Acknowledgement

Charoen Pokphand Group
Intervet
Merial
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Introduction

During the past few years we have witnessed the global emergence and re-emergence of several infectious animal diseases that have had a major impact on both animal and human health and evoked strong reactions from policy-makers and the media at the world-wide level. Fortunately, as a result of the incorporation of new scientific and technological knowledge, we now have methods to prevent many of these infectious diseases. This ‘explosion’ in technology has no precedent in the history of mankind and it comes at a time when, due to the globalisation process, the circulation of pathogens around the world is increasing.

The OIE, being the international reference organisation with regard to animal health issues and zoonoses (terrestrial and aquatic), has not remained indifferent to this new situation. Throughout time it has incorporated into its standards the best ‘state of the art’ scientific knowledge with the purpose of improving veterinary and public health conditions in all countries and protecting international trade in animals and animal products while avoiding unjustified sanitary barriers.

Avian influenza is one of the re-emergent diseases having a potential zoonotic impact, the control of which is complicated by the predominant role of the avifauna in the dissemination of the virus throughout the world. A particularly severe outbreak has hit South-East Asia since the end of 2003. Since then OIE and FAO, in close cooperation with WHO have been very active in coordinating the control of the disease and supporting the infected countries in their efforts in attempting to eradicate it. Despite these important efforts, the disease still persists. Other countries in the region and the rest of the world are at risk of becoming infected.

OIE and FAO have considered that it is timely to hold an «International Scientific Conference on Avian Influenza» to review the latest scientific knowledge and to address the different aspects of the control of the disease based on this knowledge.

This International Scientific Conference will present the valuable experience gained in the last few years in the field of epidemiology, pathogenesis, diagnosis, vaccine control and eradication of the disease. The Conference is an opportunity for the exchange of the latest scientific information at the global level that will, at the same time, assist in the evaluation and improvement of the current standards and guidelines for better control of infectious animal diseases.

It is expected that all Member Countries will benefit from this International Conference. I therefore have much pleasure in welcoming all of you to the OIE/FAO International Scientific Conference on Avian Influenza.

Dr Bernard Vallat
OIE Director General

Dr Joseph Domenech
FAO Chief Veterinary Officer
Objectives of the Conference

- To provide a multidisciplinary forum for the exchange of the latest scientific information on avian influenza
- To discuss avian influenza control and prevention, including vaccination.
- To guide OIE in setting new standards and guidelines for surveillance and international trade for adoption by Member Countries
- The main topics included on the agenda of the Conference are:
  - Ecology and epidemiology
  - Pathogenesis
  - Human health implications
  - Diagnostics
  - Control

Topics

Ecology and Epidemiology

The field to be covered includes the molecular epidemiology and antigenic profile of Avian Influenza virus isolates throughout the world and aspects of infection in domestic and wild birds. Of particular relevance is the holistic epidemiological picture of the disease and, whether the ecological and epidemiological pattern of avian influenza is changing as a result of environmental circumstances in different regions of the world and through the passages in different species of birds. Particular emphasis will be given to surveillance and the epidemiology of 'host switching' between wildlife reservoirs (avifauna) and domestic birds and the public health implications. For those concerned with policy and planning the control of infections, data on retrospective analysis of outbreaks and on going epidemic will be of particular value.

Pathogenesis

This topic will focus on the host and viral factors which determine the pathogenicity of Avian Influenza virus. It will include reviews of the behaviour of the virus in wild and domestic birds and the possible role of mammals such as pigs. Highly pathogenic strains will be compared with low pathogenic strains and the peculiarities of the H5N1 Asian stain will be reviewed. The role of wild and domestic ducks is a source of concern in many regions in the world and the scientists will review the pathogenesis of AI in this species which lead to silent infection in most of the cases. The role of pigs as a possible vessel for reassortment of avian strains and the risk of transmission to human will also be covered.

Human Health Implication of AI

The interface among animal health and public health authorities, the research and development, and field workers are the main aspects of this topic. Relevant contributions will range from basic research to applications. The potential risk of human pandemic virus originating from a reassortment of avian virus with human or pig virus will be considered and the epidemiological models for prediction will be presented. The aspects concerning the bio safety measures for the industry workers who are in contact with infected birds and the food and feed safety of poultry products will be covered. Key aspects such as the use of vaccination in birds and its possible consequences for public health, immunisation and protection in human at risk of contact with AI virus. Also means of improved collaboration between public health and veterinary authorities with respect to avian influenza prevention will be discussed.
Diagnosis

This topic principally encompasses the detection and identification of HP AI virus and the challenge of diagnosing AI in developing countries. The various diagnostic (molecular and conventional) techniques will be reviewed. Update will be made on the sensitivity and specificity of the advance molecular diagnosis test. The diagnosis and the methods for detecting virus circulation in vaccinated population using sentinel birds and DIVA strategy are of particular interest. New diagnostic methodologies for the detection, characterisation and quantification of the pathogenicity of the strains will be presented and their use in the face of an epidemic will be discussed. Furthermore, based on OIE manual, the standardisation and harmonisation of diagnostic procedures and quality assurance will be addressed.

Control

After a general overview on possible strategy for AI control, this topic will focus on the relevance of vaccination of domestic birds as a possible tool for control of the disease. The effect of vaccine on the excretion of the virus after infection (or experimental challenge) will be presented. The experience acquired in countries which use or have used vaccination for control will also be presented and critically analysed. This latter section will be of greater significance and will encompass vaccine availability, safety and efficacy. The requirements for vaccination strategies including the use of sentinel birds and post vaccination surveillance using DIVA strategy will also be discussed. The optimisation of utilisation of the vaccine and its economical aspects are of particular value. The results and data generated from those practical experiences with use of the vaccine should guide the decision of those concerned with planning vaccination campaigns. They should also help international organisations to guide the countries their control strategy and OIE to define new standards for surveillance and trade.

Improvement of management tools

This topic will focus on the practical aspects of managing HP AI outbreaks by the authorities in cooperation with the industry. It will be based on the experience acquired in countries which have already implemented the principles of compartmentalisation and of the surveillance to control AI in presence or absence of vaccination. The spirit behind the new proposed OIE standards and new code chapter on AI will be presented and discussed. The recommendations from the 2004 and 2005 FAO/OIE regional meetings for Asia and the new OIE/FAO Animal Influenza network will also be presented.
Organisation of the Conference

Steering Committee
Dr A. Schudel (OIE)
Dr J. Domenech (FAO)
Dr J.-L. Angot (OIE)
Dr S. Jutzi (FAO)
Dr D. Wilson (OIE)

Scientific Committee
Dr I. Capua
Dr D. Alexander
Mr D. Senne
Dr D. Swayne
Dr P. Selleck
Dr S. Marangon
Dr Y. Kida
Dr H. Chen
Dr J. Lubroth
Dr L. Sims

Secretariat
Dr Y. Leforban
Mr S. Berlaud
Ms S. Suárez

General information

Presentations
- Invited keynote speakers will provide presentations of each main topic.
- Final recommendations and conclusions will be presented for adoption.
- Authors (registered participants) wishing to make poster presentation on the main topics included in the programme are invited to submit abstracts of their presentations. Selected abstracts will be displayed throughout the duration of the conference. The deadline for submission of the abstracts for invited papers and poster presentations is 1st March 2005.
- All accepted abstracts will be published in the Proceedings of the Conference.

Venue
The Conference will be held at the OIE Headquarter:
12 rue de Prony
75017 Paris, France
Tel.: 33 (0) 1 44 15 18 88
Fax: 33 (0) 1 42 67 09 87

Hotel reservations
Please contact Elysées West Hôtels at www.hotels@ewh.com

Language
The Conference will be conducted in English, Spanish and French.

Conference Secretariat
The Secretariat of the Conference will be glad to assist you with any additional information about the event. Please address all correspondence to:

Saraí Suárez
Scientific and Technical Dept
OIE
12 rue de Prony, 75017 Paris, France
Tel.: 33 (0) 1 44 15 18 88
Fax: 33 (0) 1 42 67 09 87
E-mail: s.suarez@oie.int
# Programme

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20 mins  
Y. Kida (HU, Japan)

14h50 Swine influenza in China  
20 mins  
H. Chen (HVRI, China)

15h10 – Coffee break

Salon Ramon
15h10 – Poster session

Salon Vittoz
15h30 – Session 3 – Human health implications of AI  
Chairperson: G. Rodier (WHO)  
Rapporteur: G. Koch (CIDC, The Netherlands)

15h30 AI viruses and influenza in humans  
40 mins  
D. Alexander (VLA, UK)

16h10 WHO concerns about AI and influenza pandemic preparedness activities  
20 mins  
K. Stöhr (WHO)

16h30 Influenza surveillance and research priorities  
20 mins  
N. Cox (CDC, USA)

16h50 Collaboration between the health and agriculture sectors in surveillance and control of AI: challenges and opportunities  
20 mins  
F.-X. Meslin (WHO)

17h10 Occupational and consumer risks from AI viruses  
20 mins  
D. Swayne (APHIS, USDA)

Salon Vittoz
17h30 – Session 4 – Diagnostics  
Chairperson: D. Swayne (APHIS, USA)  
Rapporteur: D. Senne (APHIS, USA)

17h30 The challenge of diagnosing AI in developing countries  
30 mins  
P. Selleck (AAHL, Australia)

18h00 Advances in molecular diagnostics for AI  
15 mins  
I. Brown (VLA, UK)

18h15 Molecular diagnosis of AI during an outbreak  
15 mins  
G. Cattoli (IZS, Italy)

Salon Ramon
18h30 – Poster session

Salon Leclainche
18h30 – Press conference

Salon Ovale
18h30 Meeting of the Steering Committee, Scientific Committee, Chairpersons, Rapporteurs

19h00 – Gala Cocktail
**PROGRAMME**

**Friday, 8 APRIL 2005**

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*Chairperson: I. Capua (IZS, Italy)*  
*Rapporteur: I. Brown (VLA, UK)*

09h00  The control of HPAI in Asia  
20 mins  J. Lubroth (FAO)

09h20  Control of AI in Italy: from stamping out to emergency and prophylactic vaccination  
40 mins  I. Capua (IZS, Italy)

10h00  Quantification of the transmission characteristics of HPAI A virus in vaccinated and unvaccinated chickens  
20 mins  G. Koch (CIDC, The Netherlands)

10h20  AI vaccination in North America: strategies and difficulties  
30 mins  D. Suarez (SEPRL, USA)

10h50  Control and eradication strategies for AI in Mexico  
10 mins  C. Villareal (SAGARPA, Mexico)

11h00  Vaccines developed for H5 HPAI in China  
20 mins  H. Chen (HVRI, China)

11h20  Use of AI vaccination in Hong Kong  
20 mins  T. Ellis (AFCD, Hong Kong)

11h40  Use of strategic vaccination for the control of AI in Pakistan  
20 mins  K. Naeem (ASI, Pakistan)

12h00  Making AI vaccines available, an industry point of view (IFAH)  
20 mins  P. van Aarle (IFAH)

**Salon Ovale**

12.30  Meeting of the Steering Committee, Scientific Committee, Chairpersons, Rapporteurs

12h30 – Break

**Salon Ramon**

12h30 – Poster session

14h00 – Session 6 – Improvement of management tools  
*Chairperson: P. Vannier (AFSSA, France)*  
*Rapporteur: D. Suarez (SEPRL, USA)*

14h00  The new OIE standards:  
- The new OIE standards on AI and international trade  
20 mins  A. Thiermann (OIE)
- The new proposed Appendix for AI surveillance  
20 mins  K. Sakamoto (NIAH, Japan)
- Laboratory standards for AI  
20 mins  S. Edwards (VLA, UK)

15h00  Surveillance and compartmentalisation as a tool to control AI  
30 mins  C. Zepeda (APHIS, USA)

15h30  Compartmentalisation in Thailand  
20 mins  B. Santiwattanatam (CP, Thailand)

15h50  Social, economic and policy issues in the long–term control of HPAI  
20 mins  A. McLeod (FAO)
16h10 Conclusions and recommendations of the Second FAO/OIE Regional Conferences on Avian Influenza Control in Asia  20 mins  J. Domenech (FAO)

16h30 OIE/FAO Network on avian influenza expertise  15 mins  D. Sibartie (OIE)

16h45 – General conclusions and recommendations
Chairperson: B. Vallat (OIE)
Rapporteur: J. Domenech (FAO)
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AVIAN INFLUENZA – PAST, PRESENT AND FUTURE CHALLENGES

Ilaria Capua  
OIE and National Reference Laboratory for Newcastle Disease and Avian Influenza  
Istituto Zooprofilattico Sperimentale delle Venezie,  
Viale dell’Università 10 35020 – Legnaro, Padova, Italy

Avian influenza is an OIE listed disease, which has become a disease of great importance both for animal and for human health. Including estimations of the ongoing Asian H5N1 epidemic, in five years over 200 million birds have been affected by this disease. Some outbreaks have maintained the characteristic of minor relevance but others, such as the Italian 1999-2000, the Dutch 2003, the Canadian 2004 and the Asian 2003-2004 have led to devastating consequences for the poultry industry and negative repercussions on the public opinion. In addition, it has created significant human health concerns, including the risk of generating a new pandemic virus for humans via the avian-human link, and thus represents one of the major challenges the veterinary community will have to face.

The increased relevance of AI in the fields of animal and human health, has highlighted the lack of scientific information on several aspects of the disease, which has hampered the adequate management of some of the recent crises thus resulting in millions of dead animals and concern over loss of human lives and over management of the pandemic potential.

It is likely that the international effort that is being carried out in the medical and veterinary scientific communities in analysing data from recent outbreaks will generate significant amounts of information in the short-medium term which will broaden our current knowledge on AI, and be instrumental to the development of novel prevention and control strategies.

However, given the current situation, it is imperative that close collaboration is sought and achieved by public health officials involved in the veterinary and medical aspects of the disease. In fact, only through the exchange of data, experiences, views and information it will be possible to combat this zoonosis which represents a major threat to public health and animal wellbeing.
ECOLOGY AND EPIDEMIOLOGY OF AI WITH PARTICULAR EMPHASIS ON SOUTH EAST ASIA

V. Martin1, L. Sims2, J. Lubroth1, J. Slingenbergh1, D. Pfeiffer3,
1 Animal Health Service, FAO, Viale delle Terme di Caracalla - 00100 Rome, Italy
2 Asia Pacific Veterinary Information Services Pty Ltd PO Box 353 Marunda Qld Australia
3 Royal Veterinary College, Hawkshead Lane Hatfield AL9 7TA United Kingdom

Highly pathogenic avian influenza (HPAI) has been recognised as a serious viral disease of poultry since 1878. The number of recorded outbreaks of HPAI has increased globally in the past 10 years culminating in 2004 with the unprecedented outbreaks of H5N1 HPAI involving at least nine countries in East and South-East Asia. Apart from the geographical extent of these outbreaks and apparent rapid spread, this epidemic has a number of unique features, among which is the role that asymptomatic domestic waterfowl play in transmission of highly pathogenic H5N1.

When the extent of this disease became internationally known, with the almost simultaneous declarations in a number of Asian countries for the first time, there was considerable concern among both veterinary and public health authorities - especially as the virus also caused fatal disease in humans.

It is likely that the coincidence and grouping of the national reports declaring the outbreaks of HPAI did not truly reflect the time course of disease emergence. H5N1 HPAI viruses were known to be widespread well before the outbreak.

Field epidemiological studies are being conducted by the Food and Agriculture Organization and several collaborative centres to explore the factors that could have led to a change from infection to emergence of widespread disease in 2003-2004. Domestic waterfowl, specific farming practices and agro-ecological environment have been identified to play a key role in the occurrence, maintenance and spread of HPAI. Influenza viruses from wild aquatic birds, are likely sources of new internal genes for H5N1 HPAI viruses in terrestrial and aquatic poultry. The precise role of migratory birds in the regional spread of the disease still has to be elucidated.

Although there are numerous questions that remain unanswered regarding the origins of the 2004 outbreaks, the current understanding of the ecology and epidemiology of the disease can lead to the development of adapted targeted surveillance studies and control strategies.
ECOLOGY AND EPIDEMIOLOGY OF AVIAN INFLUENZA IN NORTH AND SOUTH AMERICA

D.A. Senne¹, D.L. Suarez², J.C. Pedersen¹ & B. Panigrahy¹
¹ U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, National Veterinary Services Laboratories, 1800 Dayton Avenue, Ames, Iowa 50010, USA
² U.S. Department of Agriculture, Agriculture Research Service, Southeast Poultry Research Laboratory, 934 College Station Road, Athens, Georgia 30605, USA

Waterfowl and shorebirds are known to be the natural reservoir for influenza A viruses. Surveillance studies in waterfowl and shorebirds in North America show that influenza A viruses are repeatedly recovered from these birds. However, the virus recovery is influenced by geography, season, age and species of birds. In addition to the natural reservoir, the live-bird marketing system (LBMS), present in certain regions of United States, has been recognized as a man-made reservoir of influenza viruses and has been linked to several outbreaks of influenza in commercial poultry. Movement of infected birds, uncleared or improperly cleaned crates, and contaminated vehicles between the LBMS and commercial poultry farms have been implicated as the source of virus in these outbreaks. However, in the majority of outbreaks involving poultry, the source of infection is unknown. Since 2002, three outbreaks of highly pathogenic avian influenza (HPAI) have occurred in the Americas; one in Chile (H7N3), one in the United States (H5N2), and one in Canada (H7N3). In each of these outbreaks, a low pathogenicity precursor virus mutated to become highly pathogenic after circulating in poultry. The HPAI viruses recovered from these outbreaks had unique molecular and phenotypic characteristics that do not conform to other known HPAI viruses. These findings emphasize the need and importance of monitoring wild and domestic bird species for presence of influenza A viruses.
RECENT EPIDEMIOLOGY AND ECOLOGY OF INFLUENZA A VIRUSES IN AVIAN SPECIES IN EUROPE AND THE MIDDLE EAST

Veterinary Laboratories Agency (Weybridge), Addlestone, Surrey KT15 3NB, United Kingdom

There have been at least ten distinct outbreaks of LPAI or HPAI in poultry caused by H5 or H7 viruses in the last 8 years in Europe and the Middle East. There appears to be an increased occurrence of such episodes consistent with global trends. As a result surveillance systems have been enhanced to facilitate early detection of infection in poultry together with active surveillance of wild bird populations. These complimentary activities have resulted in the detection of a number of viruses in wild bird populations including some with high genetic similarity to newly detected viruses in poultry, including H7N3 in Italy and H7N7 in the Netherlands. Furthermore there is evidence for continued circulation of H5 and H7 viruses in wild Anseriformes thereby presenting a real and current threat for the introduction of viruses to domestic poultry especially those reared in outdoor production systems. Viruses of H9N2 subtype continue to circulate widely in the Middle East and are associated with significant disease problems in poultry. The epidemiology has the potential to be complicated further by introduction of novel viruses through illegal importation of captive birds, such as was detected with H5N1 in Belgium in 2004. The molecular epidemiology will be discussed including the topology within genotypes of prevailing viruses in the wild bird reservoir suggesting they may not be in evolutionary stasis.
ECOLOGY AND EPIDEMIOLOGY OF AI IN OSTRICHES

A.J. Olivier
Klein Karoo Group, Research and Development: Ostrich Laboratory, PO Box 241, Oudtshoorn, 6620, South Africa

Avian Influenza is important due to its potential devastating effect on poultry health and trade. The ostrich industry of South Africa has not escaped the consequences of control and export restrictions resulting from notifiable virus infections.

Ostrich farmers first observed a syndrome of green urine in the early and mid 1980’s. A H7N1 subtype, causing high mortalities in young ostriches but with a low pathogenicity index for chickens, was first isolated in 1991. The first highly pathogenic subtype affecting ratites was reported during the 2000 epidemic of H7N1 in Italy. Low pathogenic subtypes were in South Africa isolated from 1991 to 2004, with one HPAI isolate in 2004.

International research work in ostriches with both H5 and H7 subtypes, in both low and high pathogenic pathotypes, found the severity of clinical disease was not directly correlated to the pathotype.

The ecology and epidemiology of infections in ostriches is not well understood. Surveys suggest local migratory water birds may play an important role. They have direct contact with ostrich flocks through the free-range production systems. Seasonal occurrence is seen, with the wet colder months more favorable for virus survival and detection. Management, population density, immune status and age are other important determinants of the severity of disease.

Surveillance and monitoring must be implemented to understand the ecology and epidemiology, which extends to the validation and standardization of diagnostic and serological methods for ostriches. Serious consideration should be given to vaccination, education and the use of separate production zones as part of a control program.
PATHOGENICITY OF AVIAN INFLUENZA VIRUSES IN POULTRY

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Historically, the pathogenicity of avian influenza (AI) viruses has been defined by experimental studies in the major poultry species, chickens. All AI viruses are classified into two broad categories, low pathogenicity (LP) and high pathogenicity (HP), but pathobiological changes vary by virus strain and host species within each of the categories. Typically, AI viruses that are HP for chicken produce a similar severe, systemic disease in other galliforme birds, but usually produce mild disease or no infection in ducks. The 1959-1984 H5 HPAl viruses produced longer mean death times in chickens than the newer H5 and the H7 HPAl viruses, suggesting a shift to increased virulence for chickens. All the Asian H5N1 AI viruses are HP for chickens with large quantities of virus being shed from the oropharynx and slightly lesser amount from the cloaca. The Asia H5N1 AI viruses have changed from producing inconsistent infection in ducks to some strains being HP (HK 2002 viruses) and resulting in excretion of large virus quantities from respiratory, and to lesser extent, intestinal tracts. However, the quantities are still 2log10 less than seen in chickens. Across all bird species, the ability to produce severe disease and death is associated with the virus replicating to high titers in the host. In galliforme birds, the Asian H5N1 HPAl viruses replicate in vascular endothelial cells and/or multiple parenchymal organs and the brain. This leads to production of widespread necrosis and inflammation in multiple organs. With geese, emu, house finches and budgerigars, the virus infects neurons and glial cells producing neurological signs and mortality rates ranging from 0-75%. The heart and pancreas are also affected. In domestic ducks, the lesions have been mild and usually only present in the respiratory tract, but internal organs and the brain have been affected if using the 2002 HK H5N1 viruses. With 2003-2004 Asian H5N1 AI viruses, lethality in ducks has been age dependent.
LIBRARY OF AVIAN INFLUENZA VIRUS STRAINS FOR VACCINE AND DIAGNOSTIC USE AGAINST HIGHLY PATHOGENIC AVIAN INFLUENZA (HPAI) AND HUMAN PANDEMICS

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Recent outbreaks of HPAI in Asian countries alarm to realize that there is no border for infections and give a rise to concern for human health as well as for livestock industry. This H5N1 virus has jumped the species barrier and caused severe disease with high mortality in humans in Viet Nam, Thailand, and Cambodia; 29 deaths of 37 cases, 11 of 16, and 1 of 1, respectively. It is now obvious that live bird markets play important roles for the generation of highly pathogenic strains.

Each of the known subtypes of influenza viruses perpetuates among migratory ducks and their nesting lake water in nature. Since avian viruses of any subtype can contribute genes in the generation of reassortants in pig, none of the 15 HA and 9 NA subtypes can be ruled out as potential candidates for future pandemic strains.

Surveillance study of avian influenza has been carried out since 1976 and influenza virus isolates have been characterized and stored for vaccine strain candidates and diagnostic use. Influenza virus isolates of 49 combinations of HA and NA subtypes have been isolated from fecal samples of ducks in Alaska, Siberia, Mongolia, Taiwan, China, and Japan. So far, more than 67 other combinations have been generated by the genetic reassortment procedure in chicken embryos. Thus, avian influenza viruses of 116 combinations of HA and NA subtypes have been stocked as vaccine strain candidates. Their pathogenicity, antigenicity, genetic information and yield in chicken embryo have been analyzed and registered in the database.
Swine influenza is a common infectious respiratory disease of pigs that caused by influenza A viruses, and H1N1 and H3N2 are currently the most popular subtypes of influenza viruses circulated in pig population in China. H9N2 virus was detected from pigs that imported from Mainland China in Hong Kong in 1998, and extensive viral surveillance conduced since then indicated that H9N2 viruses are widely distributed in pig population in China. Phylogenetic analysis indicated that H9N2 viruses from pigs are highly related, implying they may establish a stable lineage in pigs in China. During the routine surveillance, we also isolated 2 H5N1 viruses from pigs in Fujian province in 2001 and 2003, respectively. Molecular analysis indicated that the two viruses are origin from duck H5N1 viruses. Their replication in chickens, mice and pigs has also been investigated.
Influenza A viruses cause natural infections of humans, some other mammals and birds. Few of the 16 haemagglutinin and 9 neuraminidase subtype combinations have been isolated from mammals, but all subtypes have been isolated from birds. There are enormous pools of influenza A viruses in wild birds, especially migratory waterfowl.

In the 20th Century there were 4 pandemics of influenza due to the emergence of antigenically different strains in humans: 1918 (H1N1), 1957 (H2N2), 1968 (H3N2) and 1977 (H1N1). Influenza A viruses contain 8 distinct RNA genes and reassortment of these can occur in mixed infections with different viruses. The 1957 and 1968 pandemic viruses differed from the preceding viruses in humans by the substitution of genes that came from avian viruses, suggesting they arose by genetic reassortment of viruses of human and avian origin.

Up to 1995 there had been only three reports of avian influenza viruses infecting humans, in 1959, 1977 and 1981 [all H7N7], two were the result of laboratory accidents. Since 1996 there have been regular reports of natural infections of humans with avian influenza viruses: in England in 1996 [H7N7], Hong Kong 1997 [H5N1], 1999 [H9N2], and 2003 [H5N1], in The Netherlands 2003 [H7N7], Canada 2004 [H7N3], Vietnam 2004 [H5N1] and Thailand 2004 [H5N1]. The H5N1 virus is alarming 48 (65%) of the 74 people confirmed as infected since 1997 have died. Although there has been very little human to human transmission, these infections are a concern since if people infected with an “avian” virus were infected simultaneously with a “human” virus reassortment could result in the emergence of a virus capable of spread in the human population, but with an HA for which the human population was immunologically naive.
COLLABORATION BETWEEN THE HEALTH AND AGRICULTURE SECTORS IN SURVEILLANCE AND CONTROL OF AVIAN INFLUENZA: CHALLENGES AND OPPORTUNITIES

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Human health is multi-dimensional and its improvement requires more than the services delivered by the health services alone. Health policy and practice should therefore be both interdisciplinary and intersectoral. In the field of zoonotic diseases the two main sectors involved are public health and agriculture.

Concerted action is necessary in all countries but this is particularly critical in developing countries with weak infrastructures and limited resources. The concept of a multidisciplinary approach to health has been widely advocated and strongly promoted for many years by WHO and its Mediterranean Zoonoses Control Programme (MZCP).

Joint planning and sharing of resources between sectors has remained, however, a more-or-less self-contained exercise within the health and veterinary sectors. There are wide gaps between intention and reality, and declaration and practice. Collisions continue to occur between intersectoral and sectoral interests fuelled by administrative rules, financial selfishness or personal ambition, while rigid bureaucratic, centralized systems also create limitation to intersectoral action.

The level of collaboration between the two sectors has, however, improved lately - in particular-pathogenic Avian Influenza. The recognition of the public health risks involved, together with the enormous consequences in terms of cost and consumer confidence, have created new opportunities. Worldwide veterinary services and agriculture authorities have to face the great challenge of accepting public health as their first target. Not only do they have a great deal to contribute but they also have a great deal to gain.
OCCUPATIONAL AND CONSUMER RISKS FROM AVIAN INFLUENZA VIRUSES

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Sporadic cases of avian influenza (AI) virus infections have been reported in humans over the last 50 years. Human infections have only been reported with a few select AI virus strains. Studies with animal models suggest the risk of human infection is dependent upon the specific AI virus strain; i.e. not all AI virus strains have the same risk of infecting humans. Most human cases had infections with H7N7 high pathogenicity (HP) AI virus reported in The Netherlands (2003) and H5N1 HPAI viruses reported in several countries in Asia (1997-present). Epidemiological studies have identified direct exposure to infected poultry as the primary risk factor for human infections. In The Netherlands, veterinarians, cullers and poultry framers had an occupational risk for infection. In Asia, most of the clinical infections have involved direct exposure to poultry in the small-holding village sector or live poultry markets, and not commercial poultry. However, in Hong Kong during 1997, H5N1 infections were associated with occupational exposure (poultry farmers) based on serological evidence, but no clinical disease was reported in association with such infections. No cases of human AI infection have been linked to consumption of infected or contaminated poultry products. However, HPAI virus can be present in blood and meat of infected poultry, and therefore could be a potential source of virus for human infections if consumed raw. Cooking and pasteurization are effective methods for killing AI viruses. Proper vaccination of poultry has been shown to prevent HPAI virus from localizing in the meat.
THE CHALLENGES OF DIAGNOSING AVIAN INFLUENZA IN DEVELOPING COUNTRIES

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Diagnostic methods for avian influenza range from the observation of birds through to real-time PCR requiring expensive equipment. Tests developed for human influenza have been applied to diagnosis of animal influenza infections and adapted and modified for use specifically with avian influenza viruses. New tests are continually being developed and Luminex and microarrays represent the next generation of assays that will be applied to diagnosis of avian influenza. Countries affected by the current pandemic of avian influenza vary greatly in the standard of their facilities and infrastructure available. Whilst recent technologies provide powerful tools for AI diagnosis any training courses provided must be targeted at the facilities and equipment available, as well as the immediate needs of the country. Such training programs should develop the skills of staff, transfer the technology and provide reagents, with the ultimate goal being the establishment of an in-country diagnostic capability. The benefits of this approach are that disease control efforts are managed locally, allowing a more rapid response and minimising the economic impact of the disease. Training programs can be allied with money from funding bodies that is used to purchase the equipment necessary to establish a diagnostic capability. Reference laboratories must still play an important role. Many technologies are not available in all countries, nor are suitable containment facilities for handling live virus. Reference laboratories can offer support through follow-up visits for further training, trouble shooting, provision of reagents, quality assurance, other specialist testing, and act as repositories of viruses for future research.
ADVANCES IN MOLECULAR DIAGNOSTICS FOR AVIAN INFLUENZA

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Recent outbreaks of avian influenza (AI) have highlighted the necessity to improve existing tests and to develop new methods, in order to detect spread or new outbreaks more quickly that is vital for the early and successful implementation of control strategies. Conventionally the time between clinical suspicion and laboratory confirmation of AI can be relatively long due to the logistics of sending samples to laboratories and their capacity for providing high throughput of sensitive and specific assays. Increasingly new generation assays based on molecular diagnostics have become available and applied successfully to disease investigation or active surveillance programmes. There has been widespread application of techniques based on the amplification of specific nucleic acid sequences by polymerase chain reaction (PCR), ligase chain reaction (LCR) and nucleic acid sequence-based amplification (NASBA). The approaches generally offer high specificity and sensitivity. One of the most promising technologies is real-time PCR that enables amplification of nucleic acids and detection of the amplified products through specific probes at the same time. A rapid diagnosis can be achieved together with potential for high throughput due to process automation. Currently, microarray technology is developing rapidly and has been applied to diagnosis of influenza A virus but generally lacks the necessary sensitivity for direct application to clinical specimens. In addition, these new technologies have been increasingly applied to rapid and reliable subtyping of AI viruses. Current molecular based methodologies for AI diagnosis will be reviewed and compared.
MOLECULAR DIAGNOSIS OF AVIAN INFLUENZA DURING AN OUTBREAK

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Traditionally, laboratory protocols for the detection and the identification of avian influenza (AI) viruses were based on virus isolation in SPF eggs or in cell cultures. In recent outbreaks of major proportions, the application of these methods has been limited by the fact that they are not flexible to a sudden increase in demand, not cost-effective and often require a long processing time. These characteristics hamper the management of outbreaks which occur in densely populated poultry areas or in cases in which there has been significant spread of infection. Experience gathered during outbreaks occurring in Italy indicates that under these conditions the prompt identification of an infected flock is crucial for control and eradication purposes.

Rapid and reliable laboratory tests are now available to demonstrate direct evidence of infection in flocks located in the areas at risk of infection. These tests include RT-PCR and Real time RT-PCR, but what appears to be of vital importance in the face of an outbreak is the development of a diagnostic strategy. The strategy should encompass a variety of diagnostic tests and should be based on an adaptation of the capacities of central and peripheral laboratories in relation to the field situation. An appropriately developed diagnostic strategy including molecular diagnosis will facilitate the management of AI outbreaks by shortening reporting time, thus being more compatible with the demands of the poultry industry and will result in preservation of animal welfare.

Although molecular diagnosis has proved itself to be a valuable tool to support the management of major AI outbreaks, in due time it must be complemented with virus isolation techniques which remain the only diagnostic tool that enable a complete characterisation of the virus.
THE CONTROL OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN ASIA

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Asia’s experience with highly pathogenic avian influenza (HPAI) was limited until recently to western areas of Pakistan which had effectively managed disease through limited vaccination against H7 and H9 strains and China with H1 and H5 strains. In mid December 2003 the Republic of Korea was the first country to report the occurrence of HPAI, followed in rapid succession by ten additional countries and administrative regions by late January 2004. The control measures instituted in other countries in the recent past (Italy, Chile, USA, Canada, and The Netherlands) have relied on standstill orders, culling practices of affected flocks, culling of dangerous contacts, and purposeful surveillance with rapid response. Only Mexico employed vaccination as an additional tool in disease management. Some of the same measures were instituted in the Asian scenario with some degree of national success, but not regional or continental. The international community had never experienced this disease present itself in such a wide geographical area with the wide scope of production and marketing practices – ranging from confined commercial operations, to open systems of poultry production, rice paddies with grazing ducks, to free-scavenging chickens in villages. Though humane culling of affected flocks remains an appropriate measure to contain an outbreak from spreading and its associated environmental contamination, understanding the underlying ecology of the disease transmission and virus reservoirs, knowledge of marketing chain structures, weaknesses and limits in national capacities (in diagnosis and response), and collaboration at regional and international levels was needed. Twelve months of intense activity in country and regional assessments, field studies, raising regional and national diagnostic competence, providing guidance, and public awareness have been key in developing focussed control strategies – that although still imperfect – have kept HPAI (H5N1) in Asia without further spread to other continents. The use of quality and appropriate vaccine and vaccination tactics would benefit certain sectors in countries still unable to manage the disease effectively. Further investment and political commitment are needed to successfully control HPAI viruses in poultry and limit the risk of human disease on a wider scale. With additional resources, the international and regional organisations can engage country veterinary services in designing specific strategies for management, control, and hopefully, eradication.
CONTROL OF AI IN ITALY: FROM STAMPING OUT TO EMERGENCY AND PROPHYLACTIC VACCINATION

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Since 1997, North-eastern Italy has been repeatedly challenged by AI infections caused by viruses of the H5 and H7 subtypes. The penetration of such infections in the industrial circuit in densely populated poultry areas (DPPAs) resulted in massive spread, and early attempts to control AI only by stamping-out and restriction policies resulted in death or culling of millions of animals. The re-emergence or the introduction of AI viruses in the same DPPA indicated that another stamping out policy was not pursuable. This resulted in the development of an emergency vaccination programme based on the use of heterologous vaccination and a companion discriminatory test - known as the “DIVA” strategy. By enabling the detection of field exposure in vaccinated animals the application of this system, in conjunction to a monitoring programme and a well defined territorial strategy has resulted in the eradication of H7N1 and H7N3 epidemics which occurred between 2000 and 2004. Retrospective analysis of the AI outbreaks occurring in North-eastern Italy coupled with surveillance programmes in wild birds and in hobby flocks indicating that certain areas are at continuous high risk of infection represented the rationale to develop and implement a bivalent H5/H7 pilot vaccination programme in a restricted area of the DPPA. This prophylactic vaccination programme has been in place for 6 months and field and laboratory evidence indicate that vaccinated animals are more resistant to challenge and shed lower amounts of virus, thus acting both as a tool for prevention and for control limiting the impact of AI infections.
Devastating recent outbreaks of highly pathogenic avian influenza A (HPAI) viruses in poultry highlight the need for effective control measures to prevent and control outbreaks of HPAI. Vaccination of poultry against influenza strains of the H5 and H7 subtypes provides a potentially attractive control measure. Unfortunately, not much is known about the effect of vaccination on the epidemiologically relevant parameters (infectious period, transmissibility, virulence). To obtain estimates of these parameters we study the transmission characteristics of HPAI virus (A/chicken/Netherlands/03 H7N7) by means of transmission experiments. In a transmission experiment a number of infected animals is put in a stable with a number of uninfected contact animals and the infection chain is monitored by virus isolation and serology. The analyses of the experiments are based on a stochastic SEIR epidemic model. Our results show that vaccination can be an effective control measure. However, our results also indicate that near perfect compliance with a vaccination programme is crucial if the aim of a vaccination programme is to obtain herd immunity within a population of poultry. We discuss the implications of these conclusions for the design and use of vaccination programmes in poultry, and point out directions for future research.
AI VACCINATION IN NORTH AMERICA: STRATEGIES AND DIFFICULTIES

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Vaccines can contribute to the control of avian influenza outbreaks when used as part of a comprehensive control program that includes quarantines, animal movement controls, increased biosecurity, and enhanced surveillance. In North America both whole virus killed adjuvanted vaccines and fowlpox recombinant vaccines have been used as part of outbreak controls. The fowlpox recombinant vaccine is licensed in the U.S., but it has only been used in the field in Mexico and some Central American countries. The U.S. has considerable experience with the use of the killed vaccines, primarily in turkeys. In the state of Minnesota in the 80’s and early 90’s, outbreaks of AI in range reared turkeys were common, and vaccines were used successfully as part of a controlled marketing program. More recently, several large layer flocks in Connecticut were vaccinated as an alternative to depopulation after an H7N2 low pathogenic AI outbreak. The vaccinated flocks were intensively monitored for virus shed using sentinel birds, dead bird testing, and eventually some serologic surveillance using a neuraminidase DIVA approach. With these successes, vaccination is being considered as a more viable control option, and the U.S. has begun a vaccine bank for H5 and H7 AI viruses. Recently, experimental studies using the 1994 H5N2 Mexican vaccine strains have shown inadequate protection against 2002-2003 Mexican lineage H5N2 low pathogenicity AI viruses. This indicates antigenic drift does occur in the field and vaccines strains should be evaluated periodically with circulating field strains and replaced if they do not provide sufficient protection.
CONTROL AND ERADICATION STRATEGIES FOR AVIAN INFLUENZA IN MEXICO

Cesar Luis Villarreal Chavez

In Mexico, the poultry industry is the most developed livestock industry, the production of poultry meat and eggs is 2.3 million tons and 1.9 million tons per year, respectively, and represents 40.2% of the livestock internal net product of the country. The national poultry inventory comprises 3,800 commercial farms; the states of Jalisco, Guanajuato and the Lagunera Region are the main poultry meat producers and Jalisco, Puebla and Sonora are the main egg producers, although the remaining states also produce eggs but in smaller quantities. The annual consumption per capita is 19.22 kg eggs and 22.94 kg chicken meat.

In May 1993, low pathogenicity avian influenza virus (LPAIV) subtype H5N2 was detected for first time in commercial farms in central Mexico. Subsequently, December 1994, an outbreak of high pathogenicity avian influenza virus (HPAIV) caused by the same subtype was reported, the egg and poultry meat regions were mainly affected: Puebla and Querétaro.

The eradication activities consisted in zoning, movement control inside the country, vaccination, periodical surveillance of all commercial farms and backyard flocks, quarantine, and all the poultry on affected farms were culled and biosecurity measures were implemented. With these policies, the disease was eradicated in a relative short time, the last case was detected in June 1995.

Since then, Mexico continues to implement a LPAIV control programme based on zoning classification of free and eradication states or regions; systematic monitoring and certification of free farms; surveillance and laboratory diagnosis by haemagglutination inhibition, agar gel immunodiffusion, viral isolation and its filogenesis, virus subtipification and pathogenicity determination; movement control, vaccination under official control – authorisation only applies when LPAIV is present and for the purpose of protecting the flocks in the presence of a virus mutation to HPAIV. The current vaccine was made from the HPAIV in 1995, it is an inactivated virus emulsion subtype H5N2; in 1998, a recombinant vaccine was developed with poxvirus and avian influenza viruses.

The only avian influenza subtype detected in Mexico has been the H5N2 and since June 1995, all isolations have been of low pathogenicity.
VACCINES DEVELOPED FOR H5 HIGHLY PATHOGENIC AVIAN INFLUENZA IN CHINA

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Since the first detection of highly pathogenic H5N1 avian influenza virus from sick goose in Guangdong province in China in 1996, scientists in China started to develop vaccines for avian influenza pandemic preparedness. An H5N2 inactivated vaccine was produced from a low pathogenic virus, A/turkey/England/N-28/73, and was used for the buffer zone vaccination in the H5N1 outbreaks in 2004 in China. We also generated a low pathogenic H5N1 reassortant virus A/Harbin/Re-1/2003 (Re-1) that derives its HA and NA genes from GSGD/96 virus and 6 internal genes from the high-growth A/Puerto Rico/8/34 (PR8) virus by using plasmid based reverse genetics. The inactivated vaccine derived from Re-1 strain could induce more than 10 months protective immune response in chickens after one dose inoculation, and most importantly, this vaccine is immunogenic for geese and ducks. An H5N1 fowlpox vectored live vaccine was also generated by inserting the HA and NA gene of GSGD/96 virus in the genome of a fowlpox vaccine strain. Field test indicated that after one dose of immunization of this vaccine, chickens could develop an over than 40 weeks protective immune response against H5N1 virus challenge. This vaccine is much cheaper than the inactivated vaccines, and the application of this vaccine will not interferes the nucleoprotein based antibody avian influenza serological surveillance. The proper application of these vaccines is expected to play key role in the eradication of highly pathogenic avian influenza viruses in China.
USE OF AVIAN INFLUENZA VACCINATION IN HONG KONG

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Outbreaks of H5N1 highly pathogenic avian influenza (HPAI) that occurred in Hong Kong up until February/March 2002 were controlled by stamping out. With endemic presence of the virus in the region and large daily importation of poultry to Hong Kong the Administration considered that further risk management measures, in addition to improved biosecurity and enhanced surveillance, were necessary to prevent outbreaks. Vaccination using a killed H5N2 vaccine was evaluated over a 12-month period in the district with the last HPAI cases in the early 2002 outbreak.

The vaccination trial showed that farmer-administered killed H5N2 vaccine produced suitable flock antibody responses; vaccinated birds were protected against H5N1 HPAI virus challenge and excreted significantly less H5N1 virus; and vaccination was able to shut down virus excretion in flocks during field outbreaks.

Universal vaccination of local chicken farms was introduce in June 2003 and by the end of 2003 all chickens entering the live poultry markets in Hong Kong were vaccinated by killed H5N2 vaccine. In addition to vaccination, an enhanced biosecurity programme on farms and in live poultry markets and a comprehensive surveillance programme in poultry, wild birds, recreation park birds and pet birds were in place. Vaccination use and performance is closely monitored. This programme was successful in protecting local farms and live poultry markets from H5N1 outbreaks during the regional H5N1 outbreaks in 2004.
USE OF STRATEGIC VACCINATION FOR THE CONTROL OF AVIAN INFLUENZA IN PAKISTAN

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The first outbreak of highly pathogenic avian influenza (HPAI) caused by subtype H7N3 appeared in 1995 in Pakistan. A homologous water-based vaccine prepared from the field isolate employed as ring vaccination around the epicenter of the outbreak helped in containing the disease. Later, in 1999 an outbreak of avian influenza virus (AIV) subtype H9N2, particularly affecting the broilers and broiler-breeders, was also dealt with the same vaccination approach. However, the disease could not be successfully contained in the affected areas. Later on low path AI (H7N3) re-emerged in the broiler-breeder flocks in different parts of the Northern areas of the country during 2000-2002. This prompted introduction of a vaccine strategy, whereby a combined H7-H9 oil emulsion vaccine was routinely employed in and around the affected areas. This helped in the control of disease in this region. However, no sustainable AI-Monitoring and control strategy could be launched on national basis. In November 2003, new outbreaks of HPAI subtype H7N3 occurred specifically in commercial layers in southern part of the country, which were never vaccinated against AI in the past. In many cases subtype H9N2 was also recovered from the diseased flocks, with or without the presence of subtype H7N3. An emergency plan to control this outbreak was developed with the help of FAO. Under this plan a nation-wide AIV-monitoring and vaccination strategy was developed. The disease caused heavy losses in the south, but resulted in relatively less mortality and production losses when it spread to other parts of the country in 2004. This report discusses the role of vaccine type and vaccination schedule in the control of HPAI in this country.
MAKING AI VACCINES AVAILABLE, AN INDUSTRY POINT OF VIEW (IFAH)

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Vaccination against Avian Influenza (AI) has proven to be an efficient tool in the reduction of virus excretion and in increasing the threshold for infection. Vaccination in outbreaks, as part of a complete program, proved to be an essential component of control and eradication programs.

Avian Influenza is a serious threat to public health.

In contingency plans for outbreaks of HPAI the option of emergency vaccination, using inactivated or recombinant vaccines, should be considered. The availability of suitable vaccines has to be ensured in “peace time” in a contract for a vaccine or antigen bank.

Unlike the human influenza vaccines, poultry AI vaccines have proven to provide protection against a wide range of H5 and H7 strains. However in case new H5 or H7 strains emerge in poultry, there is no regulatory concept how to deal with a swift reaction by the vaccine industry.

Production of HPAI virus should take place in facilities with a Biosafety Level 3 (BSL3) in order to safeguard containment of virus and to prevent infection of manufacturing staff. Vaccine strains for inactivated vaccines should preferably be LPAI.

In a new outbreak it is essential to determine early, which vaccine strain will provide protection against the field virus. Sequencing does not predict the protective capacity of vaccines. Challenge studies, providing essential information, take too much time and can be carried out only in BSL 3 facilities. Serological matching of vaccine and field strains would provide a faster system. It is recommended that a matching system should be developed and validated.
THE NEW OIE STANDARDS ON AI AND INTERNATIONAL TRADE

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Based in the most recent scientific findings, the OIE has redrafted the Code on Avian influenza. The new draft Chapter was accepted by the OIE’ International Committee as ‘text under study’. Two groups of experts were convened during 2004 and suggested additional changes to the text. The resulting new draft Chapter on avian influenza has been presented by the Code Commission for adoption in May 2005. The definition of notifiable avian influenza was modified to require the notification of the presence of both highly pathogenic, as well as low pathogenic avian influenza when caused by H5 or H7 strains in poultry. The definition of poultry was also revised to limit the official notification of the disease in poultry used for domestic and commercial purposes, but excluding wildlife. This new standard, addresses both public as well as animal health concerns, providing commodity specific recommendations. In addition to recommendations on zoning, the Chapter includes the concept of compartmentalization, which is more appropriate for the separation of domestic poultry sub-populations from each other and from susceptible wildlife. The Chapter also includes recommendations on the proper use of vaccines and how to trade under these conditions. The new draft Chapter will be presented and discussed during this session.
THE NEW PROPOSED APPENDIX FOR AI SURVEILLANCE

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The Scientific Commission for Animal Health has endorsed the new Terrestrial Code Appendix on avian influenza (AI) surveillance prepared by the OIE Ad hoc Group on AI Surveillance, being proposed for adoption during the OIE General Session in May 2005. The new proposed Appendix was developed on the basis of the amended Terrestrial Code AI chapter and the new proposed Appendix on general surveillance. It takes into account the difference between highly pathogenic notifiable AI (HPNAI) and low pathogenic notifiable AI (LPNAI) and lays down guidelines for trade in poultry and poultry products in each case. The following aspects of the new AI chapter were considered relevant in the development of the surveillance guidelines:

No trade in live birds or raw products is allowed from HPNAI infected countries, zones or compartments. Trade in products from birds affected with HPNAI is allowed only if they are treated to destroy the virus and measures are taken to avoid recontamination.

The proposed definition of NAI in the Terrestrial Code chapter refers only to the infection in “poultry”, which is defined as “all birds reared or kept in captivity for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds”. This definition excludes wild birds and thus allows for the recognition of countries and zones as free from NAI.

Demonstrating the absence of infection is scientifically impossible but demonstrating the absence of virus circulation is feasible.

The OIE/FAO/WHO has issued a joint statement in which surveillance of wild bird populations is considered in the control of AI and recognising that the risk posed by wild bird populations to domestic poultry is similar throughout the world with the possible exception of countries visited by wild migratory birds where the risk may be seasonally higher. This is taken into account in the draft AI surveillance guidelines.

The Terrestrial Manual chapter on AI should be reviewed to include validation of tests for species other than chickens and also references to both HPNAI and LPNAI.
LABORATORY STANDARDS FOR AVIAN INFLUENZA

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The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (mammals, birds and bees) has from its first edition in 1989, included a chapter on Highly pathogenic avian influenza (HPAI). Chapters in the Manual provide an introductory description of the disease in question and its causal agent, followed by detailed laboratory protocols for carrying out diagnostic tests (both agent identification and serology), and finally requirements for the production of vaccines. The current chapter on HPAI includes techniques for virus isolation, with molecular and biological methods for assessment of the pathogenicity of isolates.

A version of that chapter has been prepared with minor updates to the diagnostic section, plus a new section on vaccines that discusses the appropriate use, and the limitations, of conventional inactivated vaccines, DIVA strategies and recombinant vaccines. This chapter will be proposed only if the new chapter in the Code is not adopted by the OIE International Committee.

A new version of the chapter entitled Avian Influenza has also been prepared and will be proposed to complement the new Code chapter. The laboratory techniques are essentially the same but the interpretation is adapted to the new definitions of notifiable avian influenza of both high and low pathogenicity. The new chapter includes the section on vaccines as mentioned above.
SURVEILLANCE AND COMPARTMENTALIZATION AS A TOOL TO CONTROL AVIAN INFLUENZA

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Surveillance for avian influenza can have several objectives. Generally, these are to detect the presence of infection or to declare disease freedom. Disease freedom claims can apply to an entire country, a zone within a country or a compartment. Disease freedom cannot be absolutely demonstrated; however through a multi-pronged approach employing different surveillance strategies, sufficient confidence in the absence of infection can be achieved. The recently developed OIE guidelines for surveillance for avian influenza offer different approaches to meet these goals. The guidelines are not intended to be prescriptive but rather offer options that countries may apply depending on their epidemiological situation. Compartmentalization is a new concept that allows the recognition of populations of different health status based on management as opposed to geographic factors (regionalization), a proposed approach for the application of this novel concept is presented.
COMPARTMENTALIZATION IN THAILAND

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Avian Influenza (AI) is a deadly disease in poultry throughout the world including Thailand. It does not only cause morbidity and mortality in poultry but it is also contagious and causes death in human. It further gives big impact on world food trading. To protect the consumer health and maintain confidence in internationally traded food, Charoen Pokphand Foods Public Co., Ltd (CPF) had set up the compartmentalization project according to OIE recommended measures to ensure the safety of CPF poultry product against AI. The steps of compartmentalization for AI free composed of the application of the following systems in the poultry business such as biosecurity, OIE requirement for controlling the animal diseases, risk analysis, GAP/GMP and EU directives on hygiene and sanitation, HACCP, and ISO 9001 under the supervise from Department of Livestock Development, Ministry of Agriculture and Cooperative, Thailand competent authority. Moreover, the surveillance, documentation and traceability techniques are also applied to ensure the effectiveness of this system. The CPF compartmentalization is proved to be very effective since there is no evidence of AI outbreak in CPF poultry system since the implementation in July 2004.
SOCIAL, ECONOMIC AND POLICY ISSUES IN THE LONG TERM CONTROL OF HPAI

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Prevention and control of HPAI in Asia is a long term problem with important economic and policy consequences. The macro level impact of a single national outbreak is greatest for an exporting country, ranging in 2003-5 from $65 million to over $400 million. No estimates are available for the impact of market disruption if endemic disease changes the pattern of regional and international trade. In countries with minimal exports, the total financial impact may be much smaller, but there can be serious losses to vulnerable sectors of society at several stages of the market chain. The economies of the region are growing and some countries could finance recurrent costs of AI control, but substantial investment in veterinary services is required. National and regional financing structures need to be reviewed. AI control strategies should include a broad financial support system that addresses education, credit, compensation and social relief programmes. Some strategies may result in restructuring of the industry, or affect the wider development of rural areas and local food security.
CONCLUSIONS AND RECOMMENDATIONS OF THE SECOND FAO-OIE REGIONAL MEETING ON AVIAN INFLUENZA CONTROL IN ASIA

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The second FAO-OIE Regional Meeting on Avian Influenza (HPAI) Control in Asia was held in Ho Chi Minh City – Vietnam, from 23 to 25 February 2005. It was organised by FAO and OIE, in collaboration with WHO and was attended by 160 participants including CVOs of the region, Regional Organisations, experts, officers from FAO, OIE, WHO, and donor and private companies representatives. The media coverage was very important.

The 5 Sessions allowed an assessment of the current situation, an evaluation of the achievements of control measures implemented in the last 12 months, a review the scientific advances in the understanding of avian influenza and advise on new control measures and future research needs.

The main conclusions were the following: countries have achieved positive results in improving the surveillance, detection and response to HPAI. But the situation is still worrying since the virus is epidemic in several countries and the risks for humans still persist. Eradication does not appear to be possible in the short term. Effective and practical tools and methods exist, including stamping out, biosecurity, movement controls, vaccination and compartmentalisation. Aquatic birds, especially ducks can act as reservoirs of infection. Specific research should be urgently conducted on AI surveillance on ducks and wildlife and on duck vaccination. Most human cases were acquired through direct contact with sick or dead poultry. Guidelines for risk reduction to humans should be developed jointly by FAO, OIE and WHO. The collaboration of the WHO and OIE/FAO networks of research laboratories should be strengthened. More information is required on the socio-economic impact of HPAI and on the cost of control measures. The control of HPAI may require restructuring of the poultry industry. The National and International plans should be harmonised and coordinated. The International and Regional Organisations, should continue their activities in support of the member countries. The existing FAO regional networks for surveillance and diagnosis should be sustained and the FAO/OIE Global Framework after the Control of Transboundary Animal Disease Initiative be used as foundation for the regional approach to control and eradicate AI. A master coordinated plan will be prepared with a global long term vision. A special declaration on the needs for increasing investment for HPAI control was also adopted.

Seven recommendations were adopted by the delegates attending the meeting, which covers the above mentioned issues.
OIE/FAO NETWORK ON AVIAN INFLUENZA EXPERTISE

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In view of the tremendous economic importance and the significant zoonotic potential of avian influenza (AI), OIE and FAO have proposed a joint OIE/FAO Network on avian influenza expertise called OFFLU. The main objectives of the network are to develop research on (AI), to offer veterinary expertise to Member Countries to assist in the control of AI and to collaborate with the WHO Influenza Network on issues relating to the animal-human interface.

The Network will consist of a Steering Committee, a Scientific Committee, a Secretariat and a Team of Scientific Collaborators. The Steering Committee will comprise a representative from each of the two organisations (OIE and FAO), a scientist from an OIE-FAO Epidemiology Collaborating Centre and the President of the OIE Biological Standards Commission. The Scientific Committee will comprise experts of international repute with proven laboratory expertise (diagnostics, molecular, vaccinology) and/or field experience on AI. The activities of the Scientific Committee will be supported by a Secretariat to be nominated on a rotational basis by the Steering Committee. The Team of Scientific Collaborators will comprise scientists having first hand experience of avian influenza outbreaks especially in developing countries and other scientists with proven national or regional expertise in laboratory work, epidemiology and field control programmes related to AI.

The Network will be proposed for adoption by the respective administrative bodies of the two organisations soon.
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SEPPIC VACCINE ADJUVANTS FOR POULTRY

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Avian Influenza and Newcastle disease are the 2 major diseases of poultry for which inactivated vaccines can be used. To guarantee high levels and long duration of protection, the choice of adjuvant is critical. Aluminium hydroxide adjuvant has been replaced by first generation oil adjuvants based on polysorbate formulations.

The production of the antigen is very specific and the yields of production and the purity of the final antigen is an important step. Once the antigen is defined, it is necessary to vectorised (I am not familiar with this term) as a water in oil (W/O) emulsion that need to respect the following parameters:

- Efficacy: to ensure long term as well as short term protection;
- Stability: 2 years at +4°C and about 1 month at 20°C;
- Viscosity: it must be fluid to permit easy injection of the formulation;
- Safety: to ensure that the vaccine does not produce adverse reactions in vaccinated poultry or develop blemishes in the carcass.

In order to get this quality of the vaccine, it is necessary to control the quality of the injectable oils, the quality of the surfactant (purity, oxidation, no residues, chemical definition, stability); the emulsifying properties: synergy between oil and surfactant and antigenic medium to get a perfect emulsion. The following table provides a description of the general properties of selected Montanide ISA adjuvants.

<table>
<thead>
<tr>
<th>Montanide</th>
<th>Oil</th>
<th>Adj/Antig (w/w)</th>
<th>Emulsion</th>
<th>Viscosity (mPa.s)</th>
<th>Efficacy</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Short term</td>
<td>Long term</td>
</tr>
<tr>
<td>ISA 70</td>
<td>Mineral</td>
<td>70/30</td>
<td>W/O</td>
<td>30</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>ISA 775</td>
<td>Mineral and non mineral</td>
<td>70/30</td>
<td>W/O</td>
<td>25</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>ISA 763A</td>
<td>Non mineral</td>
<td>70/30</td>
<td>W/O</td>
<td>20</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>ISA 206</td>
<td>Mineral</td>
<td>50/50</td>
<td>W/O/W</td>
<td>10</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
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COMPARISON OF VIRUS ISOLATION AND RT-PCR FOR AVIAN INFLUENZA VIRUS DETECTION IN IRAN

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Avian influenza (AI) is an economically important viral disease in Iranian poultry industry because of increased mortality and declines in egg production in affected chicken flocks. Virus isolation in embryonated SPF chicken eggs, a time consuming method, is the most commonly used diagnostic test. In this study, virus isolation (VI) and RT-PCR for detection of avian influenza virus targeting nucleoprotein of type A avian influenza virus and haemagglutinin (H9) genes were evaluated with trachea samples from 50 clinically affected flocks. Moreover, RT-PCR for detection of H5 and H7 subtypes were applied for the samples. None of the samples was found to be positive for H5 or H7 subtypes. The sensitivity and specificity rates by RT-PCR of the hemagglutinin with comparison by VI, as conventional method, were 95% and 93.3% respectively. The K value between these tests was 0.77. Compared with VI, the sensitivity and specificity of RT-PCR for nucleoprotein was 100% and 83.3%, respectively. The K value between VI and RT-PCR of nucleoprotein was 0.66. The results showed substantial agreement for the VI and RT-PCR tests in detection of AI virus. Moreover, RT-PCR for nucleoprotein was found to be more sensitive than RT-PCR for hemagglutinin. In conclusion, RT-PCR is a reliable method for rapid laboratory diagnosis of avian influenza in diseased birds because of its speed and sensitivity. Therefore, it can be used as an alternative to VI method in surveillance centres where early screening for AI virus is the goal.
In 2004, an epidemic of highly pathogenic avian influenza A (H5N1) virus among domestic poultry affected Asian countries, causing human disease in two of these.

To better understand whether there are genetic determinants of transmission of avian influenza strains, we evaluated the mutation rate in H5N1 viral sequences from birds and humans infected during the 1997 and the 2004 (first wave) outbreaks, uploaded from the Influenza Sequence Database. Phylogenetic and positive selection analyses were performed to compare the coding regions of H5N1 isolates. Positive selection analysis was performed by comparing the maximum likelihood estimates of the codon substitution models M7 and M8, implemented in the CODEML program of the PAML package developed by Yang Z. In the 1997 data set, only PB2 was found under positive selection at five positions (82, 318, 334, 727, 355), whereas in 2004, positions 140, 156 for HA and 204 for NS1 (which is involved, along with PB2, in increase of pathogenicity in mammalian hosts) were under positive selection. Positions 156 and 140 of HA are located within antigenically relevant regions of the molecule, thus positive selection at both of these sites may help the virus to evade the immune response and suggests the existence of an immunological pressure.

The occurrence of adaptive mutation in poultry, may be a crucial mechanism by which the virus could improve its transmissibility also among humans. The possibility of this event strengthens the importance of improving surveillance both in humans and poultry.
RESPONSE POLICY OPTIONS AND COMPARTMENTALIZATION FOR AVIAN INFLUENZA SURVEILLANCE IN NEW ZEALAND

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New Zealand has never recorded a case of highly pathogenic avian influenza (HPAI) in captive or free-flying birds. Low pathogenicity strains (LPAI), including subtype H5N2, have been reported in free-flying waterfowl (mallard ducks) in New Zealand. The risk profile for avian influenza (HPAI) in New Zealand is different from most other countries. New Zealand is distant from other land masses, live bird markets are uncommon and our waterfowl are not migratory. New Zealand has a number of unique and endangered bird species therefore diseases and/or disease control measures that may threaten the survival of these birds are of national significance. Response policies to outline the action to be taken in view of surveillance data from wild and captive birds in New Zealand have been developed to take account of the risks associated with different compartments (i.e. commercial poultry, back yard poultry, zoo and other captive birds, wild birds). Recommendations take account of risk to public health, native fauna and the economy. Appropriate control actions would be determined by a Technical Advisory Group (TAG) which will include representation of stakeholders and endorsed by an Independent Expert Advisory Panel. Such control actions could include depopulation, vaccination or other options depending on the clinical picture, the virus isolated and the compartment in which the virus was identified. No action would be taken to control LPAI viruses detected in wild birds but the situation would be monitored. Enhanced biosecurity to prevent the interaction of commercial poultry and wild birds is currently recommended.
CHARACTERIZATION OF H7N3 INFLUENZA VIRUSES ISOLATED DURING THE BRITISH COLUMBIA EPIZOOTIC OF 2004

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In February 2004 a highly pathogenic avian influenza (HPAI) outbreak erupted in the Fraser Valley of British Columbia, Canada. Our investigations indicated that the responsible HPAI H7N3 virus emerged suddenly from a low pathogenic avian influenza (LPAI) H7N3 virus precursor. Analysis of the hemagglutinin (HA) genes of the LPAI and HPAI viruses isolated from the index farm revealed the only difference to be the presence of a 21 nucleotide insert at the HA cleavage site of the HPAI virus. We deduced that this insert most likely arose as a result of non-homologous recombination between the HA and matrix (M1) genes of the same virus. Over the course of the outbreak, a total of 37 isolates with, and 3 isolates without inserts were characterized. Isolates with inserts could be further categorized into 7 variants based on amino acid substitutions which occurred within or adjacent to the insert. Intravenous pathogenicity indices ranged from 2.17 to 3.00 depending on the isolate, and the lesions in 4- to 6-week old and adult layer Leghorn chickens were consistent with those expected for a highly pathogenic virus. The events described here appear very similar to those which occurred in Chile in 2002 where the virulence shift of another H7N3 virus was attributed to non-homologous recombination between the HA and nucleoprotein gene.
HIGHLY PATHOGENIC AVIAN INFLUENZA IN ITALY: APPLICATION OF LOCAL SPATIAL STATISTICS TO DETECT CLUSTERS OF INFECTION

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Local spatial statistics were applied to the highly pathogenic avian influenza (HPAI) epidemic that struck northern Italy during winter of 1999 – 2000. A function of the number of IP’s that were located within discrete distances from each IP, and continuous distance-decay measures of clustering were calculated. Statistical significance of observed results was tested by a Monte Carlo simulation taking into account the spatial distribution of the population at risk. Significant clusters of HPAI were detected in the province of Brescia and Mantova (Lombardy region), and in the province of Verona (Veneto region). Subsequently, spatio - temporal clustering of HPAI was obtained as the number of new IP’s that occurred within a distance of 1.5 km from a previous IP in temporal risk window (TRW). An IP’s TRW is the time interval during which clinical signs of disease can be detected in another flock following transmission from the IP. In this way, we attempted to detect HPAI cases that most likely originated from viral transmission from neighbouring IP’s. Maximum spatio - temporal clustering was observed in Lombardy region, where up to 80% of flocks that were located within 1.5 km from certain IP’s in TRW subsequently became infected by HPAI. Our results suggest that pre-emptive slaughter and depopulation of at-risk-flocks, that were mostly carried out in Veneto, were effective in controlling the epidemic.
In order to verify the homogeneity of the Non Structural 1 (NS1) protein of representative Avian Influenza viruses of the H7 subtype (AI) isolated in Italy during 1999-2004, the gene encoding the NS1 protein from a collection of isolates obtained since 1999 in Northern Italy was analysed by sequencing. The complete sequence of the NS1 gene of 40 AI viruses (28 LPAI and 12 HPAI) isolated from a spectrum of bird species was determined and compared phylogenetically. The isolates, separated into two previously described clades designated A and B. Group A isolates were made up of H7N3 (n=11), H7N7 (n=3), H7N4 (n=1) subtypes while group B consisted of H7N1 (n= 24) and H10N4 (n=1) subtypes. In addition, analysis of the amino acid sequence of the NS1 protein from the 12 HPAI isolates revealed a previously unreported isoleucine residue at position 136 in addition to a novel C-terminal truncation.

The data presented reveals that NS1 proteins of AI viruses are not as conserved as originally believed. In addition, the novel primary structure of the NS1 protein of HPAI isolates that have circulated in Northern Italy indicate that a diagnostic test to be used in the framework of a “DIVA” strategy based on the detection of antibodies to the NS1 protein may encounter some difficulties in field validation. Work in progress is focusing on whether the variability identified confers functional or immunogenic advantages on those viruses that posses them and on the antigenic profile of the NS1 protein of a wider selection of AI viruses.
DESCRIPTIVE ANALYSIS OF THE AVIAN INFLUENZA VIRUS (H5N1) EPIDEMIC IN THAILAND IN 2004

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In January 2004, laboratory diagnosis confirmed the presence of highly pathogenic avian influenza (H5N1) virus in poultry and humans in Thailand. Control measures such as stamping-out, movement restrictions, and hygienic measures were implemented. In 2004, 1,417 villages in 60 of 76 provinces throughout Thailand were affected. Over 62 million birds were destroyed to control the disease and for animal welfare reason. H5N1 viruses transmitted from poultry to human led to hospitalization of 17; 12 of them died. Additionally, domestic cats, tigers, and leopards were killed by H5N1 viruses. About 83% of infected flocks were backyard chickens and ducks. Outbreaks were concentrated in the central, the lower part of the northern, and the eastern regions of Thailand which are wetlands, water reservoirs, and poultry densely populated areas. The relative risks (RR) of infection of poultry flocks, based on cumulative incidences over 2004, were around 4 times higher in the central region and 1.5 times higher for the eastern region, when compared to the northern region. Based on RR's, the risk of HPAI infection seemed 5 times higher in layers and broilers and 1.5 times higher in ducks, all compared to backyard chickens. However, in these observations, size of flock was considered as a confounder in relation to HPAI infection. As a result of various factors including the extent of the period between introduction of the virus into the country and its conclusive diagnosis of the initial outbreak, lack of biosecurity in certain production sector, difficulty to detect HPAI clinical signs in ducks, seasonal weather conditions, and lack of public participation and awareness, HPAI viruses disseminated widely in large areas throughout Thailand resulting in a complex situation to trace and control the disease. In early 2005, the epidemic is still on-going in Thailand.

Keywords: Avian Influenza, Epidemiology, Control measures, Thailand

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PHYLOGENETIC ANALYSES OF GENES FROM SOUTH AFRICAN LPAI VIRUSES ISOLATED IN 2004 FROM WILD AQUATIC BIRDS SUGGESTS INTRODUCTION BY EURASIAN MIGRANTS

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² University of Pretoria, Department of Veterinary Tropical Diseases, Onderstepoort, 0110, South Africa
³ University of Pretoria, Poultry Reference Laboratory, Onderstepoort, 0110, South Africa
⁴ Pfizer Animal Health, Johannesburg, 2146, South Africa

In 2004, South Africa experienced its first recorded outbreak of highly pathogenic avian influenza (HPAI) H5N2 in ostriches in the Eastern Cape province. The traditional ostrich-farming areas in the Western Cape province report outbreaks of LPAI in ostriches almost every year, which are attributed to wild birds and certain climatic patterns. During winter 2004, LPAI H3N8, H4N8, H5N2 and H5N1 avian influenza viruses were isolated from wild aquatic birds. All eight genes of the H3N8, H4N8 and H5N1 viruses were analysed. The results show that the H5N1 virus does not belong to the HPAI Z/Z+/V genotype currently circulating in Asia, but that the most recent common ancestors are Russian H5N2 and H5N3 viruses. The N1 gene lacks the stalk deletion found in current Asian H5N1 strains. Internal genes originate from a pool containing Russian, Middle Eastern and Italian viruses. The South African H3N8 and H4N8 viruses derived their genes from an ecosystem where Asian H5N1, H6N9 and H9N2; Russian N4N3, H4N9 and H6N8; and Norwegian H3N8 viruses have been circulating since 1997. All three viruses share recent nucleoprotein common ancestors with the German and Dutch HPAI H7N7 viruses from 2003. The diverse pool of genes from which local viruses are derived may reflect reassortment at the Siberian breeding grounds where migratory paths cross, or within the South African ecosystem. This data highlights the importance of surveillance in aquatic migratory birds, particularly members of the Charadriidae, for their potential roles in the introduction of avian diseases to South African poultry, particularly ostriches in the case of avian influenza.
H5 AVIAN INFLUENZA ANTIBODIES IN RURAL POULTRY OF THAI-BINH PROVINCE - VIETNAM

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Avian influenza (AI) caused by a highly pathogenic H5N1 virus was recognized in Vietnam late December 2004. Since then the disease has had enormous impact on the poultry production but also public health implications have been dramatic.

The disease appeared in Thai Binh province in the North of Vietnam in January 2004. Approximately one month after the last recognized outbreak in that province a seroprevalence study primarily for H5 virus antibodies was conducted to elucidate basic aspects of the post epidemic epidemiology of AI in rural poultry in Vietnam. In addition, virus isolation was attempted.

Blood samples and cloacal swab samples were obtained from 587 birds including 379 chickens, 76 ducks and 132 muscovy ducks from five districts of the province and representing 106 households. High levels of antibodies to H5 virus were observed in birds belonging to the three bird species which were sampled. However, a significant difference in prevalence of H5 antibodies between the three species of birds was demonstrated. A high percentage of the ducks were seropositive (78%) in contrast to the chickens of which only 6% showed seroconversion. The observation that few ducks died and only in the beginning of the outbreak in Thai Binh coupled with the fact that a very high percentage of the ducks were positive for antibodies against H5 virus demonstrate the potential of domestic ducks to act as a reservoir for AIV, including HPAI.
ISOLATION OF INFLUENZA A VIRUSES BELONGING TO THE H7N7 AND H7N4 SUBTYPES FROM WILD AND DOMESTIC WATERFOWL IN ITALY

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Following the outbreaks of Avian Influenza (AI) in Italy between 1997 and 2004, a surveillance program, funded by the Italian Ministry of Health was carried out on wild birds captured in wetlands in northern Italy. Between November 2003 and March 2005, 1512 cloacal swabs and 350 tracheal swabs were collected from wild birds captured in wetlands in northern Italy, from game-fowl and backyard flocks including waterfowl.

Cloacal and tracheal swabs were screened by Real Time RT-PCR (1) and, when positive virus isolation was attempted as described in EU Directive 92/40/EC.

Eight isolates were identified as influenza viruses belonging to the H7N7 subtype, four of which were isolated from wild mallard ducks, two from teals, one from a domestic duck and one from a domestic goose. Another isolate obtained from a mallard was subtyped as H7N4. Sequence analysis of the cleavage site of the haemagglutinin gene showed that all nine isolates are low pathogenicity avian influenza viruses. Interestingly, the H7N7 isolates from wild birds and the ones from the domestic waterfowl share a haemagglutinin sequence homology of 99.3%.

The results of this surveillance program support the theory that backyard flocks, particularly those which include domestic ducks and geese, might act as link between the natural reservoir and poultry. The evidence reported herein also indicates that Italy is continuously at risk for the introduction of H7 viruses, and supports the application of prophylactic vaccination in densely populated poultry areas (DPPA) of Northern Italy. Ongoing surveillance programmes in areas at risk, including both wild birds and poultry will improve our knowledge on the ecology, epidemiology and prevention of AI infections.

References


ECOLOGY AND MOLECULAR EPIDEMIOLOGY OF H9N2 AVIAN INFLUENZA VIRUSES ISOLATED IN ISRAEL DURING 2000-2004 EPIZOOTIC

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The first two isolates of H9N2 influenza virus from turkey and chicken in Israel were identified in May 2000. The actual epizootic of the LPAI H9N2 virus started in December 2001, followed by a 1.5-year-long period of silence, during which H10N7 and H6N3 influenza viruses were isolated sporadically. The outbreak of H9N2 influenza began in the Northern part of Israel, then the epizootic spread all over the country. Damages were relatively limited due to the widespread use of an inactivated vaccine. Since March 2003 until the last few months, the disease affected mainly broiler chicken flocks. Single isolates have been recorded in commercial ostriches, a flock of geese and in a wild pigeon. Apart from routine serological tests, the diagnostic tests were performed using RT-PCR with type-specific primers to the M and NP genes and a set of subtype-specific primers related to all the hemagglutinin (HA) and neuraminidase subtypes. All the primers were specially constructed. The part coding for N-terminus of the H1 chain of HA gene of 61 out of 400 isolates was sequenced. The isolates showed a high rate of mutability, differing distinctively from the H9 prototype strain. They belong to the same phylogenetic lineage divided into 3 sub-lineage, one of which demonstrating a unique cleavage site motif RSKR. The result indicates that two parallel evolutionary trends originated from the same local “prototype” isolate.
FIGHTING AVIAN INFLUENZA NEEDS A STEP FORWARD NOW

Harm Kiezebrink
Herman Kiezebrink Institute, Postbus 311, 8160 AH Epe, The Netherlands, www.hki-wageningen.com

HKI was responsible for culling 15 million birds on 900 farms during the AI epidemic in Europe. Animal welfare has always been most important in our approach.

1. Non – commercial poultry in Asia

Non-commercial poultry in rural areas are a high risk to human health with the potential to cause a pandemic outbreak. Addressing this is the biggest challenge to the global community. HKI has produced cheap, effective and safe systems to eradicated AI in rural areas.

2. European Model

The cost of the recent outbreaks in Europe were crippling to the poultry industry. HKI developed a risk management model to limit the consequential losses by 60% both to the industry and the EC, based upon AI insurance for farmers. A partnership is created between veterinary services and farmer’s organisations. HKI provides the equipment, training and planning to enable the disease outbreak to be attacked within 48 hours.

3. New techniques

We have employed all the techniques described by the OIE. We have developed these techniques to improve animal welfare, bio-security, cost effectiveness and reduce the risk to human health, for example;

- fast and effective culling of 10 000 adult turkeys per hour
- culling techniques for non-commercial poultry in rural areas in Asia for a capital cost of only 25 euros per device.
- revolutionary disinfecting technique using only water salt and electricity at a cost of only 250 euros per unit.
TAQMAN-MGB PROBE ONE-STEP QUANTITATIVE REAL-TIME PCR WITH INTERNAL POSITIVE CONTROL FOR AVIAN INFLUENZA VIRUS RNA DETECTION

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Development of rapid, highly specific and sensitive assays for the prompt identification of Avian Influenza Viruses (AIVs) circulating in the field to survey the emergence of potentially pandemic viruses, is one of the primary objectives of the research-management on AIV.

Though being sensitive and specific for AIV detection, conventional Reverse Transcription PCR is time-consuming, it does not allow for quantification of viral load and requires a number of manipulation of samples, thus increasing the risk of carry-over contamination.

Recently, quantitative, fluorescence-based Real-Time RT-PCR (RRT-PCR) assays have been proposed in different formats for AIV detection. In this study, a one-step RRT-PCR with Minor Groove Binder (MGB) probe was developed. A DNA probe with conjugated MGB group forms a stable duplex with single-stranded DNA target; moreover, the use of MGB probe has demonstrated low background fluorescence and improved assay specificity and sensitivity in comparison to the common non-MGB probes. Addition of an internal positive control to each sample before the processing allowed to monitor the reliability of RNA extraction and subsequent PCR amplification, eliminating the occurrence of false negative results.

A panel of avian, equine, swine and human Influenza A reference viruses were first assayed by the test. After optimization of the RRT-PCR, n°1152 cloacal swabs collected from hunted wild birds were tested and 15 samples were found positive for AIV. These results were further confirmed by a conventional RT-PCR targeting a different gene segment.
As part of our ongoing influenza A virus surveillance in wild birds in Northern Europe, Guillemots on the island Bonden in the Northern Baltic Sea, were screened. Three out of 26 sampled birds tested positive by RT-PCR. Two of these were characterized as subtype H6N2. Phylogenetic analyses showed that the PB2, PB1, HA, M and NS gene segments belonged to the American lineage of influenza A viruses, whereas the PA, NP and NA gene segments belonged to the Eurasian lineage. This is the first report of a recombinant avian influenza A virus isolated in Europe with genes of American and Eurasian origin and also the first report of an avian influenza A virus isolation from European guillemots.
HIGHLY PATHOGENIC AVIAN INFLUENZA EPIDEMIC IN ITALY: STUDY OF THE RISK FACTORS BY SURVIVAL ANALYSIS

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Background: From December 1999 to April 2000, a large epidemic of highly pathogenic avian influenza affected Lombardia and Veneto regions (north-east Italy). More than 400 outbreaks were observed, causing death or culling of 16,000,000 birds and severe economic losses. Control measures indicated in the Directive 92/40/CE were applied. In addition, pre-emptive slaughter of at risk farms was implemented mainly in one region.

The aim of the work was to evaluate the contribution of several risk factors to the spread of the infection and the efficacy of the pre-emptive slaughter to control the epidemic.

Methods: data on animal species and production, size and location of the infected farms were collected. The time of virus introduction in the farm was estimated.

A case was defined as a farm that during the observation period became infected. In the same period, uninfected or slaughtered farms were considered as censored. Cox multivariate regression model was applied for the hazard ratio (HR) evaluation.

Results: the model showed significant correlation with the animal species involved (HR=3.2 for turkey and HR=2.2 for hens), the size of the farm (HR=1.5 for larger farms) and the presence of other infected farms in a radius of 1500 and 3000 meters (HR=1.5 and HR=1.4 respectively). Comparing the two regions, the pre-emptive slaughter was protective against the infection (HR=0.4).

Discussion: raising turkey and hens, particularly in large holdings, and proximity to previously infected farms were the most relevant risk factors for a farm to be infected. The efficacy of pre-emptive slaughter was confirmed.
Efficacy of the Vaccination During the H7N3 Low Pathogenicity Avian Influenza Epidemic in Italy in 2002-2003

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Introduction: From 1997 to date, avian influenza (AI) viruses caused four epidemics in the densely populated poultry area (DPPA) in northern Italy. In 2002-2003 an epidemic due to a low pathogenicity (LPAI) virus subtype H7N3 occurred. No measures to control LPAI are provided for in the current European Union legislation, but the previously experienced epidemics moved Italian authorities to put in force measures to prevent the spread of LPAI and avoid possible re-emergence of highly pathogenic AI. Restriction measures were immediately implemented, followed after 2.5 months, by a vaccination programme. An heterologous vaccine that allowed the distinction between vaccinated and infected animals (DIVA) was used in turkey and layer flocks. The virus infected 388 farms, 86% of them were meat-type turkey farms. About 7.6 millions of birds were involved. Among the vaccinated, 88 turkey flocks were infected.

The aim of this paper is to describe the efficacy of the vaccination strategy by the estimate of the reproduction rate (R) in vaccinated and non-vaccinated poultry populations.

Methods: data on the total poultry population during the epidemic, on the time of infection and on the vaccination status were collected and entered in a stochastic model to estimate R.

Results: The estimate of R for the global poultry population at risk before and after the vaccination campaign were 2.9 (IC 95%=2.3-3.9) and 0.6 (IC 95%=0.5-0.7), respectively.

Discussion: The vaccination, together with restrictive measures, reduced significantly the diffusion of the virus among the poultry farms, allowing the control of the epidemic in DPPA.
INFLUENZA VIRUS CIRCULATION IN WILD DUCKS AND COOTS IN ITALY DURING H5N2 AND H7N1 POULTRY EPIDEMIC PERIODS (1998-1999)

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Two epidemics of avian influenza due to H5 and H7 highly pathogenic viruses occurred in poultry in Italy in 1997/98 and 1999/2000, respectively. The circulation of these serotypes in wild aquatic birds was investigated examining 488 cloacal swabs and 488 sera collected from 162 coots and 326 ducks trapped in Italian wetlands during three wintering periods, from 1998 to 1999. Seroprevalences against influenza A viruses, detected by a double antibody sandwich blocking ELISA were 16% in coots, and 44.5% in ducks. Among the Anatidae group, duck species wintering in Mediterranean areas (DWMA) showed significantly higher values than ducks wintering in South-Saharan areas (DWSSA) of Africa. In order to detect H5 and H7 antibodies, the hemagglutination-inhibition (HI) assay and two competitive ELISA tests (H5-ELISA and H7-ELISA) using monoclonal antibodies (MAbs) specific for H5 and H7 subtypes, were performed. None of the aquatic bird species were found seropositive to H7 subtype, whereas H5 positive sera were found by both HI and ELISA assays in ducks only. The highest H5 seroprevalences were detected by H5-ELISA; overall, 5% (10/201) of DWMA tested H5-seropositive by this assay, with annual seroprevalences ranging from 1.6% (2/123) to 11.8% (6/51), in 1998 and 1999 respectively. In the present study, only 5 viruses belonging to H1N1, H11N6, and H2N3 subtypes were isolated from ducks. However, the H5 seroconversion observed in one mallard duck at the beginning of 1998 indicates that H5 virus circulation also occurred in the study area.
ISOLATION AND CHARACTERISATION OF A HIGHLY PATHOGENIC H5N1 INFLUENZA VIRUS FROM EAGLES SMUGGLED FROM THAILAND INTO EUROPE

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The 2003-2004 highly pathogenic avian influenza epidemic caused by A/H5N1 viruses is now believed to be endemic in at least 6 Southeastern Asian countries and does not only affect birds. Its host range includes several mammals, including 69 laboratory confirmed human cases to date, with 46 fatal cases, after direct transmission of the virus. On October 18, 2004, two *Spizaetus nipalensis* crested hawk eagles smuggled into Europe from Thailand were seized and sacrificed at Brussels International Airport. Both eagles showed enteritis and one presented bilateral pneumonia. Samples were taken from lungs and inoculated into embryonating eggs, which died in less than 2 days. The isolated virus was denominated A/crested eagle/Belgium/01/2004. The antigenic subtyping as H5N1 was made by haemagglutination inhibition using 12 monospecific polysera and the diagnosis was confirmed by nucleoprotein gene (general for type A Influenza) and H5-specific RT-PCR. Over 600 contact birds were euthanized but all tested negative. The high pathogenicity of the virus was demonstrated by measuring the intravenous pathogenicity index in 6-week-old SPF chickens (IVPI=2.94) and confirmed in 12-week-old layers. The partial sequencing of the haemagglutinin (HA) gene indicated 6 basic amino acid residues in the cleavage site and a close relationship within the Z genotype cluster to currently circulating strains in Thailand, although some mutations were evident among which one resulted in a unique arginine (R) > lysine (K) replacement at the cleavage site. This study demonstrates that international air travel and smuggling represent significant threats for the introduction and worldwide dissemination of H5N1 viruses.
AVIAN INFLUENZA IN ASIA – THE CHALLENGES AHEAD

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Highly pathogenic avian influenza (HPAI) H5N1 viruses are still circulating in a number of Asian countries. A major challenge facing many of the infected countries is inadequate surveillance capacity, which can lead to cases of infection and disease remaining undiagnosed. This structural problem will not be overcome in the short to medium term. Therefore, it will be necessary to build HPAI control strategies around imperfect information. Data from elsewhere in the region will assist this process but may not be entirely applicable because of the marked regional differences in the various poultry production sectors and marketing systems between infected countries.

Infected countries have largely addressed HPAI as an animal health emergency. They should now design and implement integrated, long term control programs for HPAI based on reviews of likely infection pathways for each sector of the poultry industry. A suite of appropriate interventions should then be developed for those sectors considered to be playing a key role in the spread of H5N1 HPAI viruses. All available management options must be considered, covering technical as well as socio-economic and political factors of the proposed interventions before measures suited to the local situation are adopted. These pathways and measures need to be reviewed regularly.

Two countries are using vaccination widely to control HPAI as a response to endemic disease and infection. This is a valid response that appears to have significantly reduced the levels of disease, virus shedding and potential for spread. However, unless there is a significant change in the nature of circulating viruses or major structural changes in the smallholder sector in these countries it is unlikely that vaccination can be withdrawn for a considerable period of time. Managing the move towards reduced dependence on vaccination in these countries will be a major challenge.
ANCESTORS OF H5 AND H7 HIGHLY PATHOGENIC AVIAN INFLUENZA OUTBREAKS FOUND IN WILD BIRDS IN NORTHERN EUROPE

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Wild birds, in particular waterfowl, represent the natural reservoir of avian influenza A viruses (AIV). AIVs usually cause no disease signs in their natural host, but problems may arise upon introduction in non-natural host species. AIVs are occasionally transmitted to other species such as horses, pigs, marine mammals, ostriches, chickens, turkeys and humans, leading to influenza outbreaks among these animals.

In contrast with North America, information about AIVs in Eurasia is limited. However, AIVs found in Eurasian birds are of particular interest, because of their genetic differences from AIVs in the Americas, and the zoonotic potential of certain Eurasian strains is currently the cause of serious problems.

Between 1999 and 2002 we isolated 17 H5 AIVs with various NA subtypes (N2, N3, N6 and N9) and 16 H7 AIVs with various NA subtypes (N3, N7 and N9) from migrating mallards. Sequence analysis revealed high homology with recent fowl plague viruses, that caused the outbreaks in Italy in 1997 (H5N2), 1999 (H7N1), 2001 (H7N3) and in the Netherlands, Belgium and Germany in 2003 (H7N7). Phylogenetic analysis confirms the relationship of these viruses to those that caused the fowl plague outbreaks, indicating the ancestral relationship of these viruses towards the fowl plague viruses. Interestingly the majority of the H7 viruses were isolated prior to the 2003 H7N7 in The Netherlands during the fall migration of the mallards to their wintering grounds.

Our data indicate that AIV surveillance in wild birds provides an opportunity to develop reagents for diagnostic purposes and vaccine development prior to influenza outbreaks.
TROVAC AI H5, AN AVIAN INFLUENZA FOWLPOX VECTOR VACCINE, AS AN ALTERNATIVE VACCINE FOR HATCHERIES

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Inactivated vaccines are the most widely used vaccines in avian influenza (AI) controlled vaccination programs but there is currently no serology test commercially available that can differentiate infected from vaccinated birds (DIVA test). TROVAC AI H5, a fowlpox recombinant expressing the hemagglutinin of an H5 isolate, received a conditional license in US in 1998 and then registered in Mexico, Guatemala and El Salvador. More than one billion doses of TROVAC AI H5 have been used so far with no reported adverse reactions. This vaccine protects chickens against mortality and morbidity and decreases shedding after challenge with a wide panel of H5-subtype AI strains. It provided 100% protection against mortality/morbidity induced by challenge with recent Asian HP H5N1 isolates and reduced the challenge virus shedding from respiratory and gastrointestinal tracts by 2.5-4.5 log 10 of virus. TROVAC AI H5 is fully active in maternal antibody positive broiler chickens from breeder hens vaccinated with killed AI and/or fowlpox. However, after placement of chickens in the field, fowlpox vaccination or natural infection interferes with TROVAC AI-induced immunity. Birds vaccinated with TROVAC AI H5 will develop antibodies detectable by the HI test using the appropriate antigen. However, they will not produce antibodies against matrix/nucleoprotein detectable by common serological tests such as AGP and ELISA, which therefore can be used as DIVA tests. Another advantage of TROVAC AI H5 over inactivated vaccine is that it is fully efficacious when administered once in one day-old bird, and it is compatible with Marek’s disease vaccination. These features make TROVAC AI H5 an ideal vaccine to be used in hatcheries.
THE USE OF VACCINATION AS A PRIMARY ERADICATION TOOL OF HPAI OUTBREAK IN INDONESIA

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An outbreak of HPAI has been detected in Indonesia in August 2003. The epidemic continues as disease moves into new areas and less vulnerable flocks in infected areas become infected. The peak intensity of losses has passed with reduced numbers of outbreak since major losses began in November/December 2003. The disease has most probably spread rapidly through movements of live birds to most of the larger islands in Indonesia.

The primary eradication tool used has been the use of vaccination. There are eight different inactivated vaccines available (three locally manufactured; one from China; and four from Mexico). These are either H5N1 subtypes or, in the case of Mexican vaccines, H2N2 subtypes.

Vaccination has been proved to be effective in preventing local losses and in reducing the ‘virus load’ in the environment. The vaccination takes its effect as the epidemic has been slowed and the vulnerable flocks decrease in number since April 2004 up to present.

Pathogenicity and challenge testing was used to measure the vaccine efficacy. A challenge test was accomplished in order to measure the level of protection of local vaccine against field strains collected from three different locations. The result showed that the local vaccine could give a 90% protection rate. While the result of HI test showed that the mean antibody titers of vaccinated chicken at two weeks post challenge has been increased significantly.

Various field studies to monitor the antibody titers post vaccination in layer, broiler and local chicken at different ages were conducted in order to assay the duration of immunity and the effectiveness of vaccination for both local and import vaccines. The variation of protective antibody titers in the vaccinated chicken population were shown satisfactorily and booster application has given a high antibody titer in farms adopting high level of bio-security.

A molecular characterization of the isolates of AI viruses from different time and locations was conducted continuously up to present in order to be able to identify the most appropriate LP virus selected for replacing the existing master seed of local vaccine.

Keywords: vaccination, avian influenza, Indonesia
STUDY OF AVIAN INFLUENZA IN INDONESIA: MOLECULAR CHARACTERIZATION OF THE VIRUS COLLECTED FROM OUTBREAKS

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Avian influenza outbreaks in poultry have been reported in Java island since October 2003. More than 30 isolates of avian influenza virus had been isolated during October 2003 to February 2005. The virus has been identified as HPAI H5N1 subtype. Sixteen of them were then characterized further at genetic level and also for their pathogenicity. The phylogenetic analysis revealed that all avian influenza virus isolates were closely related to avian influenza virus from China (A/Duck/China/E319-2/03(H5N1). Molecular basis of pathogenicity in HA cleavage site showed that the isolates of avian influenza virus have multiple basic amino acid (B-X-B-R) indicating all of the isolates were still virulent of avian influenza virus (highly pathogenic avian influenza virus). It is concluded that there was not much change of the virus characteristics since it was first identified from outbreak of avian influenza in poultry in Indonesia.

Key words: avian influenza virus, characterization, poultry, Indonesia
MOLECULAR CHARACTERISTIC OF H5N1 AVIAN INFLUENZA A VIRUS IN THAILAND, 2004

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Influenza A virus subtype H5N1 caused a rapidly fatal systemic disease in domestic chicken, duck and other avian species as well as transmitted directly from poultry to humans. The virus also extended its host range to Felidae, causing fatal pneumonia in cats, tigers, leopards. We have sequenced the whole genome of the Thai influenza A (H5N1) viruses: A/Chicken/Nakorn-Pathom/Thailand/CU-K2/04, isolated during early 2004. H5N1 isolated from different avian species from different outbreak between January 2004 and January 2005 were also characterised. Phylogenetic analysis were performed in comparison to AI viruses from the Hong Kong 1997 and other H5N1 isolates reported during 2001–2004. Molecular characterization of HA gene of the Thai H5N1 revealed a common characteristic of a highly pathogenic AI (HPAI), with polybasic amino acid in the HA cleavage site, a 20-codon deletion in the neuraminidase gene, a 5-codon deletion in the NS gene and polymorphisms of the M2 and PB2 genes. We also evaluated an outbreak of AI in Tiger and showed evidence of probable tiger-to-tiger transmission in the tiger zoo. Sequencing and phylogenetic analysis of those viruses showed no difference to the first isolate obtained in January 2004. There was no mutation of histidine to tyrosine at position 274 of the neuraminidase molecule after Oseltamivir treatment. In both isolates, a single amino acid substitution, Glu to Lys, at the position 627 (E627K) in the PB2 protein responsible for H5N1 pathogenicity in mammals and 5 codon deletion in the NS gene were similar to the H5N1 viruses isolated in the same epidemic. Molecular characterization of H5N1 viruses from the following continuum outbreaks showed that there were no significant point mutations in critical regions.
NOVEL DIAGNOSTIC TESTS FOR THE SURVEILLANCE OF AVIAN INFLUENZA

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Novel diagnostic assays are developed for the rapid and efficient large-scale screening of Avian Influenza (AI) virus and associated antibodies. One of these tests is a rapid antigen capture assay which will detect all 15 Hemagglutinin sub-types of Type A Flu in 15 minutes. The product can be used farm side or in the laboratory for the detection of Type A Flu including South East and East Asian H5N1 isolates. This study examines the sensitivity and specificity of the assays as they compare with that of the standard Agar-Gel immuno-Precipitin (AGP) antibody test, virus isolation (VI), and other commercial products. The efficacy of the above assays for the surveillance of AI antibodies and antigens is reviewed.
SURVIVAL AND STABILITY OF HPAI H5N1 IN DIFFERENT ENVIRONMENTS AND SUSCEPTIBILITY TO DISINFECTANTS

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We studied the survival and stability of highly pathogenic avian influenza (HPAI) H5N1 isolated from affected chickens in Central Thailand. The study was done by determining the survival of HPAI H5N1 in allantoic fluid, chicken feces, cooked meat or eggs tested under local temperature in Central Thailand, including sunlight, shadow, water, and different cooking methods. Susceptibility of HPAI H5N1 to different disinfectants was also studied. The dose of HPAI H5N1 used in the experiment was 0.2 ml 10⁻⁶ EID₅₀/ml in 0.8 ml allantoic fluid, chicken feces (how much?), 1 cubic inch of meat or eggs. After tested under different environments/conditions, virus re-isolation from the residue in samples was done by chicken embryoinjection, hemagglutination and hemagglutination inhibition test. The results showed that the virus in allantoic fluid or feces was completely killed within 30 minutes after placing the sample in sunlight at 32-35 °C. The virus could survive for 10 days in allantoic fluid and 4 days in chicken feces when placed in shade at 25-32 °C. The same amount of the virus in water obtained from rice field where HPAI H5N1 affected ducks were raised, survived for 3 days. By cooking meat and eggs injected with the HPAI H5N1 virus to 70 C, for 3 minutes upward, the virus was completely killed. The HPAI H5N1 in feces was completely killed within 5 minutes with glutaraldehyde, ammonium chloride, peracetic acid, and sodium hypochloride.
CLINICAL, GROSS-HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL FINDINGS OF GRAZING DUCKS AFFECTED WITH HPAI H5N1 IN THAILAND

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During outbreak of highly pathogenic avian influenza (HPAI) H5N1 in Thailand, ducks, especially grazing ducks raised in rice fields, were also affected. We followed and observed 11 flocks of grazing ducks, containing about 2,000-3,000 ducks in each flock, in Central and Western Thailand. The grazing ducks observed were mixed breed native-khaki Campbell cross laying ducks. Clinical sign, virus shedding, pathology and immunohistochemistry were studied in the ducks. Virus shedding was studied by virus isolation from cloacal swabs. Gross and histopathology was studied in ducks found dead or moribund ducks. Immunohistochemistry for detecting the virus antigen was done in formalin fixed tissues routinely processed for histopathology. It was found that ducks at 3 weeks old, reared in houses had no clinical sign of HPAI H5N1 infection, or virus shedding. After being moved to the post-harvested rice field in contaminated areas, virus shedding was found for 7-10 days before the affected ducks showed clinical signs. The affected ducks had clinical sign including depression, circling, convulsion, dyspnea, and blindness. Gross and histopathological findings included severe pneumonia, pulmonary edema, necrotizing pancreatitis, encephalitis, keratitis, conjunctivitis, nephritis, enteritis, lymphoid depletion/necrosis and hemorrhage in serous membranes. By immunohistochemistry, viral antigen was detected in several organs including brain, trachea, lung, kidney, intestine, pancreas and liver. Our findings show that the affected grazing ducks have high potential for spreading H5N1 HPAI viruses in Thailand.
ASSESSMENT OF THE EFFICACY OF A COMMERCIAL INACTIVATED H5N2 VACCINE TO PREVENT THE EXPERIMENTAL INFECTION OF DUCKS BY AN EUROPEAN LP H5N3

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Free range domestic ducks may have direct or indirect contacts with wild birds species known as a reservoir of low pathogenic (LP) avian influenza viruses (AIV). As such, they may become infected with a wide range of LP AIV subtypes without any clinical signs. (In Asia they can also carry H5N1 HPAIV asymptotically). However, the asymptomatic infection of domestic ducks with LP H5/H7 AIV has been more or less occasionally reported in Europe. It is a matter of concern since H5/H7 infected domestic ducks may be a source of infection for other poultry species. In order to prevent such an event, the prophylactic vaccination of domestic ducks at high risk of infection could be considered provided data are established to suggest its effectiveness. Since no data are available in the vaccine files concerning the protection afforded in ducks, the first step was to determine whether commercially available vaccines can prevent the infection of ducks by recent European AIV strains.

In the present study, the efficacy of a commercial inactivated H5N2 vaccine to prevent the infection of mule ducks by a French LP H5N3 isolate (A/dk/France/02166/2002), was experimentally assessed. This vaccine is prepared with the LPAIV A/Ty/MN/3689-1751/1981 as a water in oil emulsion and given subcutaneously.

50 AI seronegative 5 week-old conventional mule ducks maintained in quarantine from one day-old were allotted in 5 groups and maintained in BL3 facilities during the study : 1) one control group, 2) one group vaccinated once with twice the chicken vaccine dose (ck dose) -recommended by the supplier- and challenged with 10^6.4 DlE50 by combined eye drop, IN and IT, 3 weeks later, 3) one group vaccinated with twice the ck dose, boosted two weeks later with one ck dose and challenged similarly as group 2 but, 2 weeks after the boost, 4 and 5) the two other groups remained unvaccinated but were challenged as previously described at 8 weeks old and 10 weeks-old together with group 2 and 3 respectively. The same schedule was followed using 50 AI sero-negative one-day old conventional mule ducks except that a unique ck dose was administrated irrespective of the group. So depending on whether ducks received one vaccination or a prime-boost, they were challenged at 3 weeks-old or 5 weeks-old respectively.

The serological response was monitored before each vaccine administration, using HI test and H5N3 antigen (homologous with the challenge strain); cloacal and tracheal shedding was monitored 3 and 5 days after challenge, using M-based real time RT-PCR (correlated with virus isolation using egg inoculation).

5 week-old vaccinated ducks displayed a higher (statistically significant) HI response in comparison with one day old vaccinated ducks. The highest HI response (7.4 log2 ± 1.9) were observed two weeks after the boost of 5 week-old vaccinated ducks. However irrespective of the vaccination scheme and the age, no statistic differences were observed between vaccinated and unvaccinated ducks with respect to i) the ratio of ducks shedding virus and ii)quantities of shed virus. Several hypotheses are considered to explain this failure, among them the phylogenetic distance between the vaccine strain and the challenge strain, the insufficient HA content of the vaccine. So, to check the first hypothesis, further trials are in progress in our laboratory using Eurasian strain-based vaccines.
PHYLOGENETIC ANALYSIS OF AVIAN INFLUENZA SUBTYPE H6 VIRUSES ISOLATED IN FRANCE BETWEEN 2000 AND 2004

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The complete sequence of the HA1 gene of 12 H6N2 and H6N8 isolates was compared. Nine isolates (7 H6N2 and 2 H6N8) were obtained from meat turkeys and turkey breeders reared in the west part of France between 2001 and 2004. They exhibited respiratory signs, decrease food consumption, increase mortality and, for breeders, egg drop production. Another H6N2 isolate was obtained from breeder ducks exhibiting mucosal nasal discharge without mortality, their holding being located close to one of the previous H6N2 infected meat turkey holding. In addition two H6N2 isolates obtained in 2000 from sentinel ducks sharing the same environment as wild birds of a bird sanctuary located in the west part of France, were also analysed. The 12 sequences were compared to some published sequences from American and Eurasian viruses isolated in 1970’s, and from contemporary aquatic and terrestrial birds, in order to determine molecular evolution among H6 viruses. The sequences of the French viruses were very similar among them although one H6N2 isolate from turkey showed a deletion of one amino acid. Six isolates possessed the amino sequence PQVETR/G at the cleavage site and 6 isolates, among them the 2 H6N2 isolates obtained in 2000 from sentinel ducks, the amino sequence PQIETR/G. The phylogenetic tree revealed that viruses isolated from France clustered in a group including german viruses isolated these last years. Phylogenetic relationships are discussed according notably to the migratory routes from Northern Europe.
A NEW NEUROKININ 1 RECEPTOR AGONIST AS POTENTIAL TREATMENT AND PROPHYLACTIC MEASURE IN POULTRY AND HUMANS FOR AVIAN FLU (AF)

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A/HongKong 8/68 influenza virus (HKV) was used in a murine model of occupational toxicology inducing Acute Respiratory Distress Syndrome (ARDS). C57BL/6 mice were exposed to JP-8 jet fuel inhalation one hour/day over seven days to wipe out pulmonary immune response, as shown previously. At day 7 mice received HKV by nasal inoculation. Mice were divided into two groups; HKV only (HKV-) and mice receiving [Sar⁹, Met(O₂)¹¹]-Substance P (SP+), 1 µM/15 minutes/day via nebulizer (HKVHS). HKV- mice developed influenza symptoms; decreased body weight, fever, dehydration, nasal discharge after 5 days and at day +7 began to die from ARDS. Remaining mice were sacrificed according to protocol. HKVHS mice did not develop influenza symptoms, however, were sacrificed at Day +7 to compare lung tissues vs HKV-. HKVHS showed leukotriene B4 levels at 33% of HKV- and normal lung cell counts after broncho-alveolar lavage. HKV- had very high lung cell counts and pathological lung injury evidence. Electron micrographs (EM) of HKV- showed absence of airway cilia, swollen airway epithelial cells, large numbers of mitochondria, and colonies of HKV. 200 EM of HKVHS did not show any lung injury evidence neither any HKV colonies.

Farmers, poultry industry, health care workers are at great risk of acquiring AF through infected poultry. We suggest collaborative community models of SP+ intervention; SP+ prophylaxis in large poultry farms and SP+ as vaccination replacement or adjuvant.

Sp+ is a potential AF treatment and prophylactic agent against all AF strains as the mechanism is strong general immunomodulation, not vaccination.
Cambodia faced 15 HPAI (H5N1) confirmed outbreaks in the different sectors of the poultry production since January 2004. The country has very limited human and financial resources and the veterinary services did not have the basic tools to collect accurate epidemiological information and to fight the disease. Therefore, different agencies under the umbrella of FAO provided a support to the Government to strengthen its capacities in terms of diagnosis, surveillance and control of the avian influenza virus. Different surveillance tools are being tested, such as market monitoring and sentinel villages’ network, in order to offset the weakness of the national passive surveillance networking. Several constraints have been identified in the implementation of this programme, such as the motivation of the provincial staff, the capacity of the central team to compile and analyse the data generated, the reluctance of the farmers to have their animals sampled, and the diagnosis capacity. The question of sustainability of such a surveillance system remains after the international support will end. Participatory epidemiology (PE) could be proposed as a complementary tool for disease searching. PE works on the principle that livestock keepers often possess detailed knowledge on animal diseases and can provide valuable diagnostics which could help locating AI outbreaks especially in remote areas. Beside usual validation methods, PE could be evaluated using Bayesian decision approaches. Finally, quantitative risk assessment methodologies are also proposed to appraise the performances - e.g. risk to do not detect in time new AI outbreaks - of surveillance systems including PE.
AVIAN INFLUENZA SITUATION IN POLAND

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Poland has never experienced highly pathogenic avian influenza. However, in 1995 an epidemic of low pathogenic avian influenza caused by AIV - H7 occurred in turkey flocks. Survey conducted between 1997 – 2002 in poultry revealed lack of active infections but two AIV - H5 were isolated in 2002 from robin and seagull. The pathogenicity of four AIV isolated from turkeys and wild birds assessed by sequencing of the HA cleavage site confirmed that the strains were low pathogenic (absence of multiple basic amino acids). These results showed concordance with in vivo studies (IVPI = 0,0).

Since 2003 a national survey programme for AIV infections in poultry and wild birds has been conducted in Poland according to the guidelines provided in the Commision Decisions 2002/649/EC (2003 and 2004) and 2004/615/EC (2004). In 2003, 8260 samples of sera or feaces from poultry and 363 samples of feaces from free-living birds were tested serologically in HI test (H5 and H7 antigens) or virologically on SPF embryonated eggs. In 2004, 11 103 sera/feaces samples (poultry) and 514 feaces samples (wild birds) were tested.

Antibodies to AIV H5 were detected in 4 samples from 3 geese flocks: 1 serum from 1 flock in 2003 and 3 sera from 1 flock (tested twice) in 2004. Antibodies to AIV H7 were found in 2 samples of sera from 2 chicken flocks in 2003. Positive flocks were retrospectively investigated at the holding with no further suspicion of AIV infection. No virus was isolated from poultry flocks, including seropositive flocks, and free-living birds.