. Spatial and spatio-temporal clusters were detected with a scan programme. A Bernoulli model was used to compare non-standard therapy (≤3 drugs) with standard therapy (≥4 drugs); and successful with non successful treatment outcomes. Logistic regression was utilised to determine associated demographic and social risk factors, with a significance level of p<0.05.

Results: 93% of records could be georeferenced. 50.7% of 2249 cases had non-standard therapy. Spatial and spatio-temporal analysis identified two significant non-overlapping geographical clusters. Logistic regression showed the likelihood of receiving non-standard therapy was significantly increased with being female, having no previous history of TB; and decreased with being non-Caucasian, born outside the United Kingdom and aged 15–24, 25–34 or 45–54 years (reference age-band 75 and over).
1345 (69.9%) of cases up to 2005 had a recorded treatment outcome. 1145 (85.1%) of cases had a successful treatment outcome. Spatial analysis identified one significant geographical cluster. Logistic regression showed the likelihood of successful treatment was significantly increased in those aged less than 75 years, non-Caucasian with no recorded risk factors for TB. The likelihood of successful treatment was significantly decreased in those over 75 years. The incidence of TB decreased during the past 10 years. The peak of TB incidence in immigrants peaked with 0.72/100,000 in 1999 and between 2002 and 2006 with 2.45/100,000 in 2004. Conclusions: Distinct spatial and demographic patterning exists in drug therapy and treatment outcome for TB in Scotland but further investigation is required to understand the public health implications.

18.105  Seroprevalence of Leptospirosis in São Paulo, Brazil
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Background: Leptospirosis is a zoonotic disease that has now been identified as an emerging infectious disease. It is caused by pathogenic spirochetes of the genus Leptospira. The natural hosts for Leptospira spp. come from a variety of species of which the rodent is the most important reservoir. Leptospirosis epidemics are often related to heavy rainfall and flooding favorable for rodent infestations. Because of its climate, São Paulo may be at high risk for leptospirosis. This study was undertaken to study the epidemiological profile of urban leptospirosis in the city of São Paulo, Brazil.

Methods: Retrospective study was undertaken from January 2003 to December 2007. A laboratory-confirmed case is defined as 4-fold rise in titer between acute and convalescent serum samples and presumptive when a single sample showed a minimum titer of 1:200 or when two or more samples did not show a four-fold increase in titer.

Results: A total of 520 cases were recorded. A peak was observed in 2004 and 2007 with 107 and 136 cases, respectively. The mean annual incidence rate was 0.53 per 100,000 population. Although leptospirosis occurred in persons of all ages, middle-aged adults were most frequently infected: 23.46% of the cases were in adults from 31 to 40 years of age, mostly males (85.58%) with an overall ratio of males to females of 5.9:1. Among the cases, 406 (78.08%) were considered serologically confirmed cases and 114 (21.92%) presumptive cases. Cross-agglutination with at least two serogroups occurred in 92 cases. Icterohaemorrhagiae was the predominant serogroup (257 cases, 57.11%), followed by Autumnalis (34 cases, 6.54%) and Cynopteri (30 cases, 5.77%). The seasonal summer distribution was evident in all years, with the peak months being December to April. Several days of heavy rainfall were followed by an increase in laboratory-confirmed cases of leptospirosis.

Conclusion: This neglected disease remains as a public health problem in the country. An understanding of the relationship between leptospirosis incidence and heavy rains is indispensable for implementing appropriate preventive measures.

18.106  Tuberculosis in Austria: An Analysis of the Past 10 Years, 1997–2006
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Background: The objectives of this surveillance data analysis were to describe the burden of tuberculosis (TB), a mandatory notifiable disease, in Austria from 1997 to 2006 and to elucidate the relevance of immigrants for TB incidence.

Methods: The annual incidence of TB and multi-drug-resistant (MDR)-TB was calculated from the cases recorded for the years 1997–2006 at the national TB-Reference Center. Changing of TB burden over time was measured by simple linear regression or log-linear regression model. The incidence estimates were compared between immigrants to Austria and Austrian citizens by chi-square test.

Results: A total of 11,496 TB-cases including 7,009 culture-positives were recorded. During the observation period, the TB-incidence decreased significantly by 0.77/100,000 inhabitants per year (total incidence percent age change: 41.3%). The incidence was significantly higher in immigrants: in 2000 with a 3.99 times higher incidence and in 2005 with a 6.06 times higher incidence. The TB-incidence in Austrian citizens decreased linearly by 0.91/100,000/year: their highest MDR-TB-incidences recorded were 0.03/100,000 in the years 1997, 1998, 2001 and 2003. The MDR-TB-incidence in immigrants was multiple times higher compared to Austrians (ratio range = 10.66–infinity) in all 10 years. Between 1997 and 2002 the MDR-TB-incidence in the immigrants peaked with 0.72/100,000 in 1999 and between 2002 and 2006 with 2.45/100,000 in 2004. Conclusions: As in other European countries, the TB-incidence in Austria decreased during the past 10 years. The peaks of TB-incidence in 2000 and 2005 and of MDR-TB-incidence in 1999 and 2004 in immigrants reflect the population mobility across Europe. The highest number of immigrants to Austria between 1997 and 2006 were recorded in 1999 and in 2004, mirroring the war in Kosovo and in Chechnya—both high TB-endemic areas. The influx of TB from abroad did not influence the burden of tuberculosis in Austrian citizens.

18.107  Invasive Pneumococcal Disease in Gran Canary Island 2004–2007
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We present the epidemiological descriptive characteristics and the temporal distribution in Invasive Pneumococcal Disease in general population and the mostly prevalents serotypes in children lower 5 years in Gran Canary Island, during the temporal period 2004–2007.

Prospective study of the Invasive Disease confirmed and notified cases to the Gran Canary Microbiological Informative Sistem, between 2004–2007, and hospitalized in Gran Canary with laboratory diagnosis as S. Pneumococcal in blood, Cerebral Spinal Fluid, or in other usually sterile places and acute disease with a compatible clinic of Pneumococcal Disease.

In the studied period, there were notified 196 cases, 40 (20%) in 2004, 63 (32%) in 2005, 43 (22%) in 2006 and 50 (26%) in 2007. Also the 29% of sickness were below 5 years old, the 30% between 5 and 65 years and the 32% older 65 years. The total number of male affected was 125 and of female was 71. Relations Male/Female: 1.76 In children lower 5 years only rested 9 processes without tipifying. Of the 48 tipified, 62.5% were not predicted by the 7-valent conjugate vaccine. The serotype most identified was the 19A (27%), and the 14 (12%) 2.

We must remark the presence of this disease in older people. The appearance of the isolated vaccinal serotypes could be conditioned by the mentioned use of the vaccine in a high percentage of lower 5 years old population. In respect to years 2004–2005, in 2006 was detected a higher number of serotypes not predictables for the vaccine (69%), over the predictables (31%). In 2007, 87.5% not predictables and 12.5% predictables. We have to increase the knowledge of this disease with a continued alertness, remarking the need of to confirmate the diagnosis and have isolations to identify the causar serotypes.
Sanitaire (InVS, France’s national institute for public health surveillance) identified Rift valley Fever (RVF) epidemics in Eastern African countries, Republic of Comoros and Madagascar in 2006–2007. Animal surveillance of Rift Valley Fever was therefore implemented in Mayotte in 2007 which found evidence of RVFV in livestock. This led to serological screening in humans.

**Method:** Patients presenting with dengue-like illness and negative for dengue, chikungunya, malaria and leptospirosis were systematically tested for direct (PCR) or indirect (IgM antibody) evidence of RVF virus infection.

**Results:** Of over 250 sera collected retrospectively or prospectively from patients with unspecified acute febrile illness (UAFI) in Mayotte from September 2007 through November 2008, 10 were positive for RVF virus RNA and for IgM. One case with underlying pathologies died. The last documented symptomatic case developed signs in May, 2008. An additional, asymptomatic case was found through screening of highly-exposed animal health professionals.

**Conclusion:** These 11 human cases are the first recorded in Mayotte. RVFV is an emerging cause of UAFI among the population in Mayotte in 2007–2008.

18.109 Zoonoses in Bosnia and Herzegovina: A Common Approach
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Global changes in reported spectrum of human infectious diseases made an impact in Bosnia and Herzegovina (B&H). Expected to be eliminated as a relevant public health problem in B&H decades ago, infectious diseases unfortunately still remain a major concern, which is even becoming more serious due to dramatic changes in the society and the environment as well as to low effectiveness of disease control and prevention measures. Social turmoil and disruptions in provision of health services as a consequence of the recent war have amplified environmental, economic and welfare impact of already present "old" diseases and "new," emerging, diseases. Based on the officially reported data through different surveillance systems on several selected zoones—Q fever, Brucellosis, Anthrax, and Trichinellosis for the last decade, as collection of data on zoonotic diseases in B&H is mandatory by law for both animal and human sectors, a review on their (re)emergence and surveillance among both humans and animals in Bosnia and Herzegovina is conducted. The data analysis shows similar trends in both populations.

18.110 Development of a Multi-Locus Sequence Typing Scheme for *Laribacter hongkongensis*, a Novel Bacterium Associated with Freshwater Fish-Borne Gastroenteritis
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**Background:** A multilocus sequence typing (MLST) system was developed for *Laribacter hongkongensis*, a novel bacterium associated with freshwater fish-borne gastroenteritis and traveler’s diarrhea.

**Methods:** A total of 146 *L. hongkongensis* strains, including 39 strains isolated from human and 107 strains from fish, were used in this study. Fragments (362 to 504 bp) of seven housekeeping genes were amplified and sequenced. The nucleotide sequences of the seven gene loci used for MLST in all the *L. hongkongensis* strains were aligned and compared with those of strain HLHK1. The genetic relatedness among isolates was then determined by comparison of the nucleotide sequence types.

**Results:** Among the 3068 bp of the seven loci, 332 polymorphic sites were observed. The median number of alleles at each locus was 34 [range 22 (IvC) to 45 (thiC)]. All seven genes showed very low dN/dS ratios of < 0.04, indicating that no strong positive selective pressure is present at all seven loci. A total of 97 different sequence types (STs) were assigned to the 146 isolates, with 80 STs identified only once. The overall discriminatory power was 0.9861. eBURST grouped the isolates into 12 lineages, with six groups containing only fish isolates and three groups only human isolates. Standardized index of association measurement showed significant linkage disequilibrium in both human and fish isolates, indicating a lack of evidence of recombination in both populations. The standardized index of association for the human and fish strains were 0.270 and 0.636, indicating the fish strains were more clonal than the human strains.

**Conclusion:** The clustering of fish and human isolates into different groups observed in both the previous pulsed field gel electrophoresis and the present MLST studies suggested that some clones of *L. hongkongensis* could be more virulent than others.

18.111 Age Distribution of Paediatric Rotavirus Gastroenteritis in Europe: The REVEAL Study
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Rotavirus is the most common cause of severe acute gastroenteritis (AGE) in infants. Almost all infants will have been infected by 5 years of age. The REVEAL Study was the first large prospective, international, observational study to investigate systematically the burden of AGE and RVGE in children <5 years old in selected areas of Europe in 3 clinical settings (primary care, emergency and hospital) using a common protocol. Nosocomial cases were not included. The study estimated annual incidences of paediatric AGE and RVGE in selected areas of Belgium, France, Germany, Italy, Spain, Sweden, and the UK during the 2004–2005 season. 2846 infants with AGE were included. ELISA results were available for 2712 infants; 1102 (40.6%) were found to be rotavirus positive and the age for 1099 infants was available. Overall, 86% of RVGE cases occurred in infants aged between 3 months and 3 years; 2% of cases were observed in infants under 3 months of age. Only 1% to 6% of cases occurred in infants over 4 years of age depending on the area. The majority of RVGE cases in all settings were observed in infants aged 3-36 months: hospital from 78.9% (Spain) to 94.9% (Belgium); emergency department from 83.3% (Sweden) to 100% (UK); and primary care from 80.4% (Italy) to 89.8% (Belgium). The majority of RVGE cases, 86%, occurred in the main risk period of 3 months to 3 years of age in children in Europe. Routine rotavirus vaccination of infants before 7 months of age, which has been shown to be effective in this main risk period, could significantly reduce the substantial burden of this potentially serious childhood disease.

*Rotavirus Gastroenteritis Epidemiology and Viral Types in Europe Accounting for Losses in Public Health and Society*
**ABSTRACTS**

**18.112** Helicobacter pylori VacA Intermediate Region and Its Clinical Relevance in Iranian Dyspeptic Patients

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Introduction: It seems that the intermediate region has a decisive role in vacuolating activity such that the i1 and i2 subtypes were described as vacuolating and non-vacuolating types respectively. Moreover, a strong association was reported between the i1-type strains and severe H. pylori associated clinical outcomes including peptic ulcer and gastric adenocarcinoma. This study was aimed to assess the status of intermediate region and its prospective value in screening high-risk patients.

Methods: This analysis was performed on a total of 207 H. pylori strains obtained from NUD (n=139), PUD (n= 34), and GC (n= 34) patients. Genotyping of the intermediate region was performed by running two PCR sets for each sample using VacF1 as forward primer for both i1 and i2 regions and C1R and C2R as reverse primers for i1-specific and i2-specific reactions respectively.

Results: More than half (53.3%) of our strains were clustered as i1 type and the remaining strains possessed the i2 genotype. Approximately 88.2% (30/34) of GC patients were colonized with the i1 type strains, compared to 43.9% of NUD patients (P=0.001) and 58.8% of PUD patients (P=0.009) who possessed this type of strains. In multivariate analysis with adjustment for patients age and sex, the association of i1 type (GC vs. NUD; P= 0.001, OR= 8.3; 95% CI: 2.5-27.30) and age (OR= 1.08; CI: 1.04-1.11) with GC remained significant.

Conclusion: The presence of the i1 type can be considered as a predictive marker for gastric cancer. This study proposes the application of intermediate region in screening of H. pylori-infected patients at risk of GC development.

**18.118** Detection of an Unknown Flavivirus in Aedes caspius Mosquitoes in Valli di Comacchio (Italy)

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**Abstract**

Mosquitoes were collected with CO2 traps in a variety of wetland locations near Comacchio (Ferrara province) at the end of mosquito season (September-October). Mosquitoes were pooled according to date, location, sex and species. Pools were tested with a Flaviivirus genus PCR targeting gene NS5 (260bp) for the simultaneous detection of all flavivirus causing important human and animal disease according to Scaramozzino et al. (2001). Positive Pools were sequenced and analyzed using the ClustalX 2 program.

**Results**

A total of 1267 mosquitoes (81 pools) belonging to the species Aedes caspius, Aedes albopictus and Culex pipiens were tested and 3 pools of Ae. caspius resulted positive for Flaviivirus genus. The 3 positive pools were sequenced and the Blast analysis revealed a certain similarity (less than 80%) with Culex Flaviivirus (CuFV) isolated from Culex pipiens in Japan. Figure 1 shows the Neighbor Joining tree analysis, performed on the same 260 bp fragment of different flavivirus put the detected virus together with flaviviruses isolated only from insects (CuFV, cell fusing agent and Kamiti River virus). This group of sequences is clearly separated from flaviviruses causing diseases in humans and animals.

**Conclusion:** The detected flaviviruses probably belong to a group of virus that are present only in mosquitoes species, so this virus does not represent a risk for human and animal populations. At the same time, these positive PCR detections demonstrate that a flavivirus causing human and animal diseases could be detected, if present, by our regional monitoring program. The presence of these mosquito flaviviruses must be taken into consideration in any future monitoring programs conducted in the same area. Finally, future experimental investigation is needed to characterize this virus.

**Figure 1.** Neighbor-Joining Phylogeny tree of the new Ae. caspius flavivirus and other diffuse flaviviruses. Abbreviation (accession number): CuFV: Culex flavivirus (AB 262759); CFA cell fusing agent (NC 001564); KRV Kamiti River virus (AY149905); TBE: tick-borne encephalitis (DQ898338); YF: Yellow fever (DQ235229); DEN: Dengue (EF105386; EU081229; AY762085); ZIK: Zika (AY632535); JE: Japanese encephalitis (NC 001437); USU: Usutu virus (NC 006551); KUN: Kunjin virus (EF536932); WN: West Nile virus (FJ159131).

**18.114** EpiSouth: From a European Project to a Mediterranean Network for the Control of Communicable Diseases

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**Background:** The Mediterranean countries share common epidemiological characteristics and public health problems. In 2005, the “Year of the Mediterranean,” some Public Health Institutes (PHI) proposed a framework of collaboration for communicable diseases surveillance and training in the Mediterranean Basin. This initiative led to EpiSouth Project, co-funded by EU Public Health Programme (DG SANCO) and by Italian MoH (EpIMed Project).

**Methods:** EpiSouth works through WorkPackages (WP) lead by PHI. The project is coordinated by the main partner (ISS, Italy) while three WPs, “Cross-border epidemic intelligence” (InVS, France), “Vaccine Preventable Diseases and migrants” (NCIPD, Bulgaria) and “Cross-border emerging zoonoses” (HCDCP, Greece), constitute the technical pillars on which the project develops. “Networking” (PTH, Italy) and “Training” (ISCIII, Spain) are WPs dedicated to capacity building. The Project Steering Committee guides the activities while all countries collaborate through WP Steering Teams and Focal Points.

**Results:** Since its starting in 2006, EpiSouth struggled to develop its Mediterranean vocation. From an initial involvement of 5 countries (Italy, Spain, France, Greece and Bulgaria) it includes now 26 countries of Southern Europe, Balkans, North Africa and Middle-East and international
mechanisms. CagA-positive clinical strains with an increased number of EPIYA tyrosine phosphorylation motifs have been associated with more severe active gastritis and atrophy. Significant heterogeneity among Iranian and western Helicobacter pylori strains, emphasizes the need for using native antigens in developing serological assays. The aim of this study was to construct a prokaryotic system for expression of variant forms of CagA gene from Iranian Helicobacter pylori isolates.

Methods and Materials: N-terminal and C-terminal fragments of cagA gene with different EPYIA types among Iranian Hp strains were amplified by designed specific primers and cloned into cloning and expression vectors, followed by confirmation through PCR, partial sequencing and restriction digestion analysis. The recombinant CagA fragments were expressed under IPTG induction. Identity confirmation was performed that Bab proteins and bab genes family could be potential risk factors for predicting HP-associated disease outcomes.

**18.115**

**Genotypic and Phenotypic Profile of the Outer Membrane Proteins BabA and BabB in Clinical Isolates of Iranian Helicobacter pylori**

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**Introduction:** The adherence of Helicobacter pylori to the gastric mucosa plays an important role in the initial colonization and long-term persistency in the human gastric mucosa which leads to various GI disorders. Helicobacter pylori BabA is the ABO blood group antigen binding adhesin, which has a closely related paralogue (BabB) whose function is unknown. These adhesins attach to the human Lewis b surface epitopes and are closely associated with Hp-colonizing capacity. The aim of this study was to investigate the status of Hp babA/babB genes and their association with BabA protein expression in various disease groups.

**Methods:** Presence of babA and babB genes and their loci were investigated by PCR on fresh single colonies from 102 Iranian patients (26GC, 22 PUD and 52 NUD). BabA and BabB immunoblotting was performed by BabB and BabA-specific antibodies.

**Result:** Collectively, 53.9% and 74.5% of the isolated strains were positive for babA and babB genes. Only 10% of isolates were babA and babB negative. In 59.8% of the cases, babA was present in A locus and in 69.6% of the cases, babB was in B locus. Prevalence of babA in GC, NUD and PUD patients was 80.8%, 44.2% and 45.5% respectively, whereas BabA and BabB protein expression was detected in 68.2%, 46.7% and 65.4%. More than 70% of GC isolates were babA/babB double-positive.

**Conclusion:** Frequency of babB is higher among high risk patients, GC and PUD cases but babA gene frequency is limited to GC cases (p=0.004). It has been demonstrated that the frequency of babB is more than babA in Iranian strains. The presence of babA might confer a stronger selective advantage than the presence of babA. In GC cases presence of babA/babB double are more prevalent. babA gene prefer to be located at locus A, some strains do not possess the babA gene, some strains possess multiple copies of the babA gene, and most strains possess the babB gene. Expressions of BabA and BabB in GC and PUD cases are higher than NUD cases. No relation is detected between genotypic patterns and protein expression. Therefore, PCR-based methods may not fully determinate the status of Bab protein expression. It appears

**18.116**

**Measles—Related Hospitalizations and Complications in Children and Adolescents in Germany in 2007 with Focus on SSPE Over the Past Five Years**

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**Background:** A major goal of WHO is elimination of measles in Europe by the year 2010. The aim of this study was to evaluate hospitalizations of children due to complications of measles in Germany in 2007 and to give an overview about newly diagnosed measles SSPE (subacute sclerosing panencephalitis), a rare, late-onset, lethal neurological complication of natural measles infection, over the past five years.

**Methods:** Since 2003 active surveillance on hospitalized measles cases up to age 16 is conducted by the German Paediatric Surveillance Unit (ESPED). Data are collected monthly in children’s clinics throughout Germany employing standardized questionnaires.

**Results:** In 2007 fourteen hospitalizations were reported. Hospitalization of 11 children (median age 14 years [range: 1.6-15.7]) was due to acute measles infection. The most frequent acute complications were pneumonia and other bacterial and viral infections. During this period 382 cases of measles were notified for Germany in this age range. Hospitalization of 3 children (median age 8.9 years [range: 7.9-14.1]) was due to measles related SSPE. Exploring SSPE further, surveillance by ESPED revealed 17 children with SSPE over the past five years (2003-2007), corresponding to approximately 3 cases per year [range: 0-7]. 71% (n=12) were male, 71% (n=12) had migrational background. All children had measles specific antibodies and were positive in cerebral fluid. The latency between measles infection and onset of SSPE were 9.1 years [range: 5.3-12.5]. 62% of them (8 of 13) were younger than one year at the time of measles infection.

**Conclusions:** Measles surveillance by means of ESPED gives a rough estimate of measles-associated complications requiring hospital treatment including SSPE. The data emphasize the importance of a widespread immunization against measles in Germany to reduce severe complications and to protect babies and other person without effective immunological system who cannot be vaccinated.

**18.117**

**Expression of Different Variants of Helicobacter pylori cagA Gene for Screening High-Risk Iranian Individuals**

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**Background:** Helicobacter pylori (Hp) CagA is highly immunogenic protein which is injected into epithelial cells, undergoes tyrosine phosphorylation, and results in cytoskeletal rearrangements. The number of repeats in tyrosine phosphorylated motifs (EPIYA) creates C-terminal polymorphisms. CagA-positive clinical strains with an increased number of EPIYA phosphorylation motifs have been associated with more severe active chronic gastritis and atrophy. Significant heterogeneity among Iranian and western Helicobacter pylori strains, emphasizes the need for using native antigens in developing serological assays. The aim of this study was to construct a prokaryotic system for expression of variant forms of CagA gene from Iranian Helicobacter pylori isolates.

**Methods and Materials:** N-terminal and C-terminal fragments of cagA gene with different EPYIA types among Iranian Hp strains were amplified by designed specific primers and cloned into cloning and expression vectors, followed by confirmation through PCR, partial sequencing and restriction digestion analysis. The recombinant CagA fragments were expressed under IPTG induction. Identity confirmation was performed
through western blotting using anti-His specific antibodies as well as true Hp positive and negative sera. Immuno- blotting against patients’ sera is under way.

Results: A 2000bp fragment of N-terminal and 1900bp, 1700bp, 1600bp and 1500bp fragments of C-terminal region were amplified and sequen- tially cloned into cloning and expression vectors. Comparison of sequenc- es retrieved from available GenBank revealed considerable heterogeneity. The recombiant fragments were expressed under 1mM IPTG as 87, 79, 76 and 72kDa proteins. Purification of the recombiant proteins and assessment of sero-reactivity toward them are being per- formed.

Conclusion: Different CagA variants representing different subtypes of EPIYA motifs, AB, ABC, ABCD and ABCD, in association with the con- served N-terminal region can be used for developing variant specific sero- logical assays which may aid in non-invasive screening of Hp infected individuals at early stages of disease.

18.118 Canine Cases of Aujeszky’s Disease in Austria Point to a Wildlife Reservoir

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Background: Suid Herpesvirus-1 (SuHV-1), the causative agent of Aujeszky’s disease (AD), is primarily a pathogen of swine but can also infect other domestic or wild animal species. The infection takes place after oral or aerogenic ingestion of infectious material. In dogs, natural cases of AD have been attributed to infections with infected wild boars. Dogs represent epizootiologically irrelevant dead-end hosts, in which cases of AD have been attributed to contact with infected wild boars. In both animals after euthanasia necropsy and histologicalexamination was performed as well as immunohistochemical staining and direct immunofluorescence for SuHV-1 antigen. Virus isolation and cultivation was attempted on PK-15 cells and consequently PCRand sequencing was conducted.

Results: Both animals showed characteristic clinical signs of AD including fever, salivation, intense pruritus and status epilepticus with lethal course. Pathohistological examination of the brain revealed severe non- suppurrative encephalitis with intranuclear inclusion bodies in brainstem neurons. The diagnosis of AD was confirmed by immunohistochemical staining and direct immunofluorescence for SuHV-1-antigen. PCR for the detection of the gb-gene of SuHV-1 virus was performed and sequencing was conducted.

Conclusion: The Austrian domestic swine population is officially declared AD-free since 1997. Up to now there are no studies available on the incidence of SuHV-1 in wild animals in Austria. Whereas in one dog contact to wild boars was evident in the other the modes of transmission were unclear. In both animals after euthanasia necropsy and histological examination was performed as well as immunohistochemical staining and direct immunofluorescence for SuHV-1 antigen. Virus isolation and cultivation was attempted on PK-15 cells and consequently PCR and sequencing was conducted.

Methods: We present two cases of AD in German hunting terriers used for hunting in the south-east of Austria. Whereas in one dog contact to wild boars was evident in the other the modes of transmission were unclear. In both animals after euthanasia necropsy and histologicalexamination was performed as well as immunohistochemical staining and direct immunofluorescence for SuHV-1 antigen. Virus isolation and cultivation was attempted on PK-15 cells and consequently PCR and sequencing was conducted.

Results: Both animals showed characteristic clinical signs of AD including fever, salivation, intense pruritus and status epilepticus with lethal course. Pathohistological examination of the brain revealed severe non- suppurrative encephalitis with intranuclear inclusion bodies in brainstem neurons. The diagnosis of AD was confirmed by immunohistochemical staining and direct immunofluorescence for SuHV-1-antigen. PCR for the detection of the gb-gene of SuHV-1 virus was performed and sequencing was conducted.

Conclusion: The Austrian domestic swine population is officially declared AD-free since 1997. Up to now there are no studies available on the incidence of SuHV-1 in wild animals in Austria. Serological surveys carried out in neighbouring countries showed that the virus is still endemic in the wild boar population. Therefore a risk of infection is evident in wild boars as well as domestic animals getting into contact with them and investigations regarding the prevalence of SuHV-1 in Austria should be attempted.

Table 1. RotaTeq™ Vaccine Effectiveness by Medical Care Site.

18.120 Lethal Infections Due to Herpes Simplex Virus of Human Origin in Domestic and Zoo Animals

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Background: Herpes simplex encephalitis (HSE) is one of the most common viral brain disorders of immunocompetent humans. Occasionally, infected humans are the source of diseases in different animal species. In the domestic rabbit (Oryctolagus cuniculus) there are two published reports of natural Herpes simplex virus (HSV) infections up to now. The first case of spontaneous HSE in the rabbit has been described in 1997 (Weissenböck et al., Vet. Pathol. 34, 44-47). In the following years we detected two further cases of HSE could be detected during standard post mortem investigation.

HSV infections have also been described in Old World primates as well as New World monkeys and prosimians. New World monkeys and prosimians are particularly susceptible to disease caused by HSV-1 and the virus is easily transmitted directly. We present three common mar- mosets (Callithrix jacchus) out of a group of four which died due to an HSV infection.
Methods and Results: All rabbits showed various neurological signs and had to be euthanized. Necropsy was performed and pathohistological examination revealed a severe non-suppurative encephalitis with neuronal cell necrosis and intranuclear inclusion bodies.

In all marmosets macroscopically gingivostomatitis was observed. Major pathohistological findings included a non purulent leptomeningitis in one case and a non suppurative encephalitis in two cases. Serum titers to HSV-1 measured by serum neutralisation test were 1:64 at the time of euthanaisa. In all cases a human herpes simplex virus was identified by immunohistochemical examination with rabbit antiserum against HSV-1 and HSV-2. In two rabbits in-situ hybridization with biotinylated probe for HSV-1 and HSV-2 DNA was conducted and showed numerous positive signals. In all marmosets and in one rabbit an HSV-1 infection could be proven by PCR.

Conclusion: HSE seems to be a sporadic disease in domestic rabbits and monkeys. Humans are the original host and the reservoir of HSV. As with some other herpesviral infections, interspecies transmission is possible in HSV-1 infections and poses a risk for certain animal species with close contact to humans.

18.123 Phylogeny of Italian 2008 Rabies Viruses
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In 2008, Italy experienced a rabies epidemic in red fox population in North-Eastern territories at the border with Slovenia. Italy was classified as rabies-free since 1997, the last case was confirmed in 1995. We have selected current and historical Italian rabies isolates dating back to 1993 and compared them to those available in public domain.

The complete open reading frame of the gene encoding the nucleoprotein was sequenced for all the isolates. The sequences obtained have been compared to those available in public database and analysed using the neighbour-joining method with 1,000 times bootstrapping (1). Phylogenetic analysis revealed that the isolates analysed belong to the Lyssavirus genotype 1, “classical” rabies virus (RV), and all clustered in the Western European group. As expected, they shared high similarity and were closely related to RVs isolated from Eastern neighbouring countries, particularly from former Yugoslavian territories, now Slovenia.

In 1990’s, the Italian epidemic of sylvatic rabies was linked to the epidemiological situation of infection in Austria and the former Yugoslavia. Phylogenetic analyses confirm that also in 2008 the introduction of sylvademiological situation of infection in Austria and the former Yugoslavian territories, now Slovenia.

Infectious disease legislation, it most likely reflects increased travel by Irish residents to malaria -infected areas. Children made up a quarter of all cases, while a further 50% of cases were aged 25–44 years. The overall species distribution was similar to that reported in Europe for imported malaria, with the majority of infections due to Plasmodium falciparum (83%).

Infection was most frequently associated with travel to Sub-Saharan Africa (71%), in particular Nigeria, and the most common reason stated for visiting a malaria -infected area was “visiting family in country of origin”; other reasons included business travel and holidays. There has been a marked change in the proportion of cases reporting “visiting family” as their reason for travel -44% between 2005 and 2007 as opposed to 14% of cases in the period 2001–2004. In the last decade there has been a rise in the number of African immigrants living in Ireland, and an important subgroup among malaria cases were Irish -born children reporting “visiting family” as their reason for exposure.

There was a difference in the peak season for notifications of malaria by reason for travel—a summer peak for cases associated with visiting family, while holidaymakers were more commonly reported in the early months of the year. This suggests that malaria prevention messages can be tailored in season for different target audiences.

18.124 The Identification of Microorganisms from Children with UTI
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Background: Urinary tract infection (UTI) is common in practice and an important cause of morbidity in people. Infected urine stimulates an immunological and inflammatory response leading to renal infection and scarring, ultimately leading to end-stage renal failure.

The present study identifies the microorganism causing urinary tract infections in suspected cases at Shohada Teaching Hospital. The aims this study were to assess the prevalence of UTI pathogen and analyze its admit ion stature.

Methods and Materials: A respective clinical and laboratory study from 2004 through 2005. 15056 suspected pediatrics from either sex, age were studied at Shohada teaching hospital. The urine specimens were sent to the laboratory from all departments of the hospital and outpatient. Urine colony count and culture were done on suspected case. Cases were divided into few groups as (outpatient, inpatient), (male, female) divided by result of the urine culture into eight groups which were compared.

Result: From 15056 urine specimens (55%), 2557 (17%) were positive. 55% of positive cultures were from female suspected cases and 64% from inpatient suspected cases. E.coli (53.3%) was the most prevalent pathogen followed by suspected cases: Staph (8.1%), klebsiella (7.9%), enterococi (5.2%), strp (3.7%), proteus (3.1%), and pseudomonas (6.4%). Proteus, klebsiella and pseudomonas were more commonly found in males.
Conclusion: UTI is a significant problem and requires a large scale study at a regular interval in order to identify organisms from time to time and recommend prompt treatment for reduced UTI related morbidity.

Incidence of Hepatitis C Virus (HCV) Infection Among Injecting Drug Users (IDU) Attending Annual Needle Syringe Program (NSP) in Australia

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Introduction: More than 225,000 people in Australia lived with HCV infection in 2005 with an average of 1000 new cases each year. Approximately ~90% of HCV infections are attributable to injecting drug use. Precise estimation of the incidence of HCV infection is difficult because most infections are asymptomatic and surveillance systems are variable. This study aimed to describe the HCV incidence rates from repeat cross-sectional sero-surveillance conducted among IDUs attending NSPs in Australia between 1998 and 2005.

Methods: Study data was collected from serial surveillance surveys conducted at NSP sites in Australia between 1998 and 2005. Incident cases defined as a negative HCV tests in first survey followed by a positive HCV test in the next 24 months. Incidence rates were compared by demographic and risk characteristics with estimation of risks.

Results: Overall incidence rate of HCV was 18.3/100 person-years with an increasing trend till 2002–2003. Infection increased with age and a higher rate observed among young injecting drug users. Incidence rate was high among recent injectors (<25 years). Victoria (24.6) and NSW(24.5) had highest incidence rates followed by Tasmania(20.0) and lowest was for Western Australia(3.6). Significant differences in incidence rates were observed among cases with history of incarceration, by survey years and by states. In multivariate analysis only incarceration remained significant.

Conclusions: This study demonstrates the use of repeated cross-sectional data to estimate incidence rates. Early sero-conversion among young IDUs suggests a risk population for intervention. Intersate variation in incidence may be due to variable attendance at NSP surveys.

Public Health Informatics Tools for Electronic Disease Surveillance in Resource-Limited Settings

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Background: Establishing early event detection capabilities in resource-limited settings is critically important given the rapidity of disease spread. While the development of these capabilities is challenging, it is not impossible. Many efforts are underway to address specific, locally-based public health informatics needs such as training, data collection, or traditional surveillance analyses. Most of these tools are independent and not easily integrated into a single system. The current efforts of JHU/APL, in collaboration with public health authorities in several countries and at DoD GEIS, aims to develop a suite of tools that could be employed to establish an end-to-end electronic disease system. This flexibility allows public health authorities to develop an inexpensive, customized system that utilizes commercial hardware and meets their specific needs.

Methods: The tools within the surveillance suite can be categorized into four areas: Data Acquisition, Data Analysis and Visualization, Communications, and Modeling and Simulation. Each area provides options for a broad spectrum of public health infrastructure, from industrialized areas with robust internet connectivity to remote settings with minimal or unreliable internet access.

Results: Currently, JHU/APL is deploying a pilot application for remote data capture and a pilot application for early event detection and desktop analysis in Peru and the Philippines, respectively. The first tool utilizes Interactive Voice Response technology. When fully deployed, this tool will enable rural health workers to report health event data to central authorities using touch-tone phones as communications platforms. The second tool, ESSENCE Desktop Edition (EDE), which runs on a personal computer, mimics the flow, functionality, and analytical power of a well established, web-based, electronic disease surveillance system widely used in the United States.

Discussion: With these two pilot activities, combined with existing automated data capture, web-enabled analysis capability, disease modeling, and information sharing, JHU/APL has begun the development of a suite of tools that allow any country to customize an end-to-end surveillance capability that meets their requirements while operating within the constraints of their infrastructure. Additionally, these tools will be open source so that a system can be self-hosted, maintained, and modified by health authorities for minimal recurring cost.

Surveillance of Chikungunya and Dengue Fever in Emilia Romagna Region, Italy, Summer 2008

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Background: Chikungunya virus epidemic outbreak occurring in Emilia-Romagna, Italy, during summer 2007, evidenced that vector-borne diseases can spread also in all those sites where the vector is present. In Italy the Emilia-Romagna Region, most cities that are located in areas below 500 m a.s.l. are infested by Aedes albopictus during the April-October period. According to 2008 regional plan, the prevention of chikungunya and dengue fever was based on Aedes albopictus control, on early detection of human suspected cases and immediate implementation of environmental control measures. The Regional Plan has been launched for the prevention of both Chikungunya and Dengue fever, even though, Ae. Albopictus is competent for the transmission of a large number of Arboviruses. This choice was based on epidemiological criteria and similarly of both diseases from a clinical point of view and the practicability of a single surveillance system. According to the Regional plan a patient with Chikungunya or Dengue clinical suspicion and positive epidemiological criteria (travelling to an endemic or epidemic area for these diseases) was considered a suspected case and the laboratory diagnosis has to be performed.

Methods: From April to November 2008, 157 suspected cases of Chikungunya fever were analyzed; 48 samples were tested only for the detection of IgM/IgG antibodies by immunofluorescence, 5 were tested for Chikungunya RNA detection by Real Time RT-PCR and 104 samples were evaluated by both tests. The presence of antibody response and virus viremia were both investigated when the blood sample was collected within 8 days from the onset of symptoms.

During the same period, blood samples of 50 suspected cases of Dengue were collected. 49 sera were analyzed by immunofluorescence for the detection of antibody response while 27 samples were pondered for Dengue RNA by Real Time RT PCR or NS1 antigen detection.

For any suspected case the laboratory diagnosis was done within 12 hours to implement immediately control measures to stop the spreading of the vector.

Results: About Chikungunya infection, six patients resulted IgG positive and two IgM/IgG positive. Only one patient, was PCR positive. About Dengue fever, RNA was detected in three patients (two serotype 1 and one serotype 3) while five patients were IgG positive and one patient IgM/IgG positive.

Conclusion: Our experience demonstrated the necessity to perform a surveillance for Chikungunya and Dengue in order to avoid the insurgence of a local epidemic outbreak originated from an imported case.

Cross Border Avian Influenza and Pandemic Preparedness in South-East Europe

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Background: South East European countries are collaborating in the field of communicable diseases control. Following a priority exercise effort have been made to establish a cross border Influenza surveillance and a laboratory influenza network. The aim of this presentation is to describe current cross border pandemic preparedness in South East Europe.
Methods: Data from Influenza surveillance and laboraotory assessment are analysed. Evaluation reports of pandemic preparednes and other common activities are reviewed. Cross border communication of avian outbreaks are analysed and presented.

Results: Influenza surveillance has been established in nine South East Europea countries and all of them can report Acute Respiratory Infections or Influenza Like Illness but sentinel flu surveillance still need to be strengthened. Six out of nine countries can provide viral diagnosis but good laboratory capacities exist in all countries.Whole society approach is missing in all countries.Low coverage of Influenza vaccine is very common. Lack of preparedness in hospitals is missing in all countries. Pharmacolog- ical and non pharmacological interventions need to be re addressed and different strategies need to be established. Table top exercises are following the outbreaks of Avian Influenza in poultry and birds in Albania, Serbia, Romania and Croatia

Conclusions: Cross border information and activities help on strengthening Influenza Pandemic Preparedness and Health alert in South East Europe.

18.129 Seroepidemiology of Herpes Simplex Type 2 (HSV2) in HIV Patients in Kermanshah-Iran 2008
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Introduction: Herpes simplex virus type 2 (HSV2) is the most common cause of genital ulcer in developed and developing countries. Many researches have shown the HSV2 and HIV relationship. On the other hand, the level of immune deficiency in HIV+ patients is an important fac- tor in reactivation of HSV2 and also, HSV2 infection plays an important role in acquisition and transmission of HIV as a concomitant factor, raising the chance of transmission with increasing the amount of HIV virus in plasma and genital secretions. This study was conducted to determine the prevalence of HSV2 and its comittance with demographic and behavioral factors in HIV+ patients, and to compare with HIV- patients.

Methods and Materials: In this descriptive study 170 HIV+ patients in Kermanshah Behavioral Disease Consult Center participated included as case group and 50 HIV- patients was admitted in Imam Reza Hospital as control group by convenience sampling method. The serum of patients was assessed for HSV2 antibody (IgG) by ELISA method. The required information collected by interview and studying patient’s records. Analysis of data was performed using SPSS software by measures, ratios, Fisher’s and x2 test.

Results: Of 170 HIV+ patients, 90% was male and 10% female. All men were IV drug users and all women were infected by their HIV affected husbands. Range of age was between 20-59 yrs with the mean age of 36.4. 54.1% of patients were single, 27% married and 18.9% divorced. Serum prevalence of HSV2 was 6.5%, 17.8% in women and 5.2% in men. The most prevalence was in the age group of 50-59 yrs (14.3%), elementary education level (10.6%), and in the drivers (18.2%) and house wives (13.8%) regarding patient’s job. The prevalence in patients who had mul-iple partners was 8.4% and 4.8% in those who did not have multiple part-ners. Patients who had concomitant HBV or HCV infection were 9.1% and 7%, respectively, much more than those who did not have hepatitis infec- tion. The prevalence in IVDU was 5.3% and 5.5% in prisoners, who had less prevalence comparing to those not addicted and not prisoned. After performing serum tests in HIV- patients who studied as control group, the prevalence in this group was zero.

Conclusion: Prevalence of HSV2 in the serum of our patients was less than the other countries. This could be beacause of life style and sexual contact restriction in Iran, and less probability of multiple sexual contacts regarding the culture and religion existed in our country. There was no statically significant relationship between our surveyed variables and serum prevalence of HSV2. Health education should be noted as an effective factor in prevention of this increasingly spreading infection.

Keywords: Herpes simplex, seroepidemiology, HIV, genital ulcer, HBV, HCV, CD4, IVDU.

18.130 Surveillance Systems and Public Health Services in Georgia: Enhanced Infrastructure and Tools for Disease Detection and Diagnosis
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The National Center for Disease Control and Public Health (NCDC) is Georgia’s central public health agency. According to the Georgian legis- lation, NCDC is responsible for reduction of the biological agent threats and proliferation. This responsibility covers surveillance, detection and response measures for diseases caused by EDPs. Strengthening of the surveillance system, and improvement of the rapid detection and timely reporting/notification of the above mentioned diseases are the most impor-tant goals.

Threat Agent Detection and Response (TADR) system implemented in Georgia by the USA Department of Defense Threat Agency is one of the most significant drivers in achieving the following goals: 1) Introduction of new and important elements in existing surveillance system of the following diseases: anthrax, botulism, plague, small-pox, tularemia, tick born encephalitis, brucellosis, viral hemorrhagic fevers (Krine-Congo, with Renal Syndrome); 2) Renovation and equipping of two NCDC laborato- ries, and 3) Development of the new electronic integrated disease surveil-lance system (EIDSS).

Thanks to the TADR program NCDC implemented a new EDP surveil-lance program, based on sensitive case definitions, and new standards for sample collection and transportation. DTRA renovated and equipped two BSL-2 national and regional level laboratories. New diagnostic tests of high specificity are now used in those laboratories. As a result, the num-ber of confirmations increased significantly. The new laboratory resources strengthened Georgia’s capabilities to rapidly detect, and respond to numerous public health threats and outbreaks. EIDSS system has been developed and introduced under the same DTRA program. It provides a near real time information flow that can be disseminated in a timely man-ner. EIDSS offers a human and veterinary surveillance integrated package with a laboratory module for tracking sample and tests results. DTRA's program has made an important contribution to Georgia’s Public Health System. All TADR components have been seamlessly integrated into the existing system, and strengthened the surveillance system of communicable diseases and improved detection of EDPs.

18.131 Malignancy and Immunosuppression as Risk Factors for Melioidosis in an Endemic Area
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Introduction: Burkholderia pseudomallei causative agent of melioidosis, is endemic in South East Asia and Northern Australia. Its presentation varies from fulminant septicemia to chronic debilitating localised infect- ion. Identified risk factors for melioidosis are diabetes, alcohol intake, male sex, renal impairment and immunosuppression. A previous publication has suggested, serological screening for melioi-dosis prior to initiation of immunosuppressive therapy in patients, as melioidosis in the immunosuppressed has higher prevalence and mortal- ity. There have been no previous studies relating to the risk of acquiring melioidosis in patients from an endemic area, who are immunocompro-mised due to malignancy or immunosuppression.

Methods: This study was carried out at The Townsville Hospital, a 500 bed tertiary level hospital in North Queensland, Australia. A retrospective, chart review was performed on all cases of melioidosis in patients with malignancies or immunosuppression that presented over a 12 year period (1996–2008). Both haematological and solid organ malignacies were considered. Immunosuppression was defined as, the use of immune-modulating agents to treat a non-malignant chronic disease. Specific demo-graphics, risk factors, management and outcomes were assessed.

Results: 164 culture confirmed cases of melioidosis were recorded. Of these 8 patients (4.9%) had a pre-existing malignancy [5 cases of solid
organ involvement and 3 cases of hematological malignancy). There were 4 patients (2.4%) that had pre-existing non malignant immunosuppression for a chronic condition. This study did not identify any predominant malignancy. All of the 12 cases occurred during the wet season (December–May). There were three cases (25%) of recurrent disease, despite adequate intravenous antibiotics.

We found that only 7.3% (n=12) of the 164 cases of melioidosis were immunosuppressed of which 4.9% (n=8) had a malignancy. Using current malignancy figures, the expected cases of melioidosis would be 37. Had serological pre-screening for melioidosis been done on all 12 cases prior to immunosuppression, only 6 (50%) would have tested seropositive with an IHA ≥40.

Conclusions: This study has demonstrated that there is no greater likelihood of a patient with malignancy or immunosuppression, acquiring melioidosis in an endemic area. Routine screening for melioidosis prior to immunosuppression is unlikely to be of value.

**18.132 Surveillance of Canine leishmaniosis in North-Eastern Italy**

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**Background:** Canine leishmaniosis (CanL) is endemic in central and southern Italy and its spread in northern Italy is an established and well documented occurrence (Maroli et al, 2008, Trop Med Int Health, 13:256–264). Following the first autochthonous focus reported in Verona province (Veneto region) in 1994, a surveillance system has been established based on autochthonous CanL cases notification and entomological and serological monitoring.

**Methods:** Entomological surveys are performed seasonally (June–October). From 2002 to 2008, 101 sites of North-Eastern Italy has been covered, using both castor-oil coated traps (sticky traps) and Centre for Disease Control miniature light traps (CDC traps). Sandflies collected were stored in 70% ethanol and identified (Romig et al, 1994, ISTISAN, 94/8: 33–42).

Focused serological surveys are carried out annually on different dog populations in areas with a suspected autochthonous case of CanL. From 2002 to 2008, 3854 samples were tested by IFAT. Whenever possible, serological positive samples were tested after one month for confirmation and analysed with PCR. Mean sand fly density differences in relation to provenance area, type of site and altitude were compared (ANOVA+Tuckey test).

**Results:** Overall, 6503 sticky (corresponding to 243.5 m²) were used and CDC traps operated for 136 nights. Fifty-four (53%) sites resulted in 70% ethanol and identified (Romig et al, 1994, ISTISAN, 94/8: 33–42).

**Conclusions:** Surveillance identified 4 new areas of CanL diffusion in North-Eastern Italy. Cumulative sandfly densities were generally low, compared with endemic areas (Rossi E et al, 2008, Acta Trop, 105;158–165). Rural and peri-urban small villages, located in hilly areas with small farms raising a variety of domestic animals (ruminants, horses, pigs, birds) can assure higher densities of sandflies, allowing even for short periods a potential transmission of CanL.
this (212,813 ha ± 10 % or about 14 % of the transect). By comparing the very humid year of 2003 with 2006 which had just below normal rainfall, the ZPOMs inter-annual variability was analyzed in a sandy-clayey ecozone with an important hydrofossil riverbed within the Ferlo region of Senegal. Very probably contributing to an increased abundance of vectors by the end of August 2003, it was shown that the aggregate pond area was already about 22 times larger than in August 2006, corresponding to an approximately five times larger total ZPOM. The results show the importance of pinpointing small ponds (sizes down to 0.1 ha) and their geographical distribution in order to assess animal exposure to the RVF vectors.

18.135 Incidence and Diagnosis of Typhoid Fever in Karachi, Pakistan
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The morbidity of typhoid fever is highest in Asia with 93% of global episodes occurring in this region. Southeast Asia has an estimated incidence of 110 cases per 100,000 population which is the third highest incidence rate for any region. Pakistan falls in this region. There is also a considerable seasonal variation of typhoid fever, carrying significant public health importance. Population based data in Pakistan is scarce.

Methods: From June 2005 to 2007 sample were collected from multiple clinical settings in slum settlements in Karachi, Pakistan.

Cases of high grade fever for three or more days were screened for typhoid clinically and blood culture, typhidot test, widal and CBC were done. Of the 5106 cases with febrile episodes of three or more days detected in the community were screened at these centers; 451 were clinically suspected of having typhoid fever. Sixty two were positive by culture whereas 212 were positive by serology. Incidence of culture proven typhoid was estimated to be less than serological based incidence. Peak incidence was noted in July and August followed by March and April. Morbidity of typhoid is quite high in Pakistan and needs public sector involvement. Hot months have high incidence of typhoid. In this study we determined incidence of typhoid fever using standard surveillance techniques.

1) i.e. collection, analysis, interpretation of patients data reporting with fever in multiple clinical settings of Karachi.

2) Important serological and microbiological methods for diagnosis in every case using: CBC, Blood Culture, Widal test, Typhidot.

This will help in evaluating a comparative efficacy and viability of each parameter with aim of early diagnosis in the field and consequently targeted treatment thus reducing the morbidity ratio in different populations with relation to gender, age and socioeconomic status classifying each category to properly assess the clinical expression of typhoid fever in Karachi.

The scientific investigation and practical implication during this project will help for the development of recent data with regard to typhoid fever in the community and to suggest preventive measures in this respect.

18.136 Prevalence of Bovine Viral Diarrhoea Virus Infection in Industrial Holstein Dairy Cattle in Suburb of Mashhad-Iran
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Background: Bovine Viral Diarrhoea Virus (BVDV) is a worldwide distributed infectious disease of cattle. This Pestivirus of the Flaviviridae family can cause economical losses. Reproductive, respiratory and enteric disorders could be associated with BVDV. The aims of this study were to estimate the prevalence of BVDV Persistency infection (PI) animals, and evaluate the significance of the associated between the PI animals and culled cows in the herds.

Methods and Materials: The study was carried out in suburb of Mashhad in Khorasan Razavi province-Iran. It is a major producer of livestock in South-east of Iran. Samples were taken true randomly using a lottery mechanism in the dairy cattle herds according to a proportional geographical distribution in various parts of suburb of Mashhad-Iran. Totally, 157 individual blood samples were taken from 18 Holstein dairy cattle herds. Forty-one (26.11%) samples were prepared from culled animals. They were examined for the presence of BVDV antigen using Pestivirus-Ag capture ELISA. Differences in proportion of PI animals between the culled and the other ones were analyzed for statistical significance (P<0.01) using the Chi-square test in SPSS software version 9. The true prevalence for infection within the herds was estimated using the Rogan and Gladen’s correction of apparent prevalence, by the equation true prevalence = (apparent prevalence + Sp - 1)(Se + Sp - 1).

Results: The observed prevalence of BVDV antigen positive animals was 5.3.18% among the herds. The calculated true prevalence of antigen positive was 0.18%. The differences between PI removed and non-removed cows were significantly higher (P<0.01%). All the PI animals were less than 17 months old.

Conclusion: It was concluded that the prevalence rate of PI cows were not more than the other studies and PI animals which were removed from the herds to be referred to slaughterhouse were significantly higher than the other ones.

H. Ahmadnia. Tehran West Health Center(IUMS), Tehran, Iran (Islamic Republic of)

Background: Tuberculosis (TB) is one of the most important health problems in the world. By integrating of DOTS surveillance system in country we hope to start treatment of infected patients early and fast.

Method: Important data’s of Tuberculosis patients analyzed by spss15.

Results: In 2007 incidence of smear positive Tuberculosis patients was around 2.27 in 100,000 population in under covers areas of Tehran west health center and in 2008 it has increased to 3.36. In Iranian patients the most frequency of age group in 2007 were patients over 65 years old but in 2008 we found that age group between 35 and 65 was predominant. In 2007 the most predominant interval between start of illness and diagnosis was between 7 to 15 months (with 44% cases) but in 2008 this interval decreased to 3 to 6 months (40%). In 2007 around 6% cases were diagnosed more than 36 months after their illness start date but in 2008 this percentage decreased to 1.5% cases.

Conclusion: Tuberculosis patients are going to find in younger ages and earlier onset of illness but it can be an alarm for our health care system because it shows that TB is going to affect younger people and we should urgently insist on case finding and focus on younger populations because it is different from latent TB that involve older people.

We expect to improve our case finding by increasing public- private relationship with education, re-education or motivation of our colleagues in private sector.

Graph 1. Comparison between registered patients in 2007 and 2008 by NATIONALITY.
Prevalence of Hepatitis C Virus Genotypes Among Chronic Infected Patients in Southern Iran

M. Ziyaeyan, A. Alborzi, M. Jamalidoust, M. Moeini, A. Kadivar. Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran (Islamic Republic of)

Background: Chronic infection with HCV is one of the major causes of cirrhosis and hepatocellular carcinoma. Since response to anti-viral therapy in HCV sufferers depends on HCV genotypes, determination of HCV genotype is of great significance for both the onset and follow-up of the treatment. The aim of the study was the estimation of the HCV genotype prevalence in Shiraz, southern Iran and by doing so to help the patients.

Methods: RT-PCR method with four specific primer sets for major HCV genotypes (1a, 1b, 2 and 3a) were used for HCV genotyping. These primers propagate different parts of 5’ un-translating region-core region of HCV genome. Genotyping test was performed for 221 patients with positive qualitative RT-PCR results.

Results: Of 221 studied subjects, 193 (87%) were males and 28 (13%) were females. One hundred nine (49%) of them were infected with 3a, 50 (22.6%) with 1a, 13 (5.9%) with 1b and 6 (2.7%) with 2 of hepatitis C genotype. Mixed infection was found in 5 patients (1a+3a in 3 (1.4%) and 1a+2 in 2 (0.9%). The extracted nucleic acid from 38 (17.2%) samples did not react to the above-mentioned primer sets. This might be due to the presence of a genotype other than the above, or no appearance of a visible band on the gel because of no sufficient copy of the virus.

Conclusion: The results revealed the highest level of infection belonging to 3a followed by 1a. Since the considerable proportion of chronic HCV infected patients were intravenous drug abuse in our region; genotype 3a appears to be more prevalent among this group. In contrast to previously reported studies in Iran, The prevalence of 1b was not significant in the present study.

Prevalence of Hepatitis C Virus Genotypes Among Chronic Infected Patients in Southern Iran

M. Ziyaeyan, A. Alborzi, M. Jamalidoust, M. Moeini, B. Pourabbas. Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran (Islamic Republic of)

Background: Herpes keratitis is one of the important causes of blindness. Early diagnosis of herpes keratitis is essential to the treatment of suspicious patients accordingly. The aim of this study was to diagnose herpes simplex DNA by qualitative PCR and quantitative analysis of its genome using TaqMan Real-Time PCR method.

Methods: Corneal swabs from HSV keratitis suspected patients were collected from September 2005 to September 2007. Upon DNA extraction the samples were analyzed by qualitative PCR and TaqMan quantitative Real-Time PCR assays. In both methods two sets of primers amplified two different parts of the common sequence of HSV-1 and HSV-2 polymerase gene. In qualitative PCR we used gel electrophoresis followed by ethidium bromide for detection of PCR products, while in quantitative PCR method the copy number of unknown samples were expressed via standard curve drawing with known amount of amplified cloned plasmid.

Results: Of 141 samples, 87 (62%) belonged to males and 54 (38%) to females. The HSV genome was detected in 43 (31%) of the subjects by PCR, consist of 22 males and 21 females. The drawn standard curve was linear in 10 to 10^5 copies of virus (R2 = 0.982). The ranges of the HSV DNA copy number in clinical samples were detected from 2.7 x 10^2 to 3.1 x 10^6.

Conclusions: The results of this study revealed that HSV load in herpes keratitis is relatively high. The detection of viral copy number in positive samples can help the physicians make correct evaluations of patients’ conditions in turn guide them to choose the best therapeutically regimes for the involved patients.

Experimental Trichinosis: Parasitological, Electrophysiological and Biochemical Studies

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Background: Trichinosis is a parasitic infection affecting the gut and the muscles causing mild gastrointestinal symptoms followed by periorbital oedema, muscle pains, fever and eosinophilia. The infection evokes functional disturbances in physiological effector systems, furthermore, several biochemical changes are associated with the infection.

Methods: To study the electrophysiological changes in intestine, striated and cardiac muscles by electromyography (EMG) and to assess the biochemical changes through measurement of serum cholinesterase and intestinal myeloperoxidase activity (MPO) in both light and heavy infected experimental animals by Trichinella spiralis (T. spiralis).

Results: Electrophysiological results showed increased contractility of the smooth muscle layers of the intestine only early in the infection, whereas both striated and cardiac muscles showed increase in the contractility with the progress of infection in both light and heavy infection. Significant myocardial dysfunction in the form of bradycardia, in addition to major histopathological changes in the heart occurred from the beginning of the infection and increased till the end of the study. Biochemical study showed gradual increase in serum cholinesterase, while, the intes-
tinal MPO showed increase only in the early stage of the infection. It was noticed that all changes were more pronounced in the heavily infected group than the lightly infected one.

**Conclusion:** All these changes could be involved in the pathogenesis of the manifestations happened at different stages of \( T. spiralis \) infection.

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**18.142 The Utility of Direct Agglutination (DAT) and Fast Agglutination Screening (FAST) Tests in Serodiagnosis of Experimental Microsporidiosis**

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The great harm occurring to human beings by microsporidiosis necessitates the consequent need for a simple technique for diagnosis. Therefore, the present study was designed to evaluate the efficiency of two serodiagnostic tests; the direct agglutination test (DAT) and the fast agglutination screening test (FAST) in the diagnosis of Microsporidia in experimentally infected mice and to differentiate between different species of the parasite. In the current study, Swiss albino mice were divided into non-infected control and infected experimental groups which were further subdivided into ten subgroups. Ten samples of microsporidial spores were isolated from ten human stool specimens and each one was used in infecting each subgroup of experimentally infected mice orally in a dose of 10 5 spores/mouse. Stool and sera were collected from each subgroup of mice weekly starting from the 1st week to the 4th week post infection. DAT and FAST agglutination tests, using antigen prepared from different species of microsporidial spores, were conducted to detect microsporidial antibodies in sera of different subgroups of mice. The cross-reactivity of microsporidial spores with antibodies of other intestinal protozoa (Cyclospora cayetanensis and Cryptosporidium parvum) was also investigated by both agglutination tests. The results proved that the two agglutination tests (DAT and FAST) were effective in detecting microsporidial antibodies in sera of infected experimental animals, starting from the 2nd week of infection till the end of the study, with no cross-reactivity with other intestinal protozoal infection. Cyclospora cayetanensis and Cryptosporidium parvum. Both tests failed to differentiate between different species of Microsporidia used, however, both tests are easy to interpret, as well as being specific and sensitive. They do not need specialized equipment, or a cold chain for storage of antigen. In addition, the FAST results can be read within three hours and not need CO2 incubator. Thus, these agglutination tests, especially FAST are very suitable tools for the serodiagnosis of microsporidiosis, they may provide a good alternative for the invasive and labor intensive methods and they are very practical under field or rural conditions.

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**18.143 Experimental Trichinosis : Parasitological, Electrophysiological and Biochemical Studies**

M. Gaafar, M. Issa, Y. Ashram, I. Diab, N. Baddour, M. El-Azzouni. Faculty of Medicine, Alexandria University, Alexandria, Egypt

**Background:** Trichinosis is a parasitic infection affecting the gut and the muscles causing mild gastrointestinal symptoms followed by periorbital oedema, muscle pains, fever and eosinophilia. The infection evokes functional disturbances in physiological effector systems, furthermore, some biochemical changes are associated with the infection.

**Methods:** To study the electrophysiological changes in intestine, striated and cardiac muscles by electromyography (EMG) and to assess the biochemical changes through measurement of serum cholinesterase and intestinal myeloperoxidase activity (MPO) in both light and heavy infected experimental animals by Trichinella spiralis (\( T. spiralis \)).

**Results:** Electrophysiological results showed increased contractility of the smooth muscle layers of the intestine only early in the infection, whereas both striated and cardiac muscles showed increase in the contractility with the progress of infection in both light and heavy infection. Significant myocardial dysfunction in the form of bradycardia, in addition to major histopathological changes in the heart occurred from the beginning of the infection and increased till the end of the study. Biochemical study showed gradual increase in serum cholinesterase, while, the tinal MPO showed increase only in the early stage of the infection. It was noticed that all changes were more pronounced in the heavily infected group than the lightly infected one.

**Conclusion:** All these changes could be involved in the pathogenesis of the manifestations happened at different stages of \( T. spiralis \) infection.

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**18.144 Role of the Molecular Diagnostics in the Control of Zoonotic Diseases in Sudan**

I.E. Elrayah. Tropical Medicine Research Institute, Khartoum, Sudan

PCR-based systems to detect the etiologic agents of disease directly from clinical samples, contributed a lot to clarify the misdiagnosis of many diseases similar in clinical signs and not easy to differentiate under microscopic (e.g. malaria and babesiosis). Sequence analysis of amplified microbial DNA allows for identification and better characterization of the parasite. Subspecies variation, identified by various techniques, has been shown to be important in the prognosis of certain diseases. Molecular tools are now used for epidemiological surveillance of drug-resistant malaria and African Trypanosomiasis which set the basis for rational drug development policies. These tools include Mutation specific (MS-PCR), PCR-RFLP and dot blot hybridization.

The study of genetic distance and phylogenetic relationships between vectors of diseases (e.g. leishmaniasis, malaria and sleeping sickness) has been introduced in the field of characterization and classification of these vectors, which help a lot in the implementation of the effective control system.

RAPD used to study the genetic structure of vector species, the amplified bands for the extracted DNA of the e.g sand flies investigated. Which help a lot to identify the genetic variations among the population of \( P. orientalis \) and accordingly their geographical origin?

In the epidemiology of sleeping sickness in southern Sudan, traditional trypanosome detection techniques rely on microscopic examination to identify parasites to the subgenus only and would not differentiate between \( B. rhodesiense \), \( B. gambiense \) or \( B. brucei \) trypanosomes. PCR succeeded to do that and even to identify and detect trypanosomes infections in tssetse and stable flies in order to estimate the role \( G. f. fuscipes \) and Stomoxys spp in the transmission of the diseases.

In Schistosomiasis the RAPD-PCR methodology represents a new approach for the analysis of genetic polymorphisms. The understanding of the genetic polymorphisms associated to resistance may contribute to the future identification of genomic sequences related to the resistance/susceptibility of \( B. mansoni \) to the larval forms of \( S. mansoni \) and to the development of new strategies for the control of schistosomiasis.

Rapid methods for the detection of infectious agents might markedly improve health care in a variety of clinical, laboratory and epidemiologic situations, and play an important role in the control.

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**18.145 WEBRIBO—an Internet Database for Characterization of \( Clostridium difficile \) Isolates by Capillary Gel Electrophoresis Based PCR Ribotyping**

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**Background:** \( Clostridium difficile \) is the most frequently identified cause of hospital-acquired diarrhea. At present PCR ribotyping is the preferred typing method for \( C. difficile \) in Europe. PCR-ribotyping lacks an interlabatory interchangeable format.

**Methods:** A \( C. difficile \) PCR ribotyping method based on capillary gel electrophoresis was developed. A web-based database (http://webribo.aeg.at) was created for capillary gel electrophoresis-based PCR ribotyping results.

**Results:** A total of 146 \( C. difficile \) isolates were studied: five isolates were reference strains (PCR ribotypes 001, 014, 017, 027 and 053); 141 were clinical isolates comprising 39 Austrian PCR ribotypes collected in the period 2006–2007 at 25 Austrian healthcare facilities. Capillary gel electrophoresis yielded up to 11 fragments per isolate and 47 ribotype patterns. All but one of the five PCR ribotypes of reference strains were
clearly reflected in the chromatograms of capillary-based typing. Capillary gel electrophoresis divided 24 isolates belonging to PCR ribotype type 014 into seven subgroups, whereas subtyping the same isolates using multiple-locus variable-number tandem-repeat analysis yielded three unrelated subgroups, without obvious correlation to the patterns generated by capillary gel electrophoresis. Using a web-based software program (http://webribotypes.at), we were able to correctly identify these 014 isolates by simply allocating the seven subgroup patterns to one ribotype, i.e. to PCR ribotype 014.

Conclusion: With the web-based application, all users are able to enter their own data and receive a ribotype identification for each submitted isolate. Any unknown PCR ribotype pattern will be accepted if the isolate's chromatogram is submitted to the database administrator for proof of quality. Capillary gel electrophoresis-based PCR ribotyping might be a way of overcoming the problems associated with inter-laboratory comparisons of typing results. Furthermore, this new method can substantially improve a laboratory's capacity for C. difficile typing by substantially diminishing the hands-on time for PCR ribotyping.

18.146 Incidence of Tuberculosis and Occurrence of MDR-TB in Sharjah, UAE: A 4-Year Retrospective Study

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Objective: To study the incidence of tuberculosis and occurrence of multidrug-resistance tuberculosis (MDR-TB) in a Ministry of Health hospital in Sharjah, United Arab Emirates.

Methods: A retrospective review of the clinical and laboratory records of one thousand four hundred sixty six suspected cases of tuberculosis was carried out between January 2004 and December 2007. The antimicrobial susceptibility pattern for each Mycobacterium tuberculosis isolate was analyzed using Rapid liquid TB culture medium: BACTECTM MGITT 960 SIRE® (automated).

Results: During the study period, two hundred sixty two culture confirmed cases of Mycobacterium tuberculosis were recorded; 189 were men while 73 were females. The majority of TB cases were seen among expatriates from South-East Asian countries (41%). Forty nine active TB cases (19%) were reported in the native residents (Emiratis) of the country. The peak age group was between 16 and 45 years. Resistance to isoniazid, with or without resistance to other antmycobacterial drugs, was most common (21%), followed by resistance to streptomycin (14%). There was non-responding to rifampicin alone among the isolates. Multidrug-resistant (MDR) tuberculosis was observed in ten cases (3.8%).

Conclusions: Incidence of tuberculosis and occurrence of MDR-TB in Sharjah, UAE is fairly low. However, an increased number of culture confirmed TB cases and emergence of MDR-TB among the native and expatriate population warrants not only strengthening of the existing screening procedure and providing effective treatment but also equipping more laboratories in the country to detect new TB cases especially those of MDR and XDR-TB in order to stop their transmission and eliminate TB from the country.

18.147 Association Between PPD and QuantiFERON Gold TB Test in TB Infection and Disease Among HIV-Infected Individuals in Shiraz, Southern Iran

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Background: Mycobacterium tuberculosis is one of the most common diseases among HIV-infected persons. A person with a positive tuberculin skin test (TST), who acquires HIV infection has a 3-13% annual risk of developing active tuberculosis. The diagnosis of TB in HIV infected patients may be difficult. QuantiFERON-TB Gold (QFT-G) test is a novel method as an aid to diagnose Mycobacterium tuberculosis infection but limited data exist regarding their performance in HIV-infected adults. We evaluated the association between TST and QFT-G test in latent TB infection (LTBI) and the TB in HIV-infected patients.

Methods: One hundred and seventy six HIV-infected subjects from Shiraz Consultation and Behavioral Modification Center (SCBMC) entered our study. Blood sample were provided for TST, using 5TU purified protein derivative (PPD), and QFT, measuring INF-γ responses to Mycobacterium tuberculosis antigen.

Results: Of 176 participants, 173 (98.30%) subjects returned for evaluation of TST results, among them 83% and 37% were negative and positive for TST respectively. All participants returned for QFT-G sampling, among them 64.77% and 27.84% were respectively negative and positive for the test and 7.39% showed undetermined results. Concordance between PPD and QFT-G in their negative results was 39.88% (69/173) and 8.1% (14/173) in their positive results and the overall concordance was 50%. Disconcordant results of TST/QFT+ were found in 19.65% of subjects and TST+/QFT- discordant result in 24.86%. CD4+ count <100 mm<sup>3</sup> was seen in 5.92%, ≥100 and <200 mm<sup>3</sup> in 17.11% and CD4+ T cell count ≥200 mm<sup>3</sup> in 76.97% of subjects.

Conclusion: The agreement rate between TST-G and TST in HIV-infected patients was fair. So a strategy of simultaneous TST and QFT-G testing would maximize the potential for LTBI diagnosis in HIV-infected subjects. Among patients with TB disease, sensitivity of PPD in 5 mm cut off was more than QFT-G but the specificity of QFT-G with excluding undetermined results was more than PPD.


V. Hasselvedt, Sykehuset Innlandet, Lillehammer, Norway

Background: Our laboratory serves Oppland an Hedmark counties, Norway. These two counties have an area that is larger than the whole of Denmark. The population is approx. 371,000. We performed 410,000 medical microbiological analyses in 2007. Our laboratory offers services to six hospitals and general practitioners, as well as nursing homes, in the area mentioned above. Our laboratory wants to perform surveillance of certain bacteria of nosocomial interest—in order to implement targeted response to reduce the risk of outbreaks.

Methods: Culture positive findings were recorded, weeks 26–through 45/2008 when it comes to the following, E. coli–ESBL, Stenotrophomonas maltophilia, Serratia marcescens and Acinetobacter baumannii. The findings were communicated to clinicians and to infection control nurses respectively. VITEK-2® was systematically used for the identification and susceptibility testing of the various bacteria.

Results: The total incidence in Sykehuset Innlandet Trust was as follows (cases): E. coli–ESBL–our prevalent strain being CTX-M-15 (15), Stenotrophomonas maltophilia (14), Serratia marcescens (13), Acinetobacter baumannii (47). Most isolates, all species of bacteria, were from urine >90%.

Conclusion: During the time span described the cases with nosocomially significant bacteria were subject to a systematic follow-up according to which micro-organism was detected. The surveillance facilitated the implementation of isolation procedures, when necessary. In addition our hospital trust regularly uses disinfection robots when decontamination of hospital departments and wards is needed—due to detection of potential for nosocomial outbreaks. In the period described above no outbreaks occurred.


M. Redberger-Fritz, T. Popow-Kraupp, Med. Univ. Vienna, Inst. of Virology, Vienna, Austria
Background: Human influenza viruses are subject to continuous antigenic drift. This phenomenon poses great problems for the vaccine recommendation.

Objectives: (a) The antigenic and genetic characterization of influenza strains obtained from the Diagnostic Influenza Network Austria and from nasopharyngeal-swabs from other sources, mainly hospitals; (b) to compare strains collected in Austria to the recommended vaccine-strains used in each season; (c) to test for the appearance of neuraminidase-inhibitor-resistant influenza strains.

Study Design: Influenza strains were collected during 6 consecutive influenza-seasons (2002/2003 to 2007/2008). Laboratory diagnosis and subtyping was done by PCR on clinical samples, followed by viral isolation in tissue-culture. The isolates were characterized antigenically by hemagglutination-inhibition-assay with post-infection ferret antiserum. Genetic characterization was performed by sequencing the HA gene. The comparison between reference and circulating strains was analyzed by the construction of phylogenetic trees. Testing for the appearance of neuraminidase inhibitor-resistant influenza strains consisted on sequencing the NA gene.

Results: Tracing changes of the HA-gene by genotyping revealed that in each season viruses started to evolve with a decreasing homology to the dominant circulating strain. These strains already showed a close relationship to the dominant strain of the following season. The circulating A/H3N2-strains matched the corresponding vaccine component only in season 2002/2003 and 2006/2007, whereas the circulating A/H1N1-strains matched the corresponding vaccine component in every season except in 2006/2007 and during the drift-situation in 2007/2008. Influenza B viruses showed a good correlation between the circulating strains and the vaccine-component in season 2004/2005 and 2006/2007. Neuraminidase inhibitor resistant strains were only detected in season 07/08 where 12 out of 165 H1N1 viruses, revealed a mutation corresponding to oseltamivir-resistance.

Conclusion: The results underscore the value of monitoring seasonal influenza strain dynamics as an instrument that can provide important and timely information on the appearance of strains with epidemiologic significance.

18.150 Chromogenic In-situ Hybridization for Specific Molecular Detection of Infectious Agents in Pathohistology

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Background: A specific molecular-based diagnostic method for the detection of pathogens in histological slides is essential in diagnostics as well as in pathogenetic studies. The development of specific antibodies for immunohistochemistry (IHC) requires a lot of effort and time. The replacement of primary antibodies by oligonucleotide probes, as is done in in-situ hybridization (ISH), reduces the elaborate preparatory work to a minimum of sequence alignment and fast production with the additional advantage of a selectable range of specificity.

Methods: A digoxigenated oligonucleotide probe is chosen to hybridize with pathogen-specific genes on species-, genus- or family-level in histological slides. The steps of digoxigenin-detection with enzymatically linked Fab-fragments, a subsequent colour-reaction and counterstaining correlate to protocols of IHC.

Results: ISH proved to give very clear and specific results especially with protozoa (e.g. Cryptosporidium sp., Giardia sp., Entamoeba sp., Leishmania sp., etc.) and viruses (West Nile virus, Usutu virus, Borna Disease virus, etc.). The host tissue can be assessed simultaneously, so that tissue distribution of the pathogen as well as surrounding tissue and inflammatory reactions can be correlated with each other.

Conclusion: Chromogenic IISH proved to be a very useful tool in diagnostics and pathogenetic studies especially regarding protozoa and viruses. This method can be used universally in samples of animal and human origin. Furthermore it can easily be adapted in its specificity to the respective needs of the study. Species-specific probes combined with double-coloured staining have successfully been used to investigate coinfections. On the other hand, probes with a broad spectrum have lead to the detection of unexpected pathogens in new host species. The production of oligonucleotide probes is considerably less elaborate than the creation of primary antibodies and cross-reactions as well as background staining, is minimized essentially. Thus chromogenic ISH shows important advantages compared to IHC.

18.151 Molecular Epidemiology of Leptospira spp by Pulsed-Field Gel Electrophoresis (PFGE)

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Background: Leptospirosis is a bacterial zoonosis disease that is emerging as a public health problem worldwide. Traditionally, microscopic agglutination test (MAT) and cross-agglutinin absorption test (CAAT) are used to identify the isolates. However, these techniques are laborious and time-consuming requiring the maintenance of a collection of live leptospires from more than 200 reference strains and correspondent rabbit antiseras. The purpose of this study was to evaluate the performance of the PFGE method with restriction enzyme NotI for discrimination of Leptospira spp isolated from human blood in São Paulo, Brazil.

Methods: Blood specimens from patients with clinical suspicion of leptospirosis were cultured in Fletcher semisolid medium at 30oC and examined for 12 weeks using a dark field microscope. Fourteen clinical isolates of Leptospira spp were analysed by MAT before being characterized by PFGE. The restriction enzyme NotI was used to generate PFGE profiles. The isolates were compared with a library of 206 different reference Leptospira serovars. Gel images were captured using a Gel Doc 2000 System. Gel analysis, Dice band-based coefficients, dendrograms and number of fragment differences were performed by BioNumerics.

Results: The PFGE profiles were clear with high resolution and the fragments were equally distributed. All the isolates gave distinct patterns by PFGE. PFGE and MAT results were in agreement for all clinical isolates evaluated. Since the patterns obtained for serovars Copenhageni and Icterohaemorrhagiae are very similar, twelve isolates were classified as serovar Copenhageni/Icterohaemorrhagiae. By MAT, these isolates were classified as serogroup Icterohaemorrhagiae with titres ranging from 12,800 to 51,200. Two isolates were classified as serovar Canicola by PFGE, and as serogroup Canicola by MAT with titres of 102,400.

Conclusion: PFGE offers the advantages of simple, reliable and reproducible results. With the exception of serovars Icterohaemorrhagiae and Copenhageni, it is possible to determine leptospires at serovar level by using PFGE. These data indicate the utility of PFGE for routine identification of clinical isolates and molecular epidemiology.

18.152 Sero-Agglutination Tests Follow-up in 17 Successfully Treated Cases of Human Brucellosis

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Background: Standard Agglutination Test (SAT) and 2-mercaptoethanol (2-ME) test are usually used for follow up of treated cases of human brucellosis. The purpose of this study was to monitor the levels of these tests after two years on successfully treated cases of brucellosis.

Methods: From April 2003 to September 2008, we were able to follow up 175 treated cases of brucellosis for 2 years. Diagnosis of brucellosis was established with SAT ≥ 1:320 and 2ME ≥ 1:80 with clinical symptoms and signs compatible with brucellosis. SAT and 2-ME were tested at the end of therapy and every 3-months interval for two years. Data from successfully treated cases were recorded.

Results: One hundred seventy five treated cases (103 males, 72 females) were followed. The mean age of the patients was 31±13.5 years. Six and 12 months after treatment, SAT titers ≥ 1:320 were seen in 41 (23.4%) and 22 (12.6%) cases, respectively. SAT titers ≤ 1:320 were seen in 7 (4%) and in 6 (3.4%) cases after 18 and 24 months of the cessation

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of therapy, respectively. Six, 12, 18 and 24 months after treatment, 2ME titers ≥1:80 were seen in 51 (29.1%), 24 (13.7%), 12 (6.9%) and 8 (4.6%) cases, respectively.

Conclusion: The results show that SAT and 2-ME may be in significant titers in less than 5% of successfully treated cases after two years of follow up. Therefore, in endemic regions one year of follow up with these tests are sufficient.

Phylogenetic Grouping and Real Time PCR Method for Differentiating Capripoxviruses

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The Genus Capripoxvirus (CaPV) of the Poxviridae family comprises three members namely, sheep poxvirus (SPPV), goat poxvirus (GTPV) and lumpy skin disease virus (LSDV) affecting sheep, goats and cattle respectively. The classification of CaPVs is based largely on the host species from which the virus has been isolated and they are considered to be host specific because they seem to cause disease in the preferential host. However, in the case of sheep and goat poxviruses there are conflicting reports on their pathogenicity: most strains produce severe clinical disease in only one host, whereas some strains are pathogenic in both species. It is evident therefore that the classification based on animal origin of virus isolation requires replacing with a system that is not simply related to those criteria. Methods relying on molecular methods offer a means for comparing strains genetically.

This was explored in our laboratories by targeting one of the capripox viral genes potentially involved in the immune system evasion, the G-protein-coupled chemokine receptor (GpCr) gene. This gene from CaPVs of different geographical origins has been cloned and sequenced. The phylogenetic reconstruction resulted in the discrimination of CaPVs strains into three groups: the SPPV group, the GTPV group and the LSDV group. Based on these findings, a real time PCR method has been designed for differentiating CaPVs. Based on Fluorescence Resonance Energy Transfer (FRET) chemistry, it was found to be able to discriminate CaPVs using the Tm value obtained after melting analyses. It can detect as few as 100 copies of viral genome.

It is anticipated that these findings will make a significant contribution to the better understanding of the epidemiology of CaPVs by enabling rapid genotyping, and allowing gene-based classification to provide unequivocal identification of viral isolates.

Community Acquired Influenza in Adult Admissions to a 1200 Bed Hospital in Singapore

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Background: Under the threat of ‘bird flu’ laboratories face demands to detect influenza ‘promptly.’ There is also a desire to control the spread of influenza in hospitals to reduce the damage that seasonal influenza infects on patients, staff and general hospital activity. Pilot data from 2007 prompted us to set up routine influenza surveillance in 2008.

Method: Respiratory samples submitted for bacterial culture within three days of admission between January and September 2008 were tested under a hospital approved initiative. RNA was extracted with an EasyMag instrument and subjected to RT-PCR for Influenza A and B with specific probe based detection using a LumineX instrument.

Results: Of 1347 samples tested, Influenza A and B were detected in 12% and 2.6% respectively. Six samples showed inhibition. The detection rate reached a maximum of 50% in week 20 and showed another peak at 40% in week 30. Repeat tests confirmed these surprising results, as did sequencing of a subset. The rates mirrored the national rates of influenza activity, lending credence to result specificity.

Conclusion: The burden of seasonal influenza amongst new inpatients is considerable. This burden may justify rapid tests to guide specific anti-influenza therapy and infection control. This would be more realistic if nose/throat swabs were taken on admission as results could then be reported within a day of admission. Against this is the cost and the potential for breeding resistance to medicines currently regarded as an important defence against ‘bird flu.’ The data suggests it may be worth looking for influenza in hospital acquired pneumonia as there is great potential for the spread of seasonal influenza in the wards with consequent morbidity and mortality amongst debilitated inpatients as well as staff.

Predominant cagA subtypes in Iranian Helicobacter pylori Infected Patients

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Introduction: The prevalence of the East Asian CagA-positive strains is associated with the mortality rates of gastric cancer in Asia. Presence of a variable number of repeat sequences contributes to the size variation of CagA protein. The sequence variation raises the possibility that the biological activity of CagA can vary from one strain to the next, which may influence the pathogenicity of different cagA positive H. pylori strains. The aim of this study was to determine the predominant cagA subtypes of Iranian H. pylori strains and their association with the infection associated diseases.

Methods: CagA variable region of 172 strains recovered from 112 NUD, 33 PUD and 27 GC cases were amplified using cag2 and cag4 primers. All the obtained partial nucleotide cagA sequences in this study were deposited in the GenBank.

Results: Eighty one percent of the examined isolates possessed cagA gene. Six CagA variants differing in length were identified. Two to six EPIYA motifs were detected among the sequenced strains. The most prevalent (45.9%) amplicon was 550bp designated as A-B-C type. All of the sequenced cagA variable regions were identified as the “Western” type. There was no statistical association between any of the cagA subtypes and gastrointestinal disorders.

Conclusion: This study demonstrated that the majority of cagA subtypes belonged to the less virulent subtype. It is suggested that circulation of A-B-C type as the most prevalent type in Iranian dyspeptic patients may have resulted in lower frequency of gastric cancer in our population compared to East Asian countries.

Detection of Galactomannan and Antibodies in Patients with Invasive Aspergillosis

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Background: In the past decades the incidence of emerging fungal infections has increased dramatically. Invasive aspergillosis (IA) represents an important cause of infectious morbidity and mortality among predominantly immunocompromised (IC) patients. However, early diagnosis of IA is difficult because of the absence of specific symptoms, blood cultures are seldom positive and biopsies often cannot be performed due to severe patient’s status. Polysaccharide galactomannan (GM), soluble egzoproduc- uct, represents major component of fungal cell wall and it can be verified in body fluids by immune reactions. The aim of this study was to investigate the clinical usefulness of sandwich ELISA test in the early diagnosis of IA.

Methods: We examined 156 patients in one year period from November 2007 until November 2008 who were clinically highly suspected for IA: 124 adult patients with hematological malignancies (group I), 11 pediatric patients with hematological malignancies (group II) and 21 patients with different pulmonary diseases (group III). Diagnosis of IA was con-
firmed by detection of GM in serum samples using sandwich ELISA test (Platelia Aspergillus, Bio-Rad, France). The results were considered as positive if the GM index was greater than 0.5. Parallel with GM, anti Aspergillus antibody classes IgA, IgM and IgG in serum samples (Serion Elisa Classic, Virion/Serion, Germany) were performed for each patient.

**Results:** In the group I GM was positive in 29.84% (37/124) patients. Nine of them (9/37) have died as a result of IA with GM index ranges from 0.83-9.61. At the same time specific antibodies were detected in almost half of the GM positive patients from I group. IgA was positive in 2.70% (1/37) while both IgM and IgG were positive in 51.35% (19/37) patients. In pediatric patients GM was positive in 21.43% (3/14). One of them was IgG positive, second one had IgG and IgM and third one had no detected antibodies. GM was positive in 14.29% (3/21) patients from the group III. All of them had IgM positive response, while IgG was negative.

**Conclusion:** In patients with suspected IA detection of GM and specific antibodies should be performed because interpretation of the results could be abstruse. Further investigations are necessary for correlation of laboratory findings with clinical diagnosis and outcome of the treatment.

**18.158** ARC Microbial Diagnostic Microarrays for Environmental and Food Analysis

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Microbial diagnostic microarrays are molecular tools used for simultaneous identification of microorganisms in food, clinical and environmental samples. The main advantages of MDMs are high throughput, parallelism and miniaturization of the detection system. Furthermore, both high specificity and high sensitivity of the detection can be achieved. Different microarray systems including microarrays based on short or long oligonucleotide probes, sequence-specific end-labelling of probes (SSELO) or approaches based on whole genome differences have been applied in our laboratory. These different approaches exhibit certain advantages and limitations and are suitable for different applications. In addition, depending in the application, various marker genes have been used for probe design including pathogenicity-related markers, housekeeping genes as well as key genes involved in environmental processes. In some cases, whole genome approaches (Diversity Arrays) have been applied as no suitable marker genes to be used for probe design could be identified.

Microbial diagnostic microarrays developed at our institute target environmental bacteria as well as human pathogens. All known (either by cultivation or by cultivation-independent analysis) methane oxidizing bacteria can be easily identified by a microarray, which is based on sequence differences within the pmoA gene encoding the key enzyme methane monooxygenase. In the field of human pathogen detection, one of our developments is able to detect and identify common water-borne pathogens and indicator organisms. This microarray is based on the combination of a unique labeling method (SSELO), a novel concept of competitive oligonucleotides and the gyrB gene as phylogenetic marker resulting in high specificity and sensitivity. Other ARC microarrays have been developed for the typing of food pathogens rather than for their detection. The ARC Salmonella Serotyping Array is able to distinguish more than 40 S. enterica serotypes prevalent in Europe and has the potential to replace classical serotyping. It is based on specific short oligonucleotides, which target two housekeeping genes (gyrB, atpD) and two flagellin genes (flIC, flJB). The development of additional microarray-based typing methods in on-going.

**18.159** Turkey’s Influenza Surveillance Results in 2007–2008 Winter Season

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**Background:** Turkey is divided into 14 provinces for the influenza surveillance. The surveillance is performed by two institutes, Refik Saydam Hygiene Center (RSHC) National Influenza Laboratory and Istanbul University, Medical Faculty, Virology Laboratory which are member of international information networks. RSHC National Influenza Laboratory is responsible for 9 provinces. With the National Influenza Surveillance System it was aimed to contribute to the detection of influenza viruses with pandemic potential.

**Methods:** To detect the influenza and influenza-like viruses (Parainfluenza virus Type I,II,III, Respiratory Syncytial Virus and Adenovirus), samples were identified and subtyped by both molecular methods and cell culture technics.

**Results:** 1157 clinical specimen collected from 9 provinces were evaluated for influenza and influenza-like viruses during November 2007–May 2008 influenza season in the laboratory. Influenza activity was measured weekly to determine the predominant strain circulating in Turkey. The results showed that the activity started around 47th week and ended around 21th week (Figure 1). In the season, 321 clinical specimen were found positive for Influenza and/or influenza-like viruses. The predominant virus strain was influenza A (55.7% of total detections), subtypes of H1 and H3 ratio was detected 61.2% and 38.8%, respectively (Figure 2). When the distribution of influenza viruses were analysed according to weeks, Influenza A H1N1 predominated in 2–4th weeks, Influenza A H3N2 predominated in 51–1st and 5–8th weeks. From the beginning of 8th week, Influenza B viruses started to increase and were also detected in 21st week.

![Figure 1. Distribution of Influenza and other respiratory viruses weekly during the 2007–2008 winter season in Turkey.](image1)

![Figure 2. The frequency of Influenza and Influenza-like viruses during 2007–2008 winter season in Turkey.](image2)
character of the circulating EV71 viruses in this outbreak. For this purpose, we sequence and analyze the whole genome of the EV71 isolates circulating in 2008.

Methods: Throat swabs were collected from HFMD/herpangina children who had participated in a longitudinal cohort study (~700 children) in Chang Gung Children Hospital, Taoyuan in 2008. These collected clinical specimens were inoculated into rhabdomyosarcoma cells for virus isolation. Virus isolates were serotyped using a panel of monoclonal antibodies. EV71 isolates were further genotyped based on VP1 nucleotide sequences (750 bp). In addition, we also conduct whole genome sequencing for EV71 isolates to detect intratypic gene recombination.

Results: Sixty throat swabs were collected from HFMD/herpangina cases from January to September 2008. Seventeen enteroviruses including eight CA2, two CA10, one CA16, one CB4, one CB5, three EV71 isolates, and one untypable enterovirus were isolated. The 3 EV71 cases (aged 19, 20, and 20 month-old, respectively) developed mild infections and all showed seroconversion against EV71 virus (neutralizing titer increased from ≥256). Based on phylogenetic analysis of VP1 nucleotide sequences, these three EV71 isolates have a >99% genetic identity and belong to B5 subgroup (bootstrap value=100%), which has not been detected in northern Taiwan before. Whole genome sequencing has been completed for one of these three EV71 isolates and no intratypic gene recombination was detected.

Conclusion: The predominant enterovirus circulating in northern Taiwan in 2008 was CA2, which is consistent with the national enterovirus surveillance data. New subgroup B5 of EV71 viruses emerged. The mechanism and epidemiological significance of genotype replacement is further studied.

18.162 Chikungunya: Monoclonal Antibodies Production and Their Employment in Serological Diagnosis

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Background: Chikungunya fever epidemic, first evidenced in Italy in 2007, represents the first autochthonous European outbreak of a tropical disease transmitted by vectors (1,2). Monoclonal antibodies (MAbs) specific to Chikungunya virus (CHIKV) were produced and used to develop a competitive ELISA test for anti-CHIKV antibody detection in animal sera from different species collected in the area of the CHIKV outbreak.

Methods: Virus used for MAbs production and as antigen in the ELISA test was strain 200395/07 isolated from an insect pool (Aedes Albopictus). Screening and characterization of MAbs were performed by indirect ELISA, immunoperoxidase, virusneutralization (VN) and Western blotting (WB). Twenty known human sera (10 positive and 10 negative) and 493 animal sera (256 dog, 123 pigeon, 79 chicken, 28 nutria and 7 rabbit sera) were analysed.

Results: Forty five specific MAbs were produced, 9 with VN activity. Two of these (1H7 and 1E10) resulted positive in WB (3). Two neutralizing MAbs (1H7 and 1A7) were further selected, cloned and conjugate with HRP for the development of a competitive ELISA test. MAb 1H7 reacted against a linear epitope while 1A7 against a conformational epitope located both within the E2 protein. The ELISA test was developed using in parallel the 2 conjugated MAbs. Nunc 96 wells plates were coated with partially purified antigen, four dilutions (from 1/5 to 1/40) of each sera were distributed, followed soon afterwards by the addition of selected MAb-conjugates. The ability of sample sera to inhibit the binding of specific MAb-conjugate to the antigen was then evaluated and results were expressed as percentage of inhibition. The human sera were correctly identified whereas all the 483 sera resulted negative.

Conclusion: The serological diagnosis represents a valid diagnostic tool in the study of the epidemiology of this disease and the effective role of animals in the virus spreading.

References

18.163 Molecular Epidemiology of Measles Virus in the Context of Global Measles Control

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Background: The genetic diversity of measles viruses (MV) in Europe and Africa was analysed in the context of regional measles elimination and global measles mortality reduction programs led by the WHO.


Results: In Europe, the prevalence and genetic diversity of indigenous MV genotypes (C2 and D6) was considerably lower than during the 1990s. However, multiple importations of MV strains from Africa and Asia as well as their introduction into highly mobile and unvaccinated communities caused a major spread of MV in Europe during recent years. Many of the imported MV genotypes originated from regions with high measles lethality but in Europe case fatality rates remained low.

In Kinshasa (DRC), different MV genotypes, B3 and B2, were found in two consecutive epidemics (2002–03 and 2004–06), suggesting that MV
circulation had been temporarily interrupted despite sub-optimal vaccination coverage in the local population. The small genetic distance (0.2%) between B2 strains from Kinshasa 2005 and those identified 20 years earlier in Gabon revealed a remarkable genetic stability of the corresponding viruses. In contrast a high genetic diversity of genotype B3 viruses (1.8–2.9%) was found within different cities of Nigeria, even though they were collected within a period of maximum 2 months. The co-circulation of many genetically distinct MV strains suggested that measles remains highly endemic in Nigeria.

Conclusion: Molecular epidemiology of MV is a powerful tool to monitor virus transmission within and between regions with different vaccination coverage and thus helps to develop optimized strategies for global measles control.

**18.164** A Rapid Detection Tool for the Diagnosis of Chikungunya Virus Directly from Aedes albopictus


In the last few years Aedes albopictus (well known as tiger mosquito) quickly and widely diffused in the Italian territory. These insects are ideal vectors for different Arboviruses, as Dengue virus (DenV), estimated to cause in the world about 50 millions of patients per year and as Chikungunya virus (ChikV), causing millions of patients per year.

The Chikungunya virus appeared for the first time in Italy in 2007, in the provinces of Ravenna, Rimini, Forli and Bologna, causing more than 300 human clinical cases and great alarm in the whole Italian population.

This situation prompted our Institute to begin a surveillance plan for this virus and a monitoring program of the vector in the Marche region, a neighbour location near the first Italian ChikV outbreak. The aim of this plan was the reduction of the vector density and a very early individuation of human illness cases to prevent viral diffusion in this territory.

For this purpose we have set up a diagnostic methodology for the rapid detection of the ChikV directly from vectors. Particularly, we have developed an end-point multiplex PCR (with an internal control for Aedes albopictus), which allows to determine the presence of the virus directly in the sampled insects (captured through the use of BG-sentinel® traps), obtained during the active entomological surveillance in the Marche region.

**18.165** Prevalence of Antiretroviral Drug Resistant HIV Among Blood Donors in Western and Eastern Parts of Democratic Republic of Congo

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Background: The use of highly active antiretroviral therapy has had a dramatic effect on the natural history of HIV disease in developed world. Morbidity and mortality associated with HIV infection has been reduced significantly among HIV-1 infected patients. Selection of drug resistant variants becomes inevitable in a setting of continued viral replication. In Democratic Republic of Congo (DRC) where the highest diversity of HIV-1 group M variants circulate, antiretroviral therapy program started n 2002. Access to antiretroviral therapy remains limited in the majority of province.

Objectives: To evaluate and compare the prevalence of antiretroviral drug resistant and the distribution of HIV subtypes HIV among blood donors in two regions of DRC.

Methods and Materials: one hundred and eighty-three HIV positive blood samples were collected using FTA card from blood donors, recruited at General reference hospitals in South Kivu and Kinshasa in DRC from July to December, 2007. RT and LTR region were amplified and RT region was then sequenced by using an automated sequencer. Sequencing analysis was done using online sequence analysis programs from Stanford University, HIV Drug Resistance Database.

Result: HIV-1 RT gene nested PCR were performed to 32 samples from Kinshasa and 34 samples from South Kivu. HXB2 DNA was used as a positive control. Positive and negative controls were included in all the PCR reactions. A total of 37 samples, 22 from South Kivu and 15 from Kinshasa showed correct size positive PCR product. Selected negative samples were further investigated for the presence of integrated HIV-1 gene by LTR PCR. All tested samples were positive indicating the presence of HIV-1 DNA in the samples. A total of 37 amplified HIV-1 RT gene PCR products were sequenced. The sequence analysis showed that 34 samples were found to be HXB2 strain, indicating contamination and only three samples, one from Kinshasa and two from South Kivu were sequences completely different from HXB2. Phylogenetic analysis revealed that the sample from Kinshasa, Kin29 was subtype G and two samples from South Kivu, Kiliba1 and Kiliba2 were subtype A1. No HIV-1 drug resistance mutations were found.

Conclusion: We found no drug resistance mutation, suggesting the limited distribution of drug resistant HIV-1 in recently infected population. Kinshasa and South Kivu subtype are different suggesting the possibility that different subtype might be prevailing in those two parts of DRC. Further study is warranted.

**18.166** Diagnosis of the Pre-Patent Schistosoma Mansoni Infection by Polymerase Chain Reaction (PCR) in Sera of Experimentally Infected Mice

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Background: This work aimed at ascertaining the role of polymerase chain reaction (PCR) in detection of Schistosoma mansoni (S. mansoni) DNA during the pre-patent period in sera of experimentally infected mice. Enzyme linked immuno-sorbent assay (ELISA) was used for detection of circulating antigen (CA) of S. mansoni as a screening method for the diagnosis.

Methods: Swiss albino mice were infected with two doses of S. mansoni (50 and 100 cercariae/mouse). ELISA and PCR techniques were performed on serum samples collected from the infected mice on the first, second, third and fourth weeks post infection (PI).

Results: The ELISA test was positive in 99.66% of samples and the readings were significantly increased with each of the dose and the duration of the infection. PCR technique was positive with all the examined samples including that was negative by the ELISA test. The minimal detectable amount of S. mansoni DNA was 1.16 fg. DNA amplification was not achieved with any of the other heminthes used to evaluate the PCR specificity including S. haematobium adult worms.

Conclusion: Both assays have proved their validity as diagnostic tools for S. mansoni infection as early as the first week PI However, PCR is a promising technique due to its higher sensitivity, specificity and since it is not affected by the immune status of the hosts. Therefore, PCR is more reliable and must be used as a confirmatory test for ELISA negative cases.

New Pathogen Discovery

18.167 – 18.176 Room: Bruckner/Mahler/Brahms – First Level

**18.167** Isolation and Identification of a Parvovirus from Monkey


Background: The monkeys emerged infectious diarrhea and died in some experimental animal center in China. The clinical symptom is similar to infection of canine parvovirus (CPV) or feline panleukopenia virus (FPV).

Methods: Collect feces and organ from ill or died monkeys to isolate and systemical identificate the virulent strain with the methods such as Hemagglutination-hemagglutination inhibition assay (HA-HI), PCR, immunohistochemistry, morphological examination by electron microscope, F81 cell culture, animal regression, and molecular biology.
Results: Specimen HA average titre is 1:29; 3 positive from 7 samples by PCR; positive intestinal chorioepithelium by immunohistochemistry; typical parvovirus-like particle in electron microscope; showed CPE by viral isolation in F81 cell line after blind passage 3. The parvovirus strain was identified by a series of tests. The VP2 gene homology of nucleotide sequences was 98.75% compared with FPV and 98.15% compared with CPV. The VP2 gene sequence see FJ231389 in GenBank. 5 kittens all died by animal regression test and the VP2 gene homology of nucleotide sequences were 99.9% compared with the challenge virus strain.

Conclusion: The results showed the isolation strain was a parvovirus velogenic strain. Whether it is a variant of FPV or how to infect monkey need to research further more.

Background: Severe vector-borne disease outbreaks originate in the tropics and are often caused by previously unknown pathogens. However, little research on new pathogen discovery is done in the last tropical rainforests.

Methods: In order to screen for novel arboviruses in tropical rainforest regions 7,067 mosquitoes were collected in and around the Tai National Park, Côte d’Ivoire. Mosquitoes were pooled according to species, gender, and habitat and investigated for viral infections on cell cultures. Putative virus positive cell cultures were screened by electron microscopy and viral genome fragments were identified by sequence independent amplification methods.

Results: From a total of 437 pools, 97 (22.2%) caused a cytopathic effect on insect cells. Selected pools were analyzed according to morphological criteria by electron microscopy. Flavivirus-rhabdovirus-, coronavirus-, bunyavirus-, orbivirus- and several uncharacterized virus-like particles were detected. We further characterized and described five isolates of different viral families and one unassigned isolate. Sequence comparison of these viruses with other viruses showed only little relationship on amino acid level to other viruses. Investigation of virus characteristics and genome organization, demonstrated that all viruses present novel members of diverse viral families. To exemplify, a novel flavivirus was isolated from Uranotaenia mashonaensis mosquitoes, a genus not known to harbour flaviviruses. Phylogenetic analyses of the whole polyprotein showed that the virus forms a cluster distinct from Aedes- and Culex-borne viruses within the clade of mosquito-borne arboviruses.

Conclusion: Our findings emphasize the importance of screenings for vector-borne pathogens at the border of remote tropical rainforests. In addition, our broad screening process provides an innovative method for discovering novel viruses that could potentially cause infections in humans. Knowledge on pathogens circulating in tropical areas will in turn help to prevent or control outbreaks of newly-emerging diseases in remote rainforest areas.

Results: All cases from the PDD group exhibited viral antigen in at least one of the investigated tissue locations. Usually, large to moderate amounts of viral antigen were present in the brain and intramural ganglia or vegetative nerve fibers of the digestive tract. Viral signals were present in the nuclei, the perikarya and processes of neurons, but also in nuclei of glial cells. The distribution of viral signals in the brain was rather diffuse and random, varied from case to case and there was no preference of certain neuroanatomical structures. From the majority of the tissues investigated, nucleic acid sequences of the N, M and L gene of avian bornaviruses were amplified.

Conclusions: In this presentation we provide evidence that viral proteins and nucleic acids are consistently present in nervous tissues of affected birds. These findings convincingly support the assumption that these viruses are the etiologic agent of PDD, which obviously caused a persistent infection of the nervous system of the affected birds and initiated the resulting immunopathological changes.

Results: From a total of 437 pools, 97 (22.2%) caused a cytopathic effect on insect cells. Selected pools were analyzed according to morphological criteria by electron microscopy. Flavivirus-rhabdovirus-, coronavirus-, bunyavirus-, orbivirus- and several uncharacterized virus-like particles were detected. We further characterized and described five isolates of different viral families and one unassigned isolate. Sequence comparison of these viruses with other viruses showed only little relationship on amino acid level to other viruses. Investigation of virus characteristics and genome organization, demonstrated that all viruses present novel members of diverse viral families. To exemplify, a novel flavivirus was isolated from Uranotaenia mashonaensis mosquitoes, a genus not known to harbour flaviviruses. Phylogenetic analyses of the whole polyprotein showed that the virus forms a cluster distinct from Aedes- and Culex-borne viruses within the clade of mosquito-borne arboviruses.

Conclusion: Our findings emphasize the importance of screenings for vector-borne pathogens at the border of remote tropical rainforests. In addition, our broad screening process provides an innovative method for discovering novel viruses that could potentially cause infections in humans. Knowledge on pathogens circulating in tropical areas will in turn help to prevent or control outbreaks of newly-emerging diseases in remote rainforest areas.
Abstracts

18.171 Virological Investigations in Native Bat Species from Germany
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Background: Despite many worldwide efforts to identify infectious agents within bat species, investigations in European bats are rather sparse, while most of such research projects concentrate on rabies viruses. A broad study regarding the occurrence of bacterial and viral infectious agents and their impact on bats from Germany is currently performed by the Leibniz Institute for Zoo and Wildlife Research, Berlin, in cooperation with the Federal Robert Koch Institute.

Methods: Carcasses of bats, which were found dead by bat researchers or protectionists, are necropsied and examined histo-pathologically while remaining organ tissues are investigated bacteriologically and virologically. For virology, tissue homogenates are inoculated onto cell culture to isolate potential viral agents. Additionally, various nucleic acid amplification and sequencing techniques are performed to identify further viral infections as i.e. adeno-, corona-, flav-, hanta-, herpes-, influenza-, parainfluenza- and paramyxoviruses. Furthermore, virology results are compared with histo-pathological findings to verify whether a detected virus causes pathological changes in the bat or whether the virus is carried by the animal without harming it.

Results: During this study, we isolated a novel adenovirus in microbats (Pipistrellus pipistrellus) from the suborder microchiroptera, visualized by electron microscopy and amplified by random PCR. Sequence analysis of the complete polymerase gene revealed a close relationship to canine adenovirus 1 and 2, genus Mastadenovirus. Tissue tropism studies indicated the new BatAdVPPV1 as an intestinal but not respiratory pathogen.

Conclusion: This is the first documentation of an adenovirus isolated from migratory microbats. The same virus was isolated from several moribund bats. Therefore, although its pathogenicity for humans is still unknown, it is strongly indicated for bats.

18.172 Unusual Gram-Negative Sepsis in Immunocompromised
V. Kolli, S. Swaminathan, D. Alcid. St Peter’s University, New Brunswick, NJ, USA, St Peter’s University Hospital, New Brunswick, NJ, USA

Case 1: A 42-year-old woman with invasive cervical cancer, on chemotherapy and radiation, past intravenous drug use, and several prior infections including Staphylococcal spinal osteomyelitis and polymicrobial bacteremia; viridans streptococcus, Acinetobacter, and Pseudomonas therapy and radiation, past intravenous drug use, and several prior infections as i.e. adeno-, corona-, flav-, hanta-, herpes-, influenza-, parainfluenza- and paramyxoviruses. Furthermore, virology results are compared with histo-pathological findings to verify whether a detected virus causes pathological changes in the bat or whether the virus is carried by the animal without harming it.

Results: During this study, we isolated a novel adenovirus in microbats (Pipistrellus pipistrellus) from the suborder microchiroptera, visualized by electron microscopy and amplified by random PCR. Sequence analysis of the complete polymerase gene revealed a close relationship to canine adenovirus 1 and 2, genus Mastadenovirus. Tissue tropism studies indicated the new BatAdVPPV1 as an intestinal but not respiratory pathogen.

Conclusion: This is the first documentation of an adenovirus isolated from migratory microbats. The same virus was isolated from several moribund bats. Therefore, although its pathogenicity for humans is still unknown, it is strongly indicated for bats.

18.173 Experimental Challenge of Cattle with H-type and L-type Atypical BSE
A. Buschmann1, U. Ziegler1, M. Keller1, R. Rogers2, B. Hills3, M.H. Groschup4, 1Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany, 2Health Canada, Bureau of Microbial Hazards, Health Products & Food Branch, Ottawa, Canada, 3Health Canada, Transmissible Spongiform Encephalopathy Secretariat, Ottawa, Canada

Background: After the detection of two novel BSE forms designated H-type and L-type atypical BSE the question of the pathogenesis and the agent distribution of these two types in cattle was fully open. From initial studies of the brain pathology, it was already known that the anatomical distribution of L-type BSE differs from that of the classical type where the obex region in the brainstem always displays the highest PrPSc concentrations. In contrast in L-type BSE cases, the thalamus and frontal cortex regions showed the highest levels of the pathological prion protein, while the obex region was only weakly involved.

Methods: We performed intracranial inoculations of cattle (five and six per group) using 10% brainstem homogenates of the two German H- and L-type atypical BSE isolates. The animals were inoculated under narcosis and then kept in a free-ranging stable under appropriate biosecurity conditions. At least one animal per group was killed and sectioned in the preclinical stage and the remaining animals were kept until they developed clinical symptoms. The animals were examined for behavioural changes every four weeks throughout the experiment following a protocol that had been established during earlier BSE pathogenesis studies with classical BSE.

Results and Discussion: All animals of both groups developed clinical symptoms and had to be euthanized within 16 months. The clinical picture differed from that of classical BSE, as the earliest signs of illness were loss of body weight and depression. However, the animals later developed hind limb ataxia and hyperesthesia predominantly and the head. Analysis of brain samples from these animals confirmed the BSE infection and the atypical Western blot profile was maintained in all animals. Samples from these animals are now being examined in order to be able to describe the pathogenesis and agent distribution for these novel BSE types.

Conclusions: A pilot study using a commercially available BSE rapid test ELISA revealed an essential restriction of PrPSc to the central nervous system for both atypical BSE forms. A much more detailed analysis for PrPSc and infectivity is still ongoing.

18.174 A New Archetype Poxvirus from an Immunosuppressed Patient in the US
Y. Liu1, J. Abraham2, D. Blair2, H. Zhao1, S. Sammons4, G. Emerson1, G. Dhawan1, D. Carroll1, M. Laken1, R. Kline1, R. Regnery1, N. Bartholoma1, M. Barczak2, C. Darnell2. 1CDC, NCZVED, DVD, PRB, Atlanta, GA, USA, 2Dept. of Pathology, SUNY Upstate Medical University, Syracuse, NY, USA, 3Infectious Disease Division, SUNY Upstate Medical University, Syracuse, NY, USA, 4CDC, NCPDCID, DSR, Biotechnology Core Facility Branch, Atlanta, GA, USA

A new poxvirus (Pox_NY014) was isolated from a patient with pustular rash, on immunosuppressives post renal allograft, whose only known animal exposure was to a neighbourhood feral cat. Biopsy histopathology revealed eosinophilic cytoplasmic inclusions, and brick-shaped poxvirus-like particles within A type inclusions were confirmed by electron microscopy. Extracted nucleic acid was not recognized by multiple PCR assays targeting Eurasian and North American orthopoxviruses. Initial DNA sequences from randomly cloned DNA fragments, and an amplicon of a highly conserved region within the RNA polymerase, showed moderate similarity to orthopoxvirus, capripoxvirus and suipoxvirus with slight
preferences to orthopoxvirus. High-throughput, massively-parallel pyrosequencing technology achieved complete genome sequencing (minus hairpin ends). Preliminary assemblage of the viral genome was completed using PCR and traditional Sanger sequencing technology. Phylogenetic analyses show Pox_NY014 forms a monophyletic node with the genus Orthopoxvirus, but is basal to the node containing all other members of the genus. Similar to other chordopoxvirus, Pox_NY014 has a conserved central genome structure and gene content, but lacks several orthopoxvirus-specific genes toward the ends, including the hemagglutinin gene. Pox_NY014 appears to have diverged from an early ancestor of the genus Orthopoxvirus.

**18.175**

A Case of Community-Acquired Pneumonia Caused by *Streptococcus Pyogenes* Without Beta-Hemolysis

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**Background:** *Streptococcus pyogenes* is relatively rare cause of community acquired pneumonia (CAP) and less than 1% of CAP is caused by this organism. Also, lack of beta-hemolysis is uncommon in this organism.

**Case:** A 41-year-old Japanese man without significant past medical history presented to Kobe University Hospital, Japan, with 10 days of fever and left side pleuritic chest pain. On presentation, he appeared well with blood pressure 104/60 mmHg, pulse rate 100/minute, respiratory rate 16/minute, body temperature 39.2°C, and SpO2 98% on room air. There was slight decrease in breath sound on the left side. Chest X-ray showed left side pleural effusion with pneumonia. Multiple blood cultures identified *streptococcus* without beta-hemolysis but was later identified as *Streptococcus pyogenes*. PYR test was positive and 16S-RNA test also confirmed *S. pyogenes*. Subsequent cultures under aerobic, microaerobic and anaerobic conditions were negative for beta-hemolysis. The patient was successfully treated with IV penicillin G followed by oral clindamycin. HIV test was negative. There was no other persons around with the same symptoms.

**Discussion:** *S. pyogenes* is uncommon cause of pneumonia and with bacteremia, mortality is high (38-47%). This patient had mild symptoms unlike typical diseases caused by this organism. Lack of beta-hemolysis, unusual in this organism, might have affected the clinical presentation. Further studies may be necessary to characterize the clinical importance of non-hemolytic *S. pyogenes*.

**18.176**

Massilia Virus, A Novel Phlebovirus (Bunyaviridae) Isolated from Sandflies in the Mediterranean

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A new virus was isolated from three independent pools of Phlebotomus perniciosus sandflies (Diptera; Psychodidae) trapped in two regions of south-eastern France, located 90 miles apart. Microscopic, antigenic and genetic analyses indicate that this novel virus belongs to the genus Phlebovirus in the family Bunyaviridae. The new virus is designated Massilia virus since the first isolate was obtained from sandflies collected in the suburban area of Marseille. The complete genome sequence was determined and used to compare the genetic and phylogenetic relationships of Massilia virus with other phleboviruses. Genetic and antigenic properties were employed to address whether or not Massilia virus should be considered a new species within the genus, or a member of a previously recognized species. Cerebrospinal fluid specimens, collected from local patients with central nervous system infections during the previous four-year period were tested for the presence of Massilia virus RNA, but gave negative results. In conclusion, Massilia virus is proposed as a member of the Sandfly fever Naples virus complex; its public health importance has yet to be determined.

**Figure 1.** Morphological identification of Massilia virus. A–D. Negative-stain electron microscopy of Vero E6 culture supernatant medium at day 6 post infection (6th passage). The bars represent 100 nm. E. Vero E6 cells infected with biological material from pool W are shown reacting with anti-TOSV human serum. F. Uninfected Vero E6 cells reacting with anti-TOSV human serum

**Agents of Bioterrorism**

18.177 MLVA and SNR Typing of *B. anthracis* Isolates Originating from the Republic of Bulgaria

M.H. Antwerpen1, D. Illin1, H. Meyer1, E. Savov2, D. Frangoulidis1. 1Bundeswehr Institute of Microbiology, Munich, Germany, 2Military Medical Academy Department of Clinical Microbiology, Sofia, Bulgaria

*Bacillus anthracis* is an aerobic growing, spore-forming soil bacterium and the causative agent of anthrax, a serious disease in animals and humans for centuries. Research on anthrax as a biological weapon began more than 80 years ago. Investigations of the “anthrax letters” in the U.S.A in 2001 led to an increased activity in forensic microbiology. To this end Multi Locus Variable Numbers of Tandem Repeats Analysis (MLVA) and Single Nucleotide Repeat (SNR) analysis are applied to characterize isolates, to determine their specific genetic “signature” and to assemble databases in order to rapidly identify the origin of a isolated strain.

Today many strain collections from different geographic parts of the world have been analysed and the results have been published. But still some geographic regions exist, where nothing is known about the genetic diversity of local *B. anthracis* strains.

In this study we investigated a collection of 40 *B. anthracis* strains isolated in the Republic of Bulgaria between 1960–80 from soil and animals. Using MLVA and SNR-analysis and subsequent clustering using UPGMA (Unweighted Pair Group Method with Arithmetic mean) methods a unique “Bulgarian” cluster could be identified and mapped within the global context.

Furthermore, two new MLVA-allele lengths were determined which underlines the need to discuss the current used nomenclature in terms of a better inter-laboratory comparison.

**18.178**

Biosafety—Accurate Risk and Vulnerability Assessment of the Release of Bioagents from Laboratories

T.M. Kollars, Jr., Jiar-Ping Hsu College of Public Health, Georgia Southern University, Statesboro, GA, USA
Bioagents and other pathogens stored within biorepositories and laboratories constitute a perceived and/or significant health risk to populations. Current methods to assess the risk posed by these pathogens include physics, weather, basic epidemiology and sometimes only insurance to cover liability.

Biotic and abiotic factors in the environment will affect the survival and amplification of bioagents and pathogens purposefully or accidentally released from these facilities. A bioagent transport and environmental modeling system (BioTEMS) was developed to provide public health and consequence management officials with a tool for pre-planning as well as a means of rapid and accurate assessment should a bioagent release occur. Risk assessment has been conducted for training exercises, laboratory facilities, government facilities and the US Republican and Democratic Presidential Conventions.

18.179 Real-Time PCR Assay for the Detection of A New Simulant for Poxvirus Biothreat Agents

L. Garnier, J.C. Gaudin, I. Rebiball, P. Bensadoun, Y. Morel. Centre d’études du Bouchet, Vert Le Petit, France

Research and financial efforts spent on biodefense technologies highlight the current concern for biothreat events preparedness. Non-hazardous but relevant “simulant” micro-organisms, because they can be easily manipulated, are typically used to simplify technological developments, testing, and staff training. The bacteriophage MS2, a small RNA virus, is classically used as the reference simulant for bioterrorism within the biodefense community. However, variola virus, considered as a major threat, displays very different features (size, envelope, and double-stranded DNA genome). The size parameter is critical for aerosol sampling, detection, and protection/filtration technologies. Therefore a panel of relevant simulants should be used to cover the diversity of bioterror agents. Thus, we investigated a new virus model, the Cydia pomonella granulovirus (baculovirus), which is currently used as a biopesticide. It displays a size similar to that of poxviruses, is enveloped, and contains double-stranded DNA. To provide a molecular tool to detect and quantify this model virus, we developed an assay based on real-time PCR, with a limit of detection ranging from about 10 to a few tens of target copies per μl according to the sample matrix. We checked the specificity of the assay against a large panel of potential cross-reactive micro-organisms and concluded that the assay is suitable for environmental samples, especially aerosol studies. In conclusion, we suggest that our PCR assay allows Cydia pomonella granulovirus to be used as a simulant for poxviruses.

18.180 Dual-Use Dangers: Biorisks and Ecohealth Perspectives

A. Gupte., Pune, India

Background: Accelerating pace of biodiversity loss, biosciences, biotechnologies and bioproduction for a bioeconomy is of concern in today’s world at risk. Most of the ancient pathogen bioweapons are now used as raw material for genetic engineering, synthetic biology, biotechnologies, nanotechnologies and so on. Low-tech, small-scale and non-lethal bioterror attacks can also be effective. The responsibility to prevent hostile uses of science and technology lies with each State. It extends beyond governments to all persons.

Methods and Materials: Literature review: scientific aspects of ecohealth, biorisks, dual use dangers, inspection and verification mechanisms, work of United Nations: genomics, proteomics, metabolomics and other “omics”; synthetic biology; vulnerability to attack of the immune system; antibiotic resistance; bioregulators; complexity of dual-use; target delivery systems; relevance of bioregulators for arms control; bioterrorism and bioinformatics; prevention, inspection and verification needs, mechanisms and ecohealth perspectives.

Discussions with Key Experts: UN, universities and governments.

Lessons Learned: from multidisciplinary education and diverse work experience mainly in India, United Nations and Canada.

Results: Underlying causes of emerging (and re-emerging) diseases are often complex and influenced by biodiversity loss, socioeconomic, culture, health, land use and global changes that can provide ideal conditions for the spread and high mortality rate of infectious diseases. Many pathogens of plants and humans have common disease mechanisms of attachment, secretion and genetic regulation. Pathogenicity and life sciences knowledge might be misused to cause harm. Despite their inherent biorisks, BS-4 labs are not specifically regulated in the USA, being subject to oversight only if they are government-funded or possess pathogen and toxins on a government’s list.

With rapid loss of biodiversity and advances in bioeconomy, agroterrorism is a major threat. The list of agrobioweapons is endless. Ecohealth plays an important role in the effectiveness of transgenic plants, agrobioweapons and food safety. Lapses in food safety inspections and surveillance caused 20 deaths due to 2008 listeriosis outbreak in Canada. Plants are used for producing almost 50% of our drugs and better environmental quality could reduce 41% of deaths due to infectious diseases. Loss of biological diversity such as the lack of genetic diversity in Tasmanian devil population is allowing facial tumor disease to spread at a rate that may lead to extinction in 20 years.

Conclusions: There is an urgent need to: (a) routinely conduct a global and national ecohealth assessment of biorisks and biotreats; and (b) establish a global biorisk information system to facilitate integrated ecohealth data and analyses for comprehensive ecohealth monitoring of biorisks of outbreaks of emerging and re-emerging infectious diseases (wildlife, plant, zoonotic and human).

Climate Change

18.181 – 18.183 Room: Bruckner/Mahler/Brahms – First Level

18.181 Modeling of Spatially Referenced Meteorological and Landscape Factors Influencing the Probability of Pathogen Isolation from Natural Environments

R. Ivanek1, Y.T. Grohn2, M.T. Wells2, A.J. Lembo2, B.D. Saunders2, M. Wiedmann2. 1Texas A&M University, College Station, TX, USA, 2Cornell University, Ithaca, NY, USA, 3Salisbury University, Salisbury, MD, USA, 4New York State Department of Agriculture and Markets, Albany, NY, USA

It is accepted that changes in climate in coming decades will likely cause important changes in the incidence and distribution of diseases caused by pathogens with free-living stages (such as Listeria monocytogenes, Salmonella, Escherichia coli, Campylobacter and Cryptococcus gattii). It is therefore important to understand how environmental factors influence the occurrence of pathogens’ free-living stages in the environment. Our objective was to develop a methodological framework to study spatially explicit meteorological and landscape factors affecting the probability of pathogen isolation from a location.

Data on isolation of Listeria spp. from the natural environment were used as a model system. From readily available spatial data models, for each sampled location we obtained potentially relevant spatially referenced covariates (grouped under soil properties, precipitation, ambient temperature, alternating freezing and thawing temperatures and geographic position). Logistic Regression (LR) and Classification Tree (CT) methods were applied and their successes in predicting Listeria presence in the environment were compared.

Analyses revealed that precipitation and alternating freezing and thawing temperatures prior to sample collection, loam soil, water storage to a soil depth of 50 cm, slope gradient and cardinal direction to the North are key predictors for Listeria isolation from a spatial location. Different combinations of factors affected the probability of Listeria isolation from the soil, vegetation and water layers of a location, indicating that the three layers represent different ecological niches for Listeria. The predictive power of CT was comparable to that of LR. However, CTS were easier to interpret, making them more appealing for field applications.

Our study identified factors affecting Listeria isolation from a location, which is valuable because Listeria is often used as an indicator microorganism for its pathogenic species L. monocytogenes. Moreover, it demonstrated the utility of the developed modeling framework in analysis of a
pathogen’s spatial distribution with the purpose of identifying predictors of the pathogen’s presence in the environment. That knowledge could be used to forecast microbial occurrence in response to changes in weather patterns anticipated in the coming years and, accordingly, to propose control strategies to reduce human and animal environmental exposure.

**18.182 Climate Change Simulations of Usutu Virus Dynamics in Vienna**

F. Rubel, K. Brugger. University of Veterinary Medicine, Vienna, Austria

**Background:** The emergence and spread of infectious diseases in mid-latitudes, so far mainly observed in the tropics, considerably increase under the current situation of climate change. A recent example is the Usutu virus (USUV) outbreak in Vienna, Austria. USUV is closely related to the West Nile virus (WNV) in the U.S. and caused mass mortalities mainly of blackbirds (*Turdus merula*). The USUV flavivirus persists in a natural transmission cycle between vectors (mosquitoes) and host reservoirs (birds) and leads—once endemic in a population—to periodic outbreaks.

**Method:** In an epidemic model to explain the USUV dynamics in Austria 2001–2005 (Rubel et al., 2008), USUV dynamics were mainly determined by an interaction of bird immunity and environmental temperature. To investigate future scenarios, we entered temperature predictions from five global climate models into the USUV model and also considered four different climate-warming scenarios defined by the Intergovernmental Panel on Climate Change, IPCC (20 different model-scenario combinations). We downscaled the 20 time series of predicted temperatures (through the year 2100) to represent the region around Vienna.

**Results:** Our simulations predict that USUV will persist in the host population after the epidemic peak observed in 2003. USUV-specific annual blackbird-mortality time series predict that the outbreak frequency increases successively from the beginning to the end of the century. Simulations of worst-case scenarios result in an endemic equilibrium with a decline of the blackbird population of about 24 percent. Additionally we calculated the annually averaged basic reproduction number for the period 1901–2010 to represent the region around Vienna.

**Conclusions:** We demonstrated that USUV epidemics are forced by environmental temperature and investigated future scenarios based on climate predictions. Our process model is proposed to investigate and explain the dynamics of the continental-scale WNV epidemics in North America.

**18.183 Rate of Change in Lyme Disease Incidence in the United States Exhibits a North-South Gradient Consistent with Climate Change Effect**

D. Fisman. Hospital for Sick Children and Ontario Public Health Laboratory, Toronto, Canada

**Background:** Tick-borne illnesses such as Lyme disease represent an important class of emerging zoonoses in North America. The northern range of ixodes tick vectors is limited by a spring-autumn interval insufficient to allow completion of the tick life cycle. Climate change is projected to increase this geographic range, resulting in increased incidence in northern areas. We evaluated this possibility using a meta-regression approach that identifies sources of between-state heterogeneity in U.S. Lyme disease trends.

**Methods:** Data on reported Lyme disease cases in the United States (1993–2007) were obtained from the U.S. Centers for Disease Control, and year-on-year incidence rate ratios (IRR) were estimated using Poisson regression methods, with state populations as offsets. We evaluated between-state heterogeneity in IRR with random effects meta-analysis; state characteristics associated with increasing incidence were identified using random effects meta-regression.

**Results:** Incidence of Lyme disease in the U.S. as a whole increased by 4.9% per year between 1993 and 2007 (95% CI 4.8% to 5.0%). There was significant between-state heterogeneity in temporal trends (P<0.001); incidence increased in 21 states, decreased in 14, and was unchanged in 15. In multivariable meta-regression models, increasing incidence showed a linear association with latitude (1% increase in IRR per degree, (95% CI 0.4% to 1.8%, P = 0.001)) and was inversely associated with per-capita health spending (2.3% decrease in IRR per $100, (95% CI 0.1% to 4.5%, P = 0.04)).

**Conclusions:** Increases in Lyme disease incidence in the U.S. are not uniform at the state level, and the rate of increase has been greatest in northern states, while stable or declining elsewhere. These differences in trends are consistent with expectations under climate change projections, and suggest that global warming trends have already impacted disease ecology. The protective effect associated with level of investment in health suggests that mitigation is possible.

![Figure 1. Mean annual basic reproduction numbers for IPCC scenario A1. Period 1901–2100.](image1)

**SESSION 19 (Parallel Session)**

**Communicating Disease Risks to the Public**

Monday, February 16, 2009
Room: Park Congress/Ground Level
08:30–10:30

**19.001 WHO’s Outbreak Communication: Guidance for Communicating During Public Health Emergencies**

D. Thompson. Consultant, Geneva, Switzerland

Infectious disease outbreaks are unique public health events. Communication with the public during an outbreak is critical not only for their rapid
control but also for reducing the social, political and economic turbulence which often attends outbreaks. Following containment of SARS, the World Health Organization began an extensive review of risk communication literature to identify critical communication components. The review identified five critical features communication. These five features were then assessed and refined at a meeting of outbreak control managers and others public, its determinants and their relationships with observed fluctuation of beef consumption, two surveys were conducted on a representative sample of the adult population; the first one in 2000 during the peak of a crisis related to an alarming event and the second one 13 months later in a quieter period. The results issued from a bivariate and multivariate analysis show that; the distribution of most of the variables significantly correlated to the perceived risk identified in the first survey had changed in the second survey, in relation with the reduction of worry and the resumption of national beef consumption; the propensity to a self protection through avoiding or ceasing beef eating was more associated to feelings of worry than to subjective vCJD probability; the main determinant of a less avoidance to beef products was the preference for beef, a feeling anchored prior to emergence of the risk of vCJD.

**19.002** Social Aspects of Risk Perception

M. Setbon\(^1\), J. Raudet\(^1\), A. Flahaut\(^1\), \(^1\)CNRS & EHESP, Aix en Provence, France; \(^2\)EHESP. Rennes, France

Risk of vCJD related to BSE-mad cow which emerged on 1996 had monopolized durably concern and attention as well of European public health authorities as of consumers consumers of beef meat. The newness of this food-borne disease and the dread suggested by its spread through beef consumption, acknowledged as the causal factor, lead to an unprecedented collapse in the beef market. Despite successive costly measures taken by authorities to reduce the risk, a global avoidance from beef was stated and assumed as the expression of risk perception, defined here as people’s cognitive and affective response to this emerging generalized threat. In order to identify the distribution of risk perception among the French public, its determinants and their relationships with observed fluctuation of beef consumption, two surveys were conducted on a representative sample of the adult population; the first one in 2000 during the peak of a crisis related to an alarming event and the second one 13 months later in a quieter period. The results issued from a bivariate and multivariate analysis show that; the distribution of most of the variables significantly correlated to the perceived risk identified in the first survey had changed in the second survey, in relation with the reduction of worry and the resumption of national beef consumption; the propensity to a self protection through avoiding or ceasing beef eating was more associated to feelings of worry than to subjective vCJD probability; the main determinant of a less avoidance to beef products was the preference for beef, a feeling anchored prior to emergence of the risk of vCJD.

**19.004** Communicating via Social Networks

P.M. Polgreen. University of Iowa, Iowa City, IA, USA

Communicating information about infectious diseases is an important part of efforts to prevent, control and treat infectious diseases. Critical information often exists but is disparate in nature. People in different geographic locations or disciplines have access to unique and valuable information not known by others. To facilitate the rapid transfer of information between groups and individuals, we need to know something about how those groups and individuals communicate and interact. One way to view how information travels between individuals is to study the “social networks” to which they belong.

Recent interdisciplinary research, inspired at least in part by the study of emerging electronic communities, finds that structural properties of real communities, expressed as contact graphs, are best represented by scale-free networks. In such networks, the number of contacts per individual is distributed according to a power law, and not a uniform distribution. Thus, while most individuals have “contact” with relatively few others, a small number of individuals serve as “hubs,” connecting a relatively large number of other individuals (the so-called “small world property”).

To increase the flow of information, we need to focus on identifying and exploiting the “hubs” in each network. We also need to identify individuals with ties or connections to different networks. These people serve can serve as bridges and introduce new information to different groups. While it might be difficult to change the social network of existing groups or communities, it might be possible to help build bridges (i.e., “loose ties”) to other networks. This might help accelerate the communication of information about infectious diseases. If such connections can be developed and maintained in a meaningful manner, they might be critical to during outbreaks especially those involving multiple countries and/or multiple species.

**SESSION 20 (Parallel Session)**

**Vaccines and Reemerging Diseases**

*Monday, February 16, 2009*

*Room: Klimt Ballroom 2 & 3/First Level*  
*08:30–10:30*

**20.001** The Pandemic Threat of Avian Influenza Viruses

K. Subbarao. NIH, Bethesda, MD, USA

Aquatic birds represent the reservoir of influenza A viruses in nature. Viruses of all known (16 HA and 9 NA) subtypes have been isolated from waterfowl and shorebirds, while a limited number of HA and NA subtypes have caused epidemics of human influenza. Antigenic shift is a rare but epidemiologically highly significant event in which a virus bearing a novel HA, or with or without an accompanying novel NA, is introduced into the human population. A virus bearing a novel HA or NA has the potential to cause a pandemic if a large proportion of the population lacks immunity to the novel HA and NA and if the virus has the ability to spread efficiently from person to person. Highly pathogenic avian influenza A H5N1 viruses have been circulating in avian species since 1997 and have caused more than 350 human infections and more than 250 deaths since 2003. The outbreaks of human infection by H5 subtype avian influenza viruses along with sporadic reports of infections by H7 and H9 subtype avian influenza viruses underscore an urgent need to develop novel prophylactic and therapeutic interventions to protect humans in the event of a pandemic. Although it is not known exactly when or which influenza A subtype will be responsible for the next influenza pandemic, the outbreaks of highly pathogenic avian influenza in poultry and an increasing number of avian influenza virus infections in humans are warning signs that should be heeded. Vaccination is the key strategy to prevent severe illness and death from pandemic influenza. We have also evaluated the efficacy of neutralizing human monoclonal antibodies for prophylaxis and treatment of H5N1 infection in BALB/c mice. While a pandemic might be inevitable, the impact of the pandemic can be mitigated by preparations to control its spread. Promising findings of efficacy suggest that active and passive immunization approaches may be useful for prophylaxis and/or adjunctive treatment of human infections by avian influenza viruses.

**20.002** Vero cell Derived Whole Virus H5N1 Vaccine: Safety, Prime-Boost and Cross-Neutralization Data


**Introduction:** The rapid spread of avian influenza (H5N1) and the transmission to humans has induced world-wide fears of a new pandemic. Vaccines are considered the most effective means to control influenza outbreaks, however, the conventional methodologies used to manufacture H5N1 vaccines have a number of disadvantages. In particular egg supplies required for vaccine production could be endangered by H5N1 infections of chicken flocks. Also clinical trials to date with non-advantaged H5N1 split vaccine formulations have demonstrated that very high antigen doses are required to induce seroconversion in immunised subjects. An alternative strategy involves the use of a wild-type virus grown in a...
continuous cell culture (Vero) system to derive an inactivated whole virus vaccine. Candidate vaccines based on clade 1 (Vietnam/1203/2004/H5N1) and clade 2 (Indonesia/05/2005/H5N1) strains have been developed and demonstrated to be highly immunogenic and protective in animal models.

Results: of clinical trials to date with clade 1 (Vietnam/1203/2004) and clade 2 (Indonesia/05/2005) strain vaccines indicate (i) the vaccines are well tolerated (ii) vaccine doses as low as 3.75 or 7.5 µg generate a robust immune response (iii) the non-adjuvanted formulation is more immunogenic than an alum adjuvanted formulation (iv) the vaccines induce antibodies which are capable of neutralising not only homologous strains but also viruses from other H5N1 clades or subclades.

Conclusion: These data indicate that this cell culture strategy allows the high yield production of a pandemic vaccine and the whole virus vaccine based on the wild-type virus.

20.003 Bluetongue Virus Vaccines: Solution or Part of the Problem?
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Bluetongue (BT) is an insect-transmitted disease of ruminants caused by BT virus (BTV). The disease was first described after European settlers introduced their fine-wool breeds of sheep to South Africa in the 17th century. BTV infection has since been described on all continents except Antarctica, coincident with the distribution of competent Culicoides vectors. Climate change is likely responsible for the remarkable recent expansion of BTV's global range, especially in Europe. Modified live (MLV) BTV vaccines were first developed in South Africa. Embryonated egg propagated MLV vaccines developed in California were teratogenic if used in pregnant sheep and were replaced by cell culture propagated vaccines. Phylogenetic analyses confirm that individual genes of field strains of BTV in California are identical to those of MLV vaccines, suggesting that vector insects naturally can acquire and transmit vaccine viruses (or reassortant viruses that include MLV genes) as also demonstrated recently in Italy. The original MLV vaccine caused characteristic brain defects in congenitally infected progeny, defects that occur sporadically in regions where these vaccines are used. Significantly, until the emergence of BTV serotype 8 (BTV8) in northern Europe, congenital BTV infection only was described in regions where MLV vaccines are used. The European strain of BTV8 commonly causes teratogenic brain defects in the progeny of cattle infected during early gestation, defects that are identical to those caused by the original MLV vaccines—a fact that raises concerns regarding the origin/history of this strain of BTV8. Inactivated BTV vaccines have been developed because of the propensity of MLV vaccines to revert to virulence, their capacity to be acquired and transmitted by Culicoides insects, and because of the capacity of BTV to generate extensive genetic diversity through reassortment and genetic drift. However inactivated vaccines also have inherent deficiencies, which has stimulated development of new generation vaccines including baculovirus-expressed virus-like particles and recombinant expression systems, most notably recombinant canarypox virus vectors that express immunogenic proteins of BTV and that induce sterilizing immunity in vaccinated ruminants.

20.004 Dengue Vaccine Development
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Dengue is an emerging mosquito-borne disease, caused by four related but different dengue viruses (DENV) that produces significant morbidity and consumes considerable health care resources in tropical and subtropical countries. An estimated 3.6 billion people (55% of world's population) live in areas at risk for DENV transmission. Each year, an estimated 36 million cases of dengue occur, which result in 2.3 million cases of severe dengue and 21,000 deaths. Vector control has been the only method to prevent dengue, a strategy that suffers from poor sustainability and effectiveness due to the unique ecology of the Aedes mosquito, the vector of DENV.

Prevention of dengue by immunization appears technically feasible and five candidate vaccines are now in preclinical or clinical evaluation. However, dengue is one of the more challenging vaccines presently under evaluation. A dengue vaccine must provide durable protection against infection by four different DENVs, which will require a tetravalent formulation. Other challenges include: 1) ensuring high levels of long lasting immunity to minimize the theoretical potential for antibody dependent enhanced disease (ADE) in partially immune persons, and 2) the need to conduct clinical trials in multiple sites to determine protection against multiple DENV's and the wide range of age groups affected by dengue.

The Pediatric Dengue Vaccine Initiative (PDVI) was established to accelerate dengue vaccine development, evaluation and introduction. The PDVI operates as a product development partnership (PDP) and supports activities in: 1) product development, 2) supportive research, 3) vaccine evaluation, and 4) vaccine access.
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