Towards the Elimination of Rabies in Eurasia
(Joint OIE/WHO/EU International Conference)

(OIE Headquarters)

Abstract book
Acknowledgements

Bio-Rad
Bioveta
Merial
Sanofi-Pasteur
Virbac
Contents

■ Introduction ............................................................................................................ 1
■ Objectives of the Conference .................................................................................. 2
■ Topics ...................................................................................................................... 2
■ Organisation of the Conference ............................................................................. 4
■ General Information .............................................................................................. 4
■ Programme .............................................................................................................. 5
■ Abstracts .................................................................................................................. 11

KEYNOTE ADDRESS
Towards the elimination of rabies in Eurasia: overview and perspectives .................. 12
J. Blancou

SESSION 1 – EPIDEMIOLOGICAL INFORMATION, RABIES IN EURASIA: REGIONAL REPORTS
Map: Areas selected for regional reports .................................................................... 13
Rabies situation in Western Europe ............................................................................ 14
A.I. Wandeler
Rabies situation in Eastern Europe ............................................................................ 15
O. Matouch
Rabies situation in Central Asia ................................................................................ 16
K.N. Gruzdev
Rabies situation in the Middle East .......................................................................... 17
A. Seimenis
Rabies situation in Far East Asia ................................................................................ 18
Z.F. Fu

SESSION 2 – RABIES PATHOGENESIS
Cytokine expression in the CNS and periphery during infection with rabies ................ 19
N. Johnson, K.L. Mansfield, D. Hicks, A. Nunez, D. Healy, S.M. Brookes, C. McKimmie,
J.K. Fazakerley & A.R. Fooks
Neuroimaging, virus and cytokine studies in rabies infected dogs ......................... 20
J. Laothamatas, S. Wacharapliesadee, B. Lumiertdacha, S. Ampawong, V. Tepsumethanon,
P. Phumesin, S. Asavaphatiboon, L. Worapruekjaru, Y. Avihingsanon, N. Israsena & T. Hemachudha
Role of Calcitonin Gene-Related Peptide (CGRP) in the pathogenesis of rabies .......... 21
Pathogenic and attenuated rabies viruses induce differential host protein expression in
the central nervous system: implication of neuronal dysfunction ............................ 22
Z.F. Fu
Dominance of a non-pathogenic over a pathogenic G protein gene in defining the pathogenicity of a rabies virus
B. Dietzschold

SESSION 3 – RABIES PREVENTION AND CONTROL STRATEGIES

Can rabies be eradicated? (Keynote speech)

Rabies Control in Dogs

Molecular epidemiology of lyssaviruses in Eurasia

Identification of novel canine rabies virus clades in the Middle East and North Africa
D. David, G.J. Hughes, B.A. Yakobson, I. Davidson, H. Un, O. Aylan, I.V. Kuzmin & C.E. Rupprecht

Rabies in South Asia - Epidemiological investigation
C.K. Singh & B.S. Sandhu

Stray dogs in Bangkok, Thailand: rabies virus infection and rabies antibody prevalence
S. Kasempimolporn, B. Sichanasai, W. Saengseesom, S. Puempumpanich, S. Chatraporn & V. Sitprija

Preference for commercially produced oral rabies baits by feral dogs

Assessment of the efficacy of oral vaccination of shepherd dogs in supplement of oral rabies vaccination of wild canids in Israel
B. A. Yakobson, R. King, N. Sheihat & D. David

Longterm immunization of recombinant rabies-canine adenovirus type-2 in dogs after intranasal or oral vaccination

New steps in the control of canine rabies in India

Rabies in Mexico, 1990-2005
C.H. Álvarez Lucas

Risk of rabies introduction by non-commercial movement of pets
P. Have, I. Alban, L.T. Berndtsson, F. Cliquet, P. Hostnik, S.C. Rodeia & M. Sanaa

Rabies Control in Wildlife

Epidemiology of rabies in South East Europe

Rabies in Mongolian steppes
A.D. Botvinkin, D. Otgonbaatar, S. Tsoodol & I.V. Kuzmin

Epidemiology and control of rabies in Iran
A.R. Janani & A. Fayaz

Rabies epidemiology in raccoon dogs and foxes
A. Singer, K. Kauhala, K. Holmala & G.C. Smith

Downside risk of wildlife translocation
R. Chipman, D. Slate, C. Rupprecht & M. Mendoza

Sylvatic rabies in Russia with special attention to perspectives of oral vaccination of wild carnivores
A. D. Botvinkin, D.V. Bankovsky & G.A. Safonov
Finnish-Russian collaboration programme on rabies control in wildlife: the outcome of five years and the future prospects  .................................................. 41
A.E. Metlin, S.S. Rybakov, K.N. Gruzdev, V.V. Mikhalishin, A. Huovilainen & E. Neuvonen

Rabies eradication programme in Estonia by means of oral vaccination of wildlife: first results  .............................................................. 42
E. Niin, M. Laine & A. Pärtel

Rabies surveillance in Poland between 1992 - 2006  ................................... 43
M. Smreczak, P. Tębas, A. Orlowska & J.F. Zmudziński

Efficacy of a square V-RG vaccine baits in red fox, domestic dog and raccoon dog  .......................................................... 44
F. Cliquet, A.L. Guiot, C. Schumacher, J. Maki & J. Barrat

Scenario-analysis evaluating emergency strategies after rabies re-introduction  ............................................. 45

The financial challenge to keep a large region free of rabies – the EU example  ...................................................... 46
C. Freuling, T. Selhorst & T. Müller

What is the future of rabies control in Europe?  ........................................ 47

The strength of 70% - revising a given threshold of rabies control  ............... 48
H.-H. Thulke & D. Eisinger

Rabies in Bats: an emerging zoonosis

A random grid based molecular epidemiological study on EBLV isolates from Germany  .................................................. 49

Asymptomatic rhabdovirus infection in meridional serotine bats (Eptesicus isabellinus) from Spain  ................................................ 50
J.E. Echevarría, S. Vázquez-Morón, C. Ibáñez, J.C. Aznar, E. Ruiz & J. Juste

Public health hazard of European bat lyssavirus, the Netherlands  ............... 51

Serological investigation of bats infected with rabies virus in China  .................. 52
L. Wang, Y. Jiang, Z. Lu, K. Sun, H. Xuan, A. Fooks & C. Tu

Epidemiology and pathogenicity of African bat lyssaviruses  ......................... 53
W. Markotter, C. Wilsenach & L.H. Nel et al.

Experimental infection of big brown bats (Eptesicus fuscus) with West Caucasian bat virus  .................................................. 54
I.V. Kuzmin, R. Franka & C.E. Rupprecht

Susceptibility of foxes (Vulpes vulpes) to European bat lyssaviruses types-1 and -2  .................................................. 55

Identification of British bat species using sequence comparison and its application to European bat lyssavirus diagnosis and surveillance  .................................................. 56
S.L. Harris, N. Johnson, S.M. Brookes, A.R. Fooks & G. Jones

Rabies Prevention in Humans

Human rabies includes early generalized vasospasm of cranial arteries that responds to tetrahydrobiopterin, L-arginine or nitroprusside  .................................. 57

Therapy of human rabies: lessons from experimental studies in a mouse model  .................................................. 58
A.C. Jackson, C.A. Scott, J. Owen, S.C. Wells & J.P. Rossiter

Rabies control and prevention in Georgia: current status and perspectives  ........ 59
P. Imnadze, V. Surguladze, T. Tushishvili & I. Baidoshvili
A simplified economical 4-site intradermal post-exposure rabies vaccine regimen is as immunogenic as the current intramuscular and intradermal methods  

Single dose for rabies pre-immunization  
P. Khawplod, H. Wilde, T. Kamolthum, Tantawichien & V. Sitprija  

Immune memory after remote pre- and post-exposure rabies vaccination: a prospective study in Thailand  
H. Wilde  

Epidemiology and prophylaxis of rabies in humans in France. Evaluation and perspectives  
Of a twenty five year surveillance program  
Y. Rotivel, M. Goudal, A. Simons de Fanti, D. Van Der Vliet & French Rabies Treatment Centres  

SESSION 4 – ADVANCES IN TECHNOLOGIES, DIAGNOSIS AND VACCINES  

Immune evasion, a critical strategy for rabies virus (Keynote speech)  
M. Lafon  

BHK-21 cell culture rabies vaccine - a candidate vaccine for humans  
D. Lalosevic & V. Lalosevic  

Factors influencing the antibody response to rabies vaccination  
V. Jakel, K. Cussler, M. König & H.-J. Thiel  

Attaining raccoon rabies management goals: history and challenges  
D. Slate, C.E. Rupprecht, D. Donovan, J. Badcock, A. Messier, R. Chipman & M. Mendoza  

Evaluation of the stability of rabies vaccine baits – field trial  
P. Mačiulskis, K. Lukauskas, E. Jacevičius, V. Kiudulas, J. Jokimas & A. Pockevičius  

Immunogenicity of ERA G 333 strain in foxes and raccoon dogs  
D. Bankovskiy, G. Safonov & Y. Kurilchuk  

Canine adenovirus-based vaccines against rabies  
N. Tordo, A. Fournier, C. Jallet, M. Szelechowski, B. Klonjkowski & M. Eloit  

Development of an edible rabies vaccine in maize, using vnukovo strain  

Genetically engineered single-chain antibody fusion proteins for detection of rabies virus antigen  
M. Mousli, I. Turki, H. Kharmachi & K. Dellagi  

Use of rabies virus as a transneuronal tracer of neuronal connections: implications for the understanding of rabies pathogenesis  
G. Ugolini  

Inhibition of rabies virus replication by Micro-RNA  
N. Israsena, N. Ratana-seyuth, P. Supavonwong, P. Virojanapirom & T. Hemachudha  

SESSION 5 – GENERAL CONCLUSIONS AND RECOMMENDATIONS  

Rabies at the Dawn of the 21st Century (Keynote speech)  
H. Koprowski  

OIE Guidelines on the control of dog populations  
S. Kahn, L. Stuardo & S.A. Rahman  

What have we achieved? The way forward (Keynote speech)  
D.J. Briggs
Don’t forget rabies when you settle abroad or travel with children
M. Goudal & Y. Rotivel

Determination of thermostability and melting point of the bait casing for the vaccine lysvulpen
V. Vrzal

Ten-day observation of live-rabid dogs
V. Tepsumethanon, H. Wilde & V. Sitprija

A simple sandwich ELISA (WELYSSA) for the detection of lyssavirus nucleocapsid in rabies suspected specimens using mouse monoclonal antibodies
G. Xu, P. Weber, Q. Hu, H. Xue, L. Audry, C. Li, J. Wu & H. Bourhy

Rabies research trends in the fgi “Federal Centre For Animal Health”

RFFIT-modified antibody-binding test (ABTmR) as a fast and reproducible test for determination of potency of inactivated purified non-adjuvanted rabies vaccines for human use
S. Stankov, V. Simin, D. Vujin, U. Ungurović, N. Vranješ & J. Desnica

Comparative analysis of the full genome sequence for EBLV -1 and -2 with other lyssaviruses

Overview of rabies in Greece from 1966 to 2006
O. Mangana, K. Nomikou, P. Lliadou & G. Anastasiadis

Bayesian estimation of rabies ab-ELISA performances in absence of a gold standard
S. Guillossou, M. Rabilloud, S. Leterme & R. Ecochard

The first case of European bat lyssavirus type 1b infection in E. sero inus in Poland
M. Smreczak, A. Orłowska, P. Trębasi & J.F. Żmudziński

Preliminary results of an active survey of bat rabies in Belgium
L. Audry, F. Kleina & H. Bourhy

Experimental immunization of cats with a recombinant rabies-canine adenovirus vaccine
R.L. Hu, Y. Liu, S.F. Zhang & F. Zhang

Profile epidemiologist and molecular characterization of the rabies virus during the period of 1996 -2006 in the estate of Mexico, MEXICO

Active monitoring of EBLV infection in natural bat colonies
B. Amengual, H. Bourhy, M. López-Roig & J. Serra-Cobo

Comparative studies of an inactivated vaccines against rabies virus in vaccinated goats

Plateletia™ Rabies II: an ELISA assay used for titration of rabies glycoprotein antibodies comparable to the reference method RFFIT
M. Feyssaguet, L. Dacheux, L. Audry, I. Blanchard & H. Bourhy

Plateletia™ Rabies II: an ELISA assay to complete current methods for titration of rabies glycoprotein antibodies in animal samples
A. Servat, M. Feyssaguet, F. Boué, I. Blanchard & F. Cliquet

Development of a sequence database for European bat variants of rabies virus identified in Eurasia
A.R. Fooks, L.M. McElhinney, D. Marston & all members of medvetnet wp05

An outbreak of pig rabies in Hunan province of China
Y. Jiang, X. Yu, L. Wang, H. Xuan, Z. Hu & C. Tu

Isolation of European bat lyssaviruses (EBLVS) on neuroblastoma cells in comparison with mouse inoculation test
Rabies surveillance, diagnosis and eradication in Austria ........................................................................ 100

A rabies cell phone advisory system: a culturally targeted tool designed to reduce human rabies suffering in the Philippines ........................................................................................................ 101
E. Adriano, M. Vinluan & S.J. Scholand

Raccoon dog rabies in Poland .................................................................................................................. 102
M. Sadkowska-Todys, M. Rosinska, M. Czerwinski, M. Smreczak & J.F. Zmudzinski

Implementation of ultra-light aircrafts in baits distribution for per-oral vaccination of venison against rabies ...................................................................................................................... 103
M. Sinkovic, D. Bosiljka & D. Lalosevic

Retrospective analysis of 174 humans who received P.E.P. (rabies immunoglobulin (RIG) and vaccine) ................................................................................................................................. 104
M. Goudal, Y. Rotivel, A. Simmons De Fantis & D. Van Der Vliet

Epidemiology of rabies in the Kyrgyz Republic ......................................................................................... 105
E.K. Akmatova, R.Z. Nurgaziev & N.T. Dzhaparaliev

Antibody response in italian dogs vaccinated with inactivated vaccines .................................................. 106
F. Monaco, G. Savini, A. Ripani, P.M. Franchi, R. Lelli

List of participants ................................................................................................................................... 107
Introduction

The theme of this second Conference on rabies hosted by the OIE in collaboration with the WHO and EU - Towards the elimination of rabies in Eurasia - signifies the commitment of the World Organisation for Animal Health to prevent and control animal diseases and zoonoses worldwide. Following the very successful first Conference on rabies, held in Kiev in 2005, this Conference aims to take the recommendations of the Kiev Conference one step further by seeking answers to questions on how we could proceed towards the eventual elimination of this most feared zoonotic disease - not only in Eurasia but all over the globe.

In spite of rapid advances in diagnostics, vaccine development and the application of novel technologies to controlling the disease, rabies remains a significant cause of death in humans following exposure to rabid animals. It is therefore the prime responsibility of the veterinary profession to apply its knowledge and skills in animal disease control to creating a buffer between the animal source of the disease and susceptible human beings. An important prerequisite to achieving this goal is the ability of the veterinary services of countries to institute and apply the international standards for the control of animal diseases and zoonoses. The OIE, fully realizing the need of many developing and in-transition countries to improve the governance and delivery of their veterinary services, has embarked on a global campaign to publicise the importance of accepting the delivery of veterinary services as an international public good. Only by assisting countries in need to apply the minimum standards for animal disease control will we be able to fully benefit from the advantages of new technologies to eventually move towards the elimination of rabies.

This Conference brings together veterinarians, researchers and regulatory officials in both animal and public health to not only share their experiences but also to confirm their common commitment to adding rabies to the list of diseases that have been successfully controlled and eliminated by the veterinary profession. The Conference will highlight important epidemiological developments on the disease in Eurasia and will share the experiences and opinions of experts on the prevention and control of rabies in dogs, wildlife and bats; the prevention of the disease in humans and cooperation between public health and animal health authorities; advances in technologies and vaccine development.

I am convinced that the veterinary profession, together with their colleagues in the public health sector, has the ability, knowledge, expertise and commitment to realise the ideal of this Conference and to conclude what Pasteur started when he developed the first rabies vaccine more than a century ago.

Dr Bernard Vallat
OIE Director General
Objectives of the Conference

- To consider the strategies and policies for the elimination of human and animal rabies in Eurasia.
- The target audience of the conference will be regulatory veterinary services, public health services, medical and veterinary practitioners, scientists and researchers.
- The main topics included on the agenda of the Conference are:
  - Epidemiology
  - Rabies pathogenesis
  - Rabies control in dogs
  - Rabies control in wildlife
  - Bat rabies an emerging zoonosis
  - Human rabies prevention
  - Advances in technologies, diagnosis and vaccines

Topics

Epidemiology

The aim of this session is to provide up-to-date information on rabies across Eurasia and to serve as a basis for the other conference topics. Therefore, the principal item of this session will be selected regional reports on the rabies situation from 5 different geographic regions in Europe and Asia, e.g. Western Europe, Eastern Europe, Middle East, Central Asia and the Far East, in recent years, which will be given by representative speakers. The regional reports will include aspects of rabies infection in man and animals, surveillance and epidemiology of rabies based on information of a questionnaire to be sent to the participating countries. Of particularly relevance is the holistic epidemiological picture of rabies in Eastern Europe and Asia.

Rabies pathogenesis

The overall aim of this session is to provide an understanding of the basic mechanisms of rabies virus-host interactions which is imperative for the prevention of rabies in humans and its control in animal populations. It includes fundamental research on the function and antigenic analysis of virus structural components, on virus genome structure and expression, on analysis of virus replication processes and on effects of virus on their host cells. Of special interest is the modulation of cellular function, the initiation of innate and humoral immune response in the early spread of rabies virus and the immune evasion. Also, the role of the blood-brain barrier in the pathogenesis and the clearance of rabies virus from the central nervous system will be addressed.

Rabies control in dogs

Domestic dogs are the principal hosts for classical rabies virus in many parts of the world, e.g. Africa, Latin America, Middle East and Asia. Genetic and antigenic tools will improve our understanding of the molecular and antigenic epidemiology of carnivora rabies in Eurasia. Past experience has clearly demonstrated that eliminating rabies in the dog population can substantially reduce human infection. The tools required to eliminate canine rabies were developed several years ago but have not been used collectively in a stepwise strategic manner to eliminate rabies in the canine population in Asia where tens of thousands of humans still die every year. Therefore, areas for discussion for this topic will mainly focus on: molecular and antigenic epidemiology of carnivora rabies in Eurasia, whether canine rabies is really independent from a wildlife reservoir, dog rabies control using parenteral vaccination, oral vaccination of stray dogs, and complementary measure to control the dog population such as
responsible dog ownership, the control of food resources, sterilization of dogs (ABC programs), establishment of animal shelters and culling. Field trials throughout Eurasia in places where dog accessibility to parenteral vaccination has been identified as the obstacle to rabies elimination are also encouraged.

**Rabies control in wildlife**

Areas for discussion for this topic will focus on sophisticated and modern methods of rabies control in various rabies wildlife reservoirs especially foxes and raccoon dogs. Rabies control by oral vaccination will be of high significance and will encompass oral vaccination programmes currently implemented, field trials, and evaluation of bait delivery systems. Also, oral vaccine safety and efficacy in target and non-target species, bait development and bait preference trials will be addressed. For those concerned with planning of vaccination campaigns, a number of requirements regarding vaccination strategies, efficacy, optimization and economics of bait delivery are of particular value. Data on field trials on the optimization of oral vaccination campaigns will be of interest. New molecular studies of rabies virus variants circulating in wildlife will complete the picture.

**Bat rabies an emerging zoonosis**

Bat variants of rabies virus are of increasing public health importance worldwide. Intriguingly, bats are reservoir and vector for six out of the seven genotypes characterized so far. Four recent isolates of Asian bat lyssaviruses are proposed as new genotypes and new isolates from bats are expected. This session, which follows OIE and the WHO recommendations for future research considerations on bat rabies, is aiming to encompass aspects of bat lyssavirus infections and to contribute to increasing international attempts to unveil the risk of bat lyssaviruses for humans. Presentations on updating existing bat rabies data and original works on (i) passive and active surveillance of bat rabies, (ii) molecular epidemiology and antigenic profile of bat lyssavirus isolates throughout Eurasia, (iii) identification of spill-over infections and (iv) studies on the pathogenicity of those viruses for bats and other terrestrial animals are welcome.

**Human rabies prevention**

This topic serves as an interface between public health authorities, those in research and development, medical practitioners and other workers in the field. Relevant contributions will range from basic research through to applications, safety and recommendations. Key aspects are vaccines for human use, vaccine responses, immunization schedules for preventative vaccination and post-exposure prophylaxis as well as the use of hyperimmune gamma-globulin or immune serum in post-exposure prophylaxis (PEP) for both terrestrial and bat rabies. Studies on the use of monoclonal antibodies to replace hyperimmune sera for PEP and recent advances in therapy will be discussed. Also, ways to improve collaboration between public health and veterinary authorities with respect to human rabies prevention should be discussed.

**Advances in technologies, diagnosis and vaccines**

Basic and technologic research should ideally find application into field problems. This pre-eminent topic is intended for those interested in the most recent developments in rabies prevention and therapy, using vaccines, antivirals as well as any basic study aiming at improving the related knowledge. Diagnosis improvement will be discussed, principally the recent advances on detection, identification and precise quantification of lyssavirus isolates and load in infected organs using molecular or other techniques. Furthermore, standardization and harmonization of diagnostic procedures and quality assurance (ring tests) will be addressed.
Organisation of the Conference

Steering Committee
G. Brückner (OIE)
F. Cliquet (AFSSA, France)
A.R. Fooks (VLA, United Kingdom)
M. Lombard (IABs, Switzerland)
F.-X. Meslin (WHO)
T. Müller (FLI, Germany)
N. Tordo (Institut Pasteur, France)
J.P. Vermeersch (European Commission)

Scientific Committee
A.D. Botvinkin (ISMV, Russia)
H. Bourhy (Institut Pasteur, France)
D. Briggs (VMC, USA)
F. Cliquet (AFSSA, France)
B. Dodet (IABs, Switzerland)
S. Edwards (VLA, United Kingdom)
A.R. Fooks (VLA, United Kingdom)
T. Hemachuda (CU, Thailand)
I. Kuzmin (CDC, USA)
O. Matouch (NRLR, Czech Republic)
M.E.G. Miranda (VPH, Philippines)
T. Müller (FLI, Germany)
Y. Rotivel (Institut Pasteur, France)
A. Seimenis (WHO)
N. Tordo (Institut Pasteur, France)
B.A. Yakobson (KVI, Israel)

Conference Organisation
A. Balmont (OIE)
F. Diaz (OIE)

General information

Presentations
- Invited keynote speakers will provide a summary overview of each main topic.
- Abstracts were selected for oral presentations.
- Selected posters will be displayed throughout the conference.
- All the abstracts selected for oral presentations, the best posters, conclusions and recommendations
  will be published in the Proceedings of the Conference.

Venue
The Conference will be held at the OIE Headquarters.

World Organisation for Animal Health (OIE)
12 rue de Prony
75017 Paris, France
Tel.: 33 (0) 1 44 15 18 88
Fax: 33 (0) 1 42 67 09 87

Language
The Conference will be conducted in English, simultaneous translation into Russian and French will be provided.
Programme

Sunday, 27 May 2007

OIE Headquarters, Salle Vittoz

16h00 - Registration

18h00 - Opening Ceremony

Chairperson: G. Brückner (OIE, France)

Welcome
B. Vallat, Director General, OIE
F.-X. Meslin, Coordinator Zoonoses and VPH, WHO
J.-P. Vermeersch, European Commission

Keynote address: Towards the elimination of rabies in Eurasia: Overview and perspectives
J. Blancou

19h00 - OIE Welcome cocktail

Monday, 28 May 2007

OIE Headquarters, Salle Vittoz

09h00 - Session 1 / Epidemiological information. Rabies in Eurasia: Regional reports
Chairperson: V. Caporale (IZS, Italy) - Rapporteur: B. Dodet (IABs, Switzerland)

09h00 Rabies situation in Western Europe  A.I. Wandeler (CFIA/ACIA, Canada)
09h12 Rabies situation in Eastern Europe  O. Matouch (NRLR, Czech Rep.)
09h24 Rabies situation in Central Asia  K.N.Gruzdev (FGI-ARRIAH, Russia)
09h36 Rabies situation in the Middle East  A. Seimenis (WHO)
09h48 Rabies situation in Far East Asia  Z.F. Fu (UG, USA)
10h00 Discussion
10h30 Coffee break

11h00 - Session 2 / Rabies pathogenesis
Chairperson: A.C. Jackson (KGH, Canada) - Rapporteur: M. Lafon (IP, France)

11h00 Cytokine expression in the CNS and periphery during infection with rabies  N. Johnson (VLA, UK)
11h10 Neuroimaging, virus and cytokine studies in rabies infected dogs  T. Hemachudha (CU, Thailand)
11h20 Role of Calcitonin Gene-Related Peptide (CGRP) in the pathogenesis of rabies  E. Weihe (UM, Germany)
11h30  Pathogenic and attenuated rabies viruses induces differential host protein expression in the central nervous system: Implication of neuronal dysfunction
Z.F. Fu (UG, USA)

11h40  Dominance of a non-pathogenic over a pathogenic G protein gene in defining the pathogenicity of a rabies virus
B. Dietzschold (TJU, USA)

11h50  Discussion

12h00  Lunch break

13h30 - Session 3 / Rabies prevention and control strategies

13h30  Keynote speech: Can rabies be eradicated?
C. Rupprecht (CDC, USA)

14h00 - Rabies control in dogs
Chairperson: F. Cliquet (AFSSA, France) - Rapporteur: B.A. Yakobson (KVI, Israël)

14h00  Molecular epidemiology of lyssaviruses in Eurasia
L.M. McElhinney (VLA, UK)

14h10  Identification of novel canine rabies virus clades in the middle east and north africa
D. David, (KVI, Israël)

14h20  Rabies in South Asia - Epidemiological investigations
C.K. Singh (RRDL, India)

14h30  Stray dogs in Bangkok, Thailand: rabies virus infection and rabies antibody prevalence
S. Kasempimolporn (QSMI, Thailand)

14h40  Preference for commercially produced oral rabies baits by feral dogs
D. Bergman (USDA, USA)

14h50  Assessment of the efficacy of oral vaccination of shepherd dogs in supplement of oral rabies vaccination of wild canids in israel
B.A. Yakobson (KVI, Israël)

15h00  Longterm immunization of recombinant rabies-canine adenovirus type-2 in dogs after intranasal or oral vaccination
R. Hu (AMMS, China)

15h10  New steps in the control of canine rabies in India
H.K. Pradhan (IVRI, India)
F.-X. Meslin (WHO)

15h20  Rabies in Mexico, 1990-2005
C.H. Álvarez Lucas (SM, Mexico)

15h30  Risk of rabies introduction by non-commercial movement of pets
P. Have (EFSA)

15h40  Discussion

16h00  Coffee break

16h30 - Rabies control in wildlife
Chairperson: A.D. Botvinkin (ISMV, Russia) - Rapporteur: I. Kuzmin (CDC, USA)

16h30  Epidemiology of rabies in South East Europe
N. Johnson (VLA, UK)

16h40  Rabies in Mongolian steppes
A.D. Botvinkin (ISMV, Russia)

16h50  Epidemiology and control of rabies in Iran
A.R. Janani (PI, Iran)
17h00 Rabies epidemiology in Raccoon Dogs and Foxes
A. Singer (CSL, UK)

17h10 Downside risk of wildlife translocation
R. Chipman (USDA, USA)

17h20 Sylvatic rabies in Russia with special attention to perspectives of oral vaccination of wild carnivores
A.D. Botvinkin (ISMV, Russia)

17h30 Finnish-Russian collaboration programme on rabies control in wildlife: the outcome of five years and the future prospects
A. Metlin (FGI ARRRAIH, Russia)

17h40 Rabies eradication programme in Estonia by means of oral vaccination of wildlife: first results
M. Laine (FB, Estonia)

17h50 Rabies surveillance in Poland between 1992 – 2006
M. Smreczak (NVRI, Poland)

18h00 Efficacy of a square presentation of V-RG vaccine baits in red fox, domestic dog and raccoon dog
F. Cliquet (AFSSA, France)

18h10 Scenario-analysis evaluating emergency strategies after rabies re-introduction
H.-H. Thulke (HCER, Germany)

18h20 The financial challenge to keep a large region free of rabies – the EU example
T. Müller (FLI, Germany)

18h30 What is the future of rabies control in Europe?
G. Smith (CSL, K)

18h40 The strength of 70% - Revising a given threshold of rabies control
H.-H. Thulke (HCER, Germany)

18h50 Discussion

09h00 - Rabies in bats: an emerging zoonosis
Chairperson: A.R. Fooks (VLA, UK) - Rapporteur: T. Müller (FLI, Germany)

09h00 A random grid based molecular epidemiological study on EBLV isolates from Germany
C. Freuling (FRIAHI, Germany)

09h10 Asymptomatic rhabdovirus infection in meridional serotine bats (Eptesicus isabellinus) from Spain
J.E. Echevarría Mayo (ISC, Spain)

09h20 Public health hazard of European bat lyssavirus, the Netherlands
W.H.M. Van der Poel (WUR, Netherlands)

09h30 Serological investigation of bats infected with rabies virus in China
C. Tu (AMMS, China)

09h40 Epidemiology and pathogenicity of African bat lyssaviruses
L.H. Nel (UP, South Africa)

09h50 Experimental infection of big brown bats (Eptesicus fuscus) with West Caucasian bat virus
I. Kuzmin (CDC, USA)

10h00 Susceptibility of foxes (Vulpes vulpes) to European bat lyssaviruses types-1 and -2
E. Picard, (AFSSA, France)

10h10 Identification of british bat species using sequence comparison and its application to european bat lyssavirus diagnosis and surveillance
S.L. Harris (UB, UK)

10h20 Discussion

10h30 Coffee break
<table>
<thead>
<tr>
<th>Time</th>
<th>Session Title</th>
<th>Chairperson</th>
<th>Rapporteur</th>
<th>Speaker</th>
</tr>
</thead>
<tbody>
<tr>
<td>11h00</td>
<td>Rabies prevention in humans</td>
<td>F.-X. Meslin (WHO)</td>
<td>Y. Rotivel (IP, France)</td>
<td>R. Willoughby (CHW, USA)</td>
</tr>
<tr>
<td>11h00</td>
<td>Human rabies includes early generalized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>vasospasm of cranial arteries that responds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>to tetrahydrobiopterin, l-arginine or nitroprusside</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11h10</td>
<td>Therapy of human rabies: lessons from experimental</td>
<td></td>
<td>A.C. Jackson (KGH, Canada)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>studies in a mouse model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11h20</td>
<td>Rabies control and prevention in the republic of</td>
<td></td>
<td>P. Imnadze (NCCD, Georgia)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Georgia: current status and perspectives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11h30</td>
<td>A simplified economical 4-site intradermal</td>
<td></td>
<td>M. Warrell (UO, UK)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-exposure rabies vaccine regimen is as</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>immunogenic as the current intramuscular and</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>intradermal methods</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11h40</td>
<td>Single dose for rabies pre-immunization</td>
<td></td>
<td>P. Khawplod (QSMI, Thailand)</td>
<td></td>
</tr>
<tr>
<td>11h50</td>
<td>Immune memory after remote pre- and</td>
<td></td>
<td>H. Wilde (CU, Thailand)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>post-exposure rabies vaccination: a prospective</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>study in thailand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h00</td>
<td>Epidemiology and prophylaxis of rabies in humans</td>
<td></td>
<td>Y. Rotivel (IP, France)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>in France. Evaluation and perspectives of a twenty</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>five year surveillance program</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h10</td>
<td>Discussion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12h30</td>
<td>Lunch break</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14h00</td>
<td>Session 4 / Advances in technologies, diagnosis</td>
<td>N. Tordo (IP, France)</td>
<td>T. Hemachuda (CU, Thailand)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>and vaccines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14h00</td>
<td>Keynote speech: Immune evasion, a critical</td>
<td></td>
<td>M. Lafon (IP, France)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>strategy for rabies virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14h30</td>
<td>BHK-21 cell culture rabies vaccine- a candidate</td>
<td></td>
<td>D. Lalosevic (FMNS, Serbia)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vaccine for Humans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14h40</td>
<td>Factors influencing the antibody response to</td>
<td></td>
<td>V. Jakel (JLU, Germany)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rabies vaccination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14h50</td>
<td>Attaining raccoon rabies management goals:</td>
<td></td>
<td>D. Slate (USDA, USA)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>history and challenges</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15h00</td>
<td>Evaluation of the stability of rabies vaccine baits</td>
<td></td>
<td>P. Maciulskis (LVA, Lithuania)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>field trial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15h10</td>
<td>Immunogenicity of ERA G 333 strain in foxes and</td>
<td></td>
<td>D. Bankovskiy (ARRI, Russia)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>raccoon dogs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15h20</td>
<td>Canine adenovirus-based vaccines against raccoons</td>
<td></td>
<td>N. Tordo (IP, France)</td>
<td></td>
</tr>
<tr>
<td>15h30</td>
<td>Coffee break</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16h00</td>
<td>Development of an edible rabies vaccine in maize,</td>
<td></td>
<td>E. Loza-Rubio (NCVM, Mexico)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>using vnukovko strain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16h10</td>
<td>Genetically engineered single-chain antibody</td>
<td></td>
<td>M. Mousli (IP, Tunisia)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>fusion proteins for detection of rabies virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>antigen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
16h20 Use of rabies virus as a transneuronal tracer of neuronal connections: Implications for the understanding of rabies pathogenesis  
G. Ugolini (CNRS, France)

16h30 Inhibition of rabies virus replication by Micro-RNA  
N. Israsena (CUHR, Thailand)

16h40 Discussion

17h00 Keynote speech: Rabies at the dawn of the 21st Century  
H. Koprowski (TJU, USA)

Wednesday, 30 May 2007

09h00 - Session 5 / General conclusions and recommendations  
Chairperson: N. Belev (OIE) - Rapporteur: T. Müller (FLI, Germany) and A.R. Fooks (VLA, UK)

10h00 Coffee break

10h30 Report of the questionnaire on stray dog control  
S. Kahn (OIE, France)

10h40 Keynote speech: What have we achieved? The way forward  
D. Briggs (VMC, USA)

11h15 Announcement and video of the next conference

11h30 Closing of the conference

13h00 Press point
TOWARDS THE ELIMINATION OF RABIES IN EURASIA: OVERVIEW AND PERSPECTIVES

J. Blancou
11, rue Descombes, 75 017 Paris, France

After a short overview of the present and past epidemiological situation regarding animal and human rabies in Eurasia, the general characteristics of the disease are described in each vector.

Three main rabies cycles are presently established in Eurasia: in dogs, in wild carnivores and in insectivorous bats. Because of the strong barrier that exists between species-adapted rabies viruses and various potential hosts, these cycles are quite independent. They are perceived in many countries in Eurasia not to have a significant impact on animal health or the rural economy in general: losses of dog (or cattle) are therefore also not priority animal health issue at the national level; wildlife rabies have been almost eliminated from western Eurasia by oral vaccination campaigns; and bats do not represent a real threat for a well informed public.

Rabies is thus essentially a public health issue. Human rabies of canine origin has continued unabated since centuries in eastern Eurasia, despite the Pasteur treatment and subsequent improvements of rabies post-exposure prophylaxis, modalities and biological products. This as dog rabies, which is the main source of human contamination, remains practically uncontrolled. The Ministries of Health of all infected countries of Eurasia, should focus their attention on canine rabies first as it incurs endless expenses treating humans that have been exposed to dog bites.

These Ministries should use the competences of Veterinary Services and all the other national bodies involved in dog rabies control, and contribute all necessary resources to support them to control this reservoir. This goal seems achievable in less than five years in a country, provided that cost-shared, well-planed and mass dog oral vaccination campaigns are organised and coordinated at regional and international levels. The conditions for the success of such campaigns are presented.
AREAS SELECTED FOR THE REGIONAL REPORTS:
THE RABIES SITUATION IN WESTERN EUROPE

A.I. Wandeler
Canadian Food Inspection Agency, Ottawa Laboratory Fallowfield, Ottawa, Canada

The analysis of the present rabies situation in Western Europe is based on completed questionnaires from 16 countries. The questionnaire is focusing on the years 2005 and 2006. Additional information was obtained from the “Rabies Bulletin Europe”, and from OIE’s publication on “Historical perspective of Rabies in Europe and the Mediterranean Basin”. Rabies in domestic animals and wild carnivores has become very rare in Western Europe. Only Germany reports a fair number of rabid foxes for 2005. Bat rabies was observed in 4 countries. One imported human case was seen in the UK, while Germany reported 4 cases in transplant recipients. Wildlife vaccination campaigns were conducted by Austria, Germany and France. Reporting of human postexposure prophylaxis is inconsistent due the lack of information. Similarly, data on domestic animal rabies prophylaxis seem to be difficult to collect.
THE RABIES SITUATION IN EASTERN EUROPE

O. Matouch
State Veterinary Institute, National Reference Laboratory for Rabies, Liberec 30, Czech Republic

An overview of the rabies situation in the 19 countries of Central and Eastern Europe covering more than 2.5 million km² is presented. A total of 6,500 cases of rabies were reported in this region in 2005, 31% in domestic animals, and 69% in wild animals. Rabies was found in a limited number of dogs in 10 countries, and sporadically in 7 bats in 3 countries. The situation for the year 2006 was similar. In general, rabies cases in humans are very rare; however, two human rabies cases were reported in Byelorussia in 2006. Around 40,000 people receive post-exposure prophylaxis annually due to exposure with rabies suspect animals. The red fox (Vulpes vulpes) is the main vector and reservoir of rabies in Eastern Europe representing 50% of all rabies cases. The raccoon dog (Nyctereutes procyonoides) is another significant vector, mainly in the Baltics. Oral vaccination is used in varying degrees in 10 countries with promising results. Two countries are currently rabies free, and several others are close. An unfavorable situation remains mainly in the Baltics and nearby eastern countries, and also in some Balkan regions. Rabies as a notifiable disease in all countries studied. Those countries have implemented appropriate national legislation on rabies control as well as national reference laboratories.
THE RABIES SITUATION IN CENTRAL ASIA

K.N. Gruzdev
FGI “Federal Centre for Animal Health” (FGI “ARRIAH”) Vladimir, Russia

Rabies is the most important zoonosis in Central Asian countries. In the years 2005 and 2006, a total of 615 animal rabies cases were reported of which 78%, 17% and 5% were found in dogs and cats, domestic animals and wildlife, respectively. Foxes accounted for 78% of all wildlife rabies cases. For the reporting period about 31 cases of human rabies were confirmed by laboratory testing. Eighty four and 16% of the cases dogs were due to contact with dogs and wildlife, respectively.

Rabies is a notifiable disease in all central Asian countries and national legislations on animal rabies control and human rabies prevention are in place. Rabies data collation and reporting is established on a monthly and annual basis. Based on a rough estimate between 30 and 50% of the dog population are considered owned dogs, whereas 10-20% and 30-50% of dogs either having owners but run wild or are stray dogs. There is no information on the real stray dog population density and on stray dog control measures available. The issue of rabies control in wildlife is not considered yet.

Rabies poses a serious problem for public and animal health despite existing national rabies control programs suggesting an urgent need for improvement. To make rabies control more effective in Central Asian countries it is necessary to adapt the national programs accordingly and to increase the overall vaccination coverage in dogs and cats. To tackle the rabies problem in wildlife oral vaccination of foxes might be a promising option. The worldwide experience of oral rabies vaccination, however, needs to result in an adequate vaccination strategy under the prevailing ecological and climatic conditions.
THE RABIES SITUATION IN THE MIDDLE EAST

A. Seimenis
Director, Mediterranean Zoonoses Control Centre of the World Health Organization, 24, Stournari str., 10682 Athens, Greece

Rabies is a public health problem of significant importance in the majority of southern and eastern Mediterranean and Middle East countries. In some of them the number of deaths keeps on a considerable rate. The dog is the main source of human infection while cats constitute the second most important group of domestic animals followed by cattle, sheep, goat, camels, donkeys as well as wild animals.

The number of reported human cases in these regions range from 800 to 1000 annually. Moreover, tens of thousands people need post-exposure prophylaxis in each country every year. Laboratory confirmation of rabies cases is not always performed. In most countries there is only one central laboratory for rabies diagnosis with trained staff while the diagnosis capability of district laboratories is still relatively poor and insufficient.

Animal rabies control consists mainly on sporadic vaccination of dogs and cats, elimination of stray animals, health education of the public, etc. Unfortunately, mass vaccination of dogs is not implemented yet, because the effective vaccination coverage rate is not exactly known. Culling of stray dogs and other animals using both shooting and poisoning is still in place in certain countries, however, with either minimal or no effect on rabies transmission.

Certain countries of the Middle East region, e.g. Saudi Arabia, Oman, Yemen, Israel, Iran and Turkey, are facing new problems due to emerging wildlife rabies. Red foxes (vulpes vulpes) and golden jackals (canis aureus) might play an important role.

Coordinated actions should be undertaken by affected countries with the assistance of International Organizations, to confront the serious rabies public health and economic problems under suitable conditions according to the country.
THE RABIES SITUATION IN FAR EAST ASIA

Z.F. Fu
Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

Aims/Objectives: This study was to evaluate human rabies epidemiology in Far East Asia.

Materials and Methods: Questionnaires were sent by OIE to relevant countries and recent publications were reviewed to gather information as to rabies epidemiology in these countries.

Results / Discussion / Conclusions: Most of the human rabies in the world occurs in Far East Asia and more than 28,000 deaths has been reported each year, which represents more than 50% of all human rabies cases around the globe. There are only a few countries or regions from which no human rabies has been reported such as Japan, Singapore, South Korea, Hong Kong, and Taiwan. In many of these rabies endemic countries, the number of human rabies cases has not changed much during the past decade. The only country that has seen steady decline is Thailand and the number of cases has decreased from around 200 to about 20 cases per year. The most dramatic changes are observed in China. Human rabies cases decline from around 5,000 cases per year in the 1980s to about 160 in the middle of 1990s. However, these trends were reversed since then. A steady increase has been reported during the past 10 years with more than 3,200 cases reported in 2006. Although there are many factors that contribute to the epidemic or endemic nature of rabies in these countries, the single most important factor is the failure to immunize domestic dogs that transmit rabies to humans. However, dog vaccination is at or below 5%, which will not be able to stop the transmission from dogs to dogs, thus to humans. It is thus most important for these countries to initiate mass vaccination campaigns in dog populations in order to stop the occurrence of human rabies.
Aims/Objectives: Rabies virus causes severe encephalitis that is invariably fatal for the victim. However, the contribution of the virus and the host to the damage of the Central Nervous System (CNS) is unclear. In order to investigate this we have studied the cytokine expression patterns within a murine model of rabies using a street isolate derived from a human case of disease.

Materials and Methods: In the murine model, infection of the CNS progresses rapidly following inoculation in the periphery, and virus replication can be detected in the spinal cord, brain and salivary gland at 11 days post-infection (p.i.). Rabies virus (isolate RV61 and Challenge Virus Standard) were detected by end-point PCR and immunohistochemistry. Murine transcripts were detected by real-time reverse transcriptase PCR.

Results / Discussion / Conclusions: By the time severe clinical symptoms have appeared at day 11 p.i., there is a large magnitude up-regulation in the brain of a range of inflammatory cytokines including IL-6 and TNF-alpha, along with an up-regulation of TLR-3 and 2'-5' OAS1. Transcription of certain chemokines (CCL5 and CXCL10) with chemotactic properties for T cells was also observed to increase. Furthermore there is an increase in the transcription of type-2 interferon (gamma) during the late stages of infection. However, this increase is not matched by the development of T cell infiltration into the brain and subsequent anti-viral responses that might prove deleterious to the host. Instead it seems that it is the actions of the virus that cause the loss of nervous function. This is in contrast to the observations made with a fixed strain of rabies virus, which shows distinct inflammatory changes within the CNS.
NEUROIMAGING, VIRUS AND CYTOKINE STUDIES IN RABIES INFECTED DOGS

J. Laothamatas¹, S. Wacharapluesadee², B. Lumlertdacha³, S. Ampawong³, V. Tepsumethanon³, P. Phumesin², S. Asavaphatiboon¹, I. Worapruekjaru¹, Y. Avihingsanon², N. Israsena² & T. Hemachudha²

¹ Department of Radiology, Ramathibodi Hospital, Mahidol University
² Department of Medicine, Chulalongkorn University Hospital
³ Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand

Objective: Furious and paralytic rabies differ in terms of clinical manifestations and sites of anatomical involvement. We studied whether there were specific patterns of magnetic resonance imaging (MRI), cytokine and virus distribution in rabies infected dogs of both clinical types.

Methods: MRI of the brain and upper spinal cord were performed in 2 furious and 2 paralytic dogs during early stage. Rabies virus RNA and mRNA of 19 cytokines were determined at each of 12 brain regions of early (2 furious, 2 paralytic) and late (one each) stage rabid dogs.

Results: Two different MR imaging patterns were evident. Abnormal hypersignal T2 changes were found at hippocampus, hypothalamus, brainstem and spinal cord in 2 paralytic dogs. More widespread changes but of less intensity were noted in 2 furious dogs. During early stage, virus RNA was found mainly in the midline structures in all but one furious dog that had virus RNA in most regions. IL-1 beta (2/2) and interferon-gamma (2/2) mRNAs were found at most regions starting at the early stage only in paralytic dogs. During early stage of both clinical types, TNF-alpha (3/4), TGF-beta (4/4), IL-12 (3/4) and -18 mRNAs (4/4) were found in the midline structures.

Conclusions: Two distinct patterns of MRI abnormalities were found in furious and paralytic rabies. Rabies virus RNA was distributed mainly in the midline structures. This does not explain limbic symptoms. It remains to be determined whether cytokine responses at particular regions influence the development of clinical aggression and extent of virus distribution in the brain.
ROLE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN THE PATHOGENESIS OF RABIES

E. Weihe¹, M. Bette¹, M.A.R. Preuss¹,², M. Faber², M.K. Schäfer¹, M.J. Schnell² & B. Dietzschold²

¹ Inst. of Anatomy, Dept. of Mol. Neuroscience, Philipps Univ. Marburg, Germany
² Dept. of Microbiology & Immunology, Thomas Jefferson Univ., Philadelphia

Here we test whether rabies virus (RV) infection enhances CNS expression of CGRP and other immunomodulatory neuropeptides to escape immune responses. To investigate the effect of RV infection on cerebral CGRP in relation to microglial and inflammatory responses, we studied the effect of TNFa using recombinant RVs that contained either an active (SPBN-TNFa(+)) or an inactive (SPBN-TNFa(-)) TNFa gene.

While RV infection did not significantly affect the expression of most neuropeptides, it caused a strong upregulation of CGRP in non-infected cortical and hippocampal neurons. Infection with SPBN-TNFa(+) resulted in reduced upregulation of CGRP as compared to that seen in SPBN-TNFa(-)-infected brains. The induction of the invariant chain of MHC-II, the activation of microglia, and the infiltration of inflammatory cells was strongly enhanced in SPBN-TNFa(+) as compared to SPBN-TNFa(-)-infected brains. Mice infected with SPBN-TNFa(+) showed decreased virus spread compared to mice infected with SPBN-TNFa(-). While none of the SPBN-TNFa(+) infected mice died, 80% of mice infected with SPBN-TNFa(-) succumbed to the infection.

Given the known inhibitory effect of CGRP on antigen presentation, we conclude that CGRP induction in brain after RV infection represents a potential mechanism by which RV escapes immune detection. The protective activity of TNFa may be due to reduced upregulation of CGRP with concomitant enhancement of CNS inflammation. This in turn, might prevent the escape of RV from innate and adaptive immune responses. Thus, the use of CGRP antagonists to unleash the inhibition of antigen presentation during RV infection could represent a useful strategy in post-exposure prophylaxis of rabies.
PATHOGENIC AND ATTENUATED RABIES VIRUSES INDUCE DIFFERENTIAL HOST PROTEIN EXPRESSION IN THE CENTRAL NERVOUS SYSTEM: IMPLICATION OF NEURONAL DYSFUNCTION

Z. F. Fu
Department of Pathology, College of Veterinary Medicine, University of Georgia, Athens, GA 30602, USA

Aims/Objectives: This study was to understand how rabies virus (RV) infection results in neuronal dysfunction.

Materials and Methods: We employed proteomics technology to profile host responses to RV infection in the mouse model.

Results / Discussion / Conclusions: In mice infected with pathogenic RV, the expression of proteins involved in ion homeostasis was altered. H+ ATPase and Na+/K+ ATPase were up-regulated while Ca2+ ATPase was down-regulated, which resulted in reduction of the intracellular Na+ and Ca2+ concentrations. Furthermore, infection with pathogenic RV resulted in down-regulation of SNAREs such as -SNAP, syntaxin, and pallidin, all of which are involved in docking and fusion of synaptic vesicles to and with presynaptic membrane. As a consequence, accumulation of synaptic vesicles was observed in the presynaptic membrane in mice infected with pathogenic RV. On the other hand, attenuated RV did not induce changes in the expression of these proteins. Together, our data demonstrate that infection with pathogenic RV results in alteration of host protein expression, particularly those involved in ion homeostasis and docking and fusion of synaptic vesicles to presynaptic membrane, which may provide the structural and metabolic basis by which RV infection causes neuronal dysfunction.
DOMINANCE OF A NON-PATHOGENIC OVER A PATHOGENIC G PROTEIN GENE IN DEFINING THE PATHOGENICITY OF A RABIES VIRUS

B. Dietzschold
Department of Microbiology and Immunology, Thomas Jefferson University, Philadelphia, PA 19107, USA

The non-pathogenic phenotype of the rabies vaccine virus SPBNGAN is determined by a G protein designated as GAN that has an Arg → Glu exchange at position 333. We have recently shown that after several passages of SPBNGAN in newborn mice an Asn → Lys mutation occurred at position 194 of GAN resulting in GAK, which was associated with a reversion to the pathogenic phenotype. To investigate whether the presence of two GAN genes within the genome of the nonpathogenic double G RV variant SPBNGAN-GAN increases or decreases the probability for reversion to the pathogenic phenotype, double G RV variants were constructed that contain both, the non-pathogenic GAN and the pathogenic GAK gene (SPBNGAN-GAK or SPBNGAK-GAN). Additionally, we constructed an RV containing two pathogenic RV G genes (SPBNGAK-GAK). While SPBNGAK-GAK was pathogenic, SPBNGAN-GAK was completely non-pathogenic and the pathogenicity of SPBNGAK-GAN was strongly reduced. Furthermore, virus loads in SPBNGAN-GAK-and SPBNGAK-GAN-infected brains were significantly lower than those detected in SPBNGAK-GAK-infected brains, indicating that the non-pathogenic phenotype determined by GAN is dominant over the pathogenic phenotype associated with GAK. Virus production and viral RNA synthesis were markedly higher in SPBNGAN-, SPBNGAK-GAN- and SPBNGAN-GAK-infected NA cells than in SPBNGAK- and SPBNGAK-GAK-infected NA cells, suggesting that the dominance of GAN over GAK is controlled at the level of viral RNA synthesis. These data not only confirm the concept that the pathogenicity of an RV correlates inversely with its capacity to replicate in vitro but also support the conclusion that a recombinant RV carrying two identical GAN genes bears a lower risk of a reversion to the pathogenic phenotype than an RV carrying only a single GAN gene.
Rabies is the most significant viral zoonosis at large today. From public health, veterinary and economic standpoints, the disease requires renewed consideration in relationship to eradication. Given wildlife involvement on all major continents, and in particular the evolutionary and ecological role of bats as reservoirs, rabies is not a candidate for true eradication at this time, from a classical definition. Nevertheless, the opportunity for shared scientific expertise, multi-disciplinary leadership, technology transfer, creative vision, and political willingness, offers the promise of significant outcome-oriented collaborations in an unprecedented fashion during the 21st century. Laboratory-based surveillance, combined with active health education and enhanced public awareness, and the strategic utilization of potent inexpensive vaccines, are basic requirements for effective rabies prevention and control. Human rabies can be eradicated by eliminating exposures, expanding primary vaccination for those at risk, and proper, timely application of modern postexposure prophylaxis. Canine rabies transmission can be eliminated, as documented in developed and developing countries, especially as exemplified throughout Latin America. Wildlife rabies can be controlled significantly, and among certain carnivore populations suppressed, as demonstrated by regional elimination over major portions of Europe and North America. Rather than concentrate random initiatives concomitantly on a worldwide basis, coordinated steps should encompass selection of areas or regions with the ideal biological, social, and political underpinnings to maximize the greatest probability for success as exemplary showcases for gradual expansion. Ultimately, major financial commitment and serious long term partnering with interested member countries and organizations are perquisites for appreciable recognition of global rabies eradication goals.
MOLECULAR EPIDEMIOLOGY OF LYSSAVIRUSES IN EURASIA

L.M. McElhinney¹, D. Marston², S. Stankov², C. Tu³, C. Black¹, N. Johnson¹, Y. Jiang³, N. Tordo⁴, T. Müller⁵ & A.R. Fooks¹

¹ Veterinary Laboratories Agency (Weybridge), Surrey, United Kingdom
² Pasteur Institute Novi Sad, Yugoslavia
³ Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China
⁴ Unité de la Rage, Institut Pasteur, Paris, France
⁵ Federal Research Centre for Virus Diseases of Animals, D16868 Wusterhausen, Germany

Advances in molecular epidemiological methods over the past 15 years have resulted in an increase in genetic data on lyssaviruses circulating in a range of host species worldwide. The molecular diversity of classical rabies viruses (Genotype 1, RABV) has been studied at the global level and reference has been made to the existence of a number of European strains in a range of mammalian species. They are believed to be clustered within a ‘Cosmopolitan Lineage’ having ancestral roots in Europe in the 17th century before its widespread dispersal to Asia, Africa and the Americas as a result of European exploration and colonization. To further investigate the existence of such a lineage in Eastern Europe and Asia, a molecular epidemiological review of new and existing data was undertaken for Eurasian RABV isolates. A phylogenetic study has been performed on a unique panel of viruses from the Former Republic of Yugoslavia, isolated from a wide range of hosts over a 30 year period. A number of divergent strains have been identified in this region. In contrast, when we performed targeted surveillance of stray dogs in the poor socioeconomic areas of three Chinese provinces (Sichuan, Hunan and Guangdong) and within Tianjin City, very little genetic diversity was identified. In addition, we will report on a targeted surveillance programme of insectivorous bats in the Guangdong, Guandxi, Yunan and Hainan provinces of China, provoked by the emergence of the new bat lyssaviruses (Aravan, Irkut, Khujand and West Caucasian Bat Virus) in Eurasia.
IDENTIFICATION OF NOVEL CANINE RABIES VIRUS CLADES IN THE MIDDLE EAST AND NORTH AFRICA

D. David¹, G.J. Hughes², B.A. Yakobson³, I. Davidson¹, H. Un³, O. Aylan³, I.V. Kuzmin⁴ & C.E. Rupprecht⁴

¹ Kimron Veterinary Institute, Bet Dagan, 50250, Israel
² The University of Edinburgh, Edinburgh, EH9 1QH, UK
³ Etilk Central Veterinary Controls and Research Institute, Etilk, Ankara, Turkey
⁴ Centers for Disease Control and Prevention, Atlanta, GA, 30333, USA

Rabies is endemic in the Middle East, however so far only Israel has implemented an oral rabies vaccination (ORV) control program. Molecular analysis of a 328 bp long fragment of the rabies virus (RABV) nucleoprotein (N) gene was used to identify variants of rabies viruses obtained in a previous wildlife control program. We identified rabies virus isolates from years 1993-1998 into 4 clades (I-IV) occurring in 4 geographical regions of Israel.

Objectives: To conduct ORV and rabies surveillance along Israeli borders.

Materials and Methods: We analysed 90 Israeli and 28 RABV isolated in border regions from neighbouring countries; seven from the Jordan border region, three from Lebanon border region, and 18 from Syrian border region. We also analysed three RABV isolates from Egypt, six from Jordan and seven from Turkey.

Results: An antigenic and phylogenetic analysis of the Middle Eastern RABV isolates was conducted. Also, the chronological evolution of the RABV variants was estimated using molecular clock. The phylogenetic analysis of the rabies virus sequences revealed four new genomic clades, with clades V, VI and VII occurring in the Middle East and clade Africa 4 circulating in North Africa. The molecular clock application indicated that the current diversity of canine RABV variants from the Middle East emerged at about the same time (~1870) as in Europe, following their divergence from established lineages in Africa and Asia.

Discussions and Conclusions: There is evidence that the RABV circulating in the Middle East belong to novel canine clades V, VI, VII, which appeared on the borders and invaded Israel from Jordan and Syria. A strict surveillance is required along borders of countries where ORV control program is performed to avoid further spread of these RABV variants.
RABIES IN SOUTH ASIA - EPIDEMIOLOGICAL INVESTIGATION

C.K. Singh¹ & B.S. Sandhu²
¹ Veterinary Pathologist
² Assistant Professor, Department of Veterinary Pathology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India

A five year study was conducted in northern India to investigate epidemiological aspects of rabies in different animal species. Brain tissues were collected from a total of 384 rabies suspect animals including 231 dogs, 79 buffaloes, 46 cows and 28 wild species. Out of the 384 suspected cases, 55.98% were positive for rabies as shown by direct fluorescent antibody test (FAT). Among the various species of animals, the maximum rabies cases were found in dogs (59.53 %) followed by buffaloes (20.46%), cows (13.48%) and wild animals (6.51%). Increased incidence was observed during January to April and during August to November. Analysis of the age and sex of different species revealed that adult male dogs (39%) and adult female bovines were more affected by rabies.

Concerning the major clinical presentations 30% of the rabid animals demonstrated the paralytic form of rabies whereas 61 % of the rabid dogs showed the furious form of rabies with aggressiveness as the predominant clinical symptom. There was proven evidence that the majority of rabid dogs (61%) had bitten humans or other animals. In buffaloes and cows, anorexia, salivation and bawling were the main clinical symptoms. Histopathological demonstration of Negri bodies was less sensitive than FAT in detecting rabies in both domestic and wild animals. In 27% and 24% of the cases owners were able to identify rabies related clinical symptoms in buffaloes and cows, respectively. In contrast, 47% of dog rabies cases were discerned by dog owners due to obvious clinical signs. The accumulated history records revealed that 78% of owned rabid dogs were not prophylactically vaccinated against rabies.
STRAY DOGS IN BANGKOK, THAILAND: RABIES VIRUS INFECTION AND RABIES ANTIBODY PREVALENCE

S. Kasempimolporn¹, B. Sichanasai², W. Saengseesom¹, S. Puempumpanich¹, S. Chatraporn² & V. Sitprija¹

¹ Queen Saovabha Memorial Institute (WHO Collaborating Center for Research on Rabies), Thai Red Cross Society, Bangkok, Thailand
² Veterinary Public Health Division, Health Department, Bangkok Metropolitan Administration, Bangkok, Thailand

In Thailand, rabies incidence is highest in stray dogs. Dog bite-related rabies cases account for 70% to 95% of the reported human rabies deaths in Thailand. The incidence of dog bites is highest in the central part of the country, e.g. the Bangkok metropolis where an estimated 900,000 dogs live. Approximately 17% of the estimated dog population is considered ownerless or stray dogs. In a previous epidemiologic survey it could be shown that no less than 5 canine rabies virus variants are circulating among dogs of the Bangkok metropolis. According to WHO recommendations, at least 70% of the dog population has to be continuously vaccinated to control rabies effectively. However, little is known about the vaccination status in stray dogs. The objective of our study was to investigate the rabies incidence and rabies antibody prevalences among stray dogs in Bangkok. Both saliva and serum samples from a total of 3,314 stray dogs captured between 12/2003 and 6/2004 were collected and subsequently tested for the presence of rabies virus and rabies specific antibodies, respectively. Rabies virus antigen could be detected in a one 2-year-old female dog by latex agglutination test and was also confirmed by reverse transcription-polymerase chain reaction. The overall antibody seroprevalence as detected by enzyme-linked immunosorbent assay was 62% (95% CI: 54, 70%). Antibody seroprevalence was higher in dogs captured within central Bangkok (86% of 1208 dogs captured) than in dogs captured in the outskirts of the greater metropolitan area (49% of 2106 dogs captured). Provided the sample size of stray dogs is representative, the results obtained would indicate that the seroprevalence achieved from previous vaccination campaigns is too low to protect both the dog and human population.
PREFERENCE FOR COMMERCIAL PRODUCED ORAL RABIES BAITS BY FERAL DOGS

D. Bergman1, S. Bender2, D. Slate3, C.E. Rupprecht4, K. Wenning1, C. Heuser1, S. Joe1 & T. Deliberto5

1 USDA/APHIS/Wildlife Services, Phoenix, Arizona, USA 
2 Navajo Nation Veterinary Program, Chinle, Arizona, USA 
3 USDA/APHIS/Wildlife Services, Concord, New Hampshire, USA 
4 Centers for Disease Control and Prevention, Atlanta, Georgia, USA 
5 USDA/APHIS/Wildlife Services, Ft. Collins, Colorado, USA

Domestic dogs are the leading vector for rabies throughout the world. On United States Indian Reservations rabies vaccinations for dogs may be as low as 5 to 20%. At the aforementioned rates, it would be nearly impossible to break the rabies cycle through vaccination of dogs. The US Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services and the Centers for Disease Control and Prevention were invited by the Navajo and Hopi Nations to determine the acceptance of commercially available rabies baits by tribal dogs. During 2003 and 2004, we have offered Artemis Ontario Slims, Merial raccoon baits, Merial gray fox baits and Merial fish meal coated sachets. Baits were hand tossed to each individual animal. We recorded whether the animal ignored the bait, swallowed the bait whole, chewed and discarded the bait without puncturing the placebo packet, chewed and punctured the placebo packet and discarded the bait, and chewed and punctured the placebo packet and swallowed the contents. Preference among the baits from least favorable to most favorable were Artemis Ontario Slims < Merial raccoon baits < Merial gray fox baits < Merial coated sachets. For 2003 and 2004, we had an acceptance rate of 78% for coated sachets. During 2005 and 2006, we used the Merial fish meal coated sachets with Raboral V-RG in Chinle and Window Rock, respectively. Acceptance by feral dogs was 85% acceptance rate in 2005 and a 74% acceptance rate in 2006. Coated sachets are a strong candidate for ORV in dogs.
ASSSESSMENT OF THE EFFICACY OF ORAL VACCINATION OF SHEPHERD DOGS IN SUPPLEMENT OF ORAL RABIES VACCINATION OF WILD CANIDS IN ISRAEL

B.A. Yakobson¹, R. King², N. Sheihat¹ & D. David¹
¹ Kimron Veterinary Institute, Bet Dagan, Israel
² Nature & park Authority, Jerusalem, Israel

Since 1956, red foxes (Vulpes vulpes) and golden jackals (Canis aureus) have been the primary vectors maintaining wildlife rabies in Israel. Oral rabies vaccination of wild canids initiated in 1998 resulted in near-elimination of the disease in wildlife by 2005. During 2005 and 2006, an outbreak of rabies was observed in stray dogs in the vaccination area of the Golan Heights, with no cases in foxes or jackals. Epidemiological investigations showed that the infected dogs were from territories across the border. This was confirmed by molecular analysis, which showed that the rabies virus (RABV) was different from RABV isolates endemic to this area. The objective of this study was to determine bait acceptance and feasibility of oral rabies vaccination in semi-wild shepherd dogs. Coated sachets and fishmeal polymer baits of Raboral V-RG (Merial, USA) were tested in five different test zones. Both formats were hand-fed to individual dogs and to animals belonging to packs. Bait uptake and consumption were observed for each dog. Uptake of either bait format was >90%. Vaccine delivery problems were observed in dogs belonging to packs; dominant animals ate multiple baits and in competitive situations dogs gulped the bait on the whole. Because of the uncertainty associated with the oral vaccination of dogs other methods were needed to control this outbreak: stray dogs were removed and herd dogs were vaccinated parenterally. This study showed that oral rabies vaccination of dogs in packs using vaccine baits designed for wildlife would not be effective. Perhaps the search for different baits or for solutions to circumvent competition within the dog pack can help to make this approach feasible.
LONGTERM IMMUNIZATION OF RECOMBINANT RABIES-CANINE ADENOVIRUS TYPE-2 IN DOGS AFTER INTRanasal OR ORAL VACCINATION

S.F. Zhang¹, Y. Liu¹, A.R. Fooks², F. Zhang¹, R.L. Hu¹
¹ Laboratory of Epidemiology, Veterinary Institute, Academy of Military Medical Science, 1068 Qinglong Road, Changchun 130062, P.R.China
² Veterinary Laboratories Agency (Weybridge), Surrey, United Kingdom

Rabies cause about 55 000 human deaths worldwide each year, up to 98% of the cases were caused by exposure to rabid dogs. Prevention of dog rabies would be the most effective way to stop rabies transmission to humans. However, vaccinating stray dogs in urban and rural areas using conventional vaccines is always difficult and is not cost-effective for use in China. In this paper, we utilized a recombinant rabies vaccine, in which the canine adenovirus type-2 served as a live vector and the rabies virus glycoprotein as the protective antigen, performed a non-parenteral trial in dogs. This vaccine was orally or intranasally administrated once to 136 dogs in solution form (46) with 1×10⁸.⁵ PFU or in a specially designed bait (90) with 3×10⁸.⁵ PFU recombinant adenovirus. About 87.5% (119/136) of the immunized dogs developed neutralizing antibodies. The protective immune response against rabies in dogs was detectable at 2-3 weeks after administration, reaching a peak by 5-6 weeks. Among the seroconverted animals, 90.8% (108/119) elicited a neutralizing antibody response for over 24 months. The antibody titre during the two years was above 0.5IU/ml while showing a gradual but slow decline from 6 weeks after vaccination. In a challenge experiment of 10 dogs 2 years after the vaccination with 60,000 mouses LD50 of CVS-24, all of the dogs survived. It is hoped to further improve the bait design for commercial production. Nevertheless, the results demonstrated that the recombinant vaccine and its bait were useful for oral immunization of dogs.
NEW STEPS IN THE CONTROL OF CANINE RABIES IN INDIA

H.K. Pradhan¹, J.P. Gurbuxani², F. Cliquet³, B. Pattnaik¹, S.S. Patil¹, A. Regnault⁴, H. Begouen⁴, A.L. Guiot⁵, R. Sood¹, P. Mahi⁴, R. Singh¹, E. Picard³, M.F.A. Aubert⁶, J. Barrat³, F.-X. Meslin⁷

¹ HSADL-High Security Animal Disease Laboratory, Indian Veterinary Research Institute, Anand Nagar, Bhopal 462021, Madhya Pradesh, India
² Petswill, Kesar Complex, Malhar Road, Gurudev Nagar, Ludhiana, Punjab, India
³ AFSSA, National Research Laboratory on Rabies and Wildlife Diseases, WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory on Rabies, EU Reference Institute for Rabies Serology, Technopole Agricole et Vétérinaire, BP 40009, Malzéville, France
⁴ VIRBAC Laboratories, B.P. 27, 06511 Carros Cedex, France
⁵ Conseils en Pharmacie et Biologie, Santé, 69110 Sainte Foy les Lyon, France
⁶ AFSSA, Small Ruminants and Bee Research Laboratory, 105 route des Chappes, BP 111, F-06902 Sophia Antipolis Cedex, France
⁷ World Health Organization, Department of Communicable Diseases, 1211 Geneva, Switzerland

In India, about 20,000 people die of rabies every year. The dog is the main reservoir and vector of the disease. A pilot rabies control programme has been launched in 5 Indian federal states in February 2007. This initiative is led by the Animal Welfare Board of India (AWBI) federating many animal welfare organizations and the Ministry of Agriculture. It aims at creating a “Rabies Free India”.

The programme combines parenteral vaccination of accessible owned dogs, stray dog population, spaying/neutering followed by parenteral vaccination and oral vaccination of unaccessible dogs. The freeze-dried vaccine SAG2 including the bait casing has recently been registered in India following successful evaluation of vaccine-bait safety and efficacy (by survival after virulent challenge) in captive Indian stray dogs in the Bhopal high security laboratory. Furthermore, bait acceptance was tested under both experimental and field conditions. Results of the experimental studies will be presented, which finally led to registration and field use of SAG2 baits in India. Furthermore, the AWBI strategy for rabies control and elimination will be discussed.
Rabies in dogs was unknown in the Americas before the arrival of the Spanish ‘conquistadores’. Until the middle 1980s rabies in animals and, in turn, in humans, changed little from year to year, when the number of canine vaccinations reported annually rarely reached one million. In Mexico, the national rabies control program using parenteral mass vaccination of dogs started in 1990 with about 7 million dogs vaccinated in the same year. The number of vaccinated dogs exceeded 10 and 15 million in 1995 and 2005, respectively. Modern cell culture based inactivated rabies virus vaccines were used. A key factor for the success of the dog rabies control program was the supply of potent canine rabies vaccines. Between 1990 and 2005 more than 150 million vaccine doses from 300 lots were administered. Each lot was tested for potency prior to use in the field. The required minimum content of rabies virus antigen of vaccines was 2 I.U according to WHO. Testing revealed antigen contents ranging from 3.28 to 5.59 IU. As a result of the mass dog vaccination campaigns human rabies cases due to dog-mediated rabies decreased from 60 in 1990 to 0 in 2000. The number of rabies in dogs decreased from 3,049 cases in 1990 to 70 cases last year.
In response to a request of the Commission of the European Union (EU) the European Food Safety Agency (EFSA) has carried out a quantitative assessment of the risk of rabies introduction into the UK, Ireland, Sweden, and Malta as a possible result of movements of pets incubating rabies at the time of movement.

The risk that a pet is incubating rabies at the time of first vaccination is equal to the prevalence of rabies-incubating pets in the population of origin. Despite induction of immunity by vaccination, animals already incubating rabies will still develop clinical disease as a function of time after vaccination (termed type A risk). Waiting time reduces this risk.

A small percentage of animals may not be protected after single-shot primary vaccination and hence, may become infected after vaccination. The risk of becoming infected after the first vaccination (termed type B risk) depends on the prevalence and efficiency of vaccination. Serological testing can be used to identify non-immune pets and will therefore reduce this risk accordingly. Type A and B risk were modelled as a function of waiting time after vaccination and fitted to a non-linear model incorporating test specificity and vaccination efficiency.

Within the EU, the prevalence and hence the type A risk is so low, that for most countries the risk is negligible. However, for some countries the risk is non-negligible. In this case inclusion of a waiting time is necessary to reduce this risk. Here, serological testing is appropriate, because type B risk becomes relatively more important as type A risk is reduced with extended waiting times (over 100 days).
EPIDEMIOLOGY OF RABIES IN SOUTH EAST EUROPE

N. Johnson¹, C. Freuling², A. Vos³, H. Un⁴, R. Valtchovski⁵, M. Turcitu⁶, F. Dumitrescu⁶, V. Vlad⁶, R. Velić⁷, V. Sandrac⁸, O. Aylan⁴ & A.R. Fooks¹

¹ Veterinary Laboratories Agency (Weybridge), Surrey, United Kingdom
² Federal Research Centre for Virus Diseases of Animals, D-16868 Wusterhausen, Germany
³ Impfstoffwerk Dessau – Tornau GmbH, Rosslau, Germany
⁴ Etilik Central Veterinary Control and Research Institute, Etilik, Ankara, Turkey
⁵ Institute for Diagnosis and Animal Health, Doctor Staicovici Street no. 63, District 5 050557, Bucharest, Romania
⁶ National Diagnostic and Research Veterinary Institute, 15A P.Slaveikov Blvd, BG-1606, Sofia, Bulgaria
⁷ Department of infectious diseases, Veterinary faculty, University of Sarajevo, Sarajevo, Bosnia and Hercegovina
⁸ Veterinary Institute of the Republik of Srpska, Banjy Luka, Bosnia and Hercegovina

Aims/Objectives: Rabies remains endemic within a number of countries in southeast Europe including Romania, Bulgaria and Turkey. With the probable expansion of the European Union eastwards it is likely that rabies elimination programs will be increased to reduce the burden of this disease in new accession countries. A clear understanding of the epidemiology of the virus in this area of Europe is vital before such programs are introduced. With the exception of Turkey, the red fox (Vulpes vulpes) is the principal disease reservoir in this region. However, cases of rabies in the dog (Canis familiaris) are regularly reported. This study summarises the current rabies situation in southeast Europe and demonstrates the phylogenetic relationships between the viruses in a number of the countries within the region.

Materials and Methods: Rabies virus RNA was extracted from original samples and a fragment of the nucleoprotein gene amplified by reverse-transcriptase PCR. Automated sequencing was used to derive nucleoprotein gene sequences and these were used to prepare a molecular phylogeny of rabies viruses in southeast Europe.

Results / Discussion / Conclusions: In Bulgaria, the dog is the main vector bringing rabies into contact with humans and livestock. However, other species also act as reservoirs for the disease, complicating the development of elimination strategies. The fox is the principal reservoir species of rabies in Romania although cases in dogs are regularly reported. Despite a gradual decline in dog rabies, urban pockets of the disease remain in many regions of Turkey. Furthermore, there is some evidence that the fox has been a significant vector for rabies and may be responsible for increases in rabies in cattle in the Aegean region of the country. Throughout the region there is evidence for cross-border movement of rabies by both wildlife and canine vectors.
RABIES IN MONGOLIAN STEPPES

A.D. Botvinkin¹, D. Otgonbaatar², S. Tsoodol² & I.V. Kuzmin³

¹ Irkutsk State Medical University, Russia
² Center of Infectious Diseases with Natural Foci, Ulaanbaatar, Mongolia
³ Center for Diseases Control and Prevention, Atlanta, USA

The presence of rabies in Mongolia is associated with its characteristic landscape, e.g. the steppes and forest-steppes, known as the Mongolian steppes. Main reservoirs of rabies virus (RABV) are the red fox, corsac fox and wolf. Fox rabies has been reported in Mongolia since the early 1960s. Eleven human rabies cases were reported during 1994-2004, approximately one case per year resembling about 0.4 cases per one million inhabitants. Of the human rabies death 5 persons died after wolf bites, 2 people due to exposed to foxes, and 4 after dog bites. Children of cattle-breiders aged 4-7 years comprised about 50% of the human rabies cases. Biting attacks from herding dogs represent the main cause for post-exposure prophylaxis (PEP) in Mongolia with most people subjected to PEP in rural areas difficult to assess. During 1996-2004, a total of 1273 rabid animals were reported (about 140 cases per year). More than 80% of all reported cases occurred in cattle. The rabies epizootic is cyclic, with 10 to 12 year periods. In the years 2000 to 2002 a major rabies outbreak occurred in the north-western part of the country.

The Mongolian steppes extend to the Chita region of Russia, the autonomous Republics of Buryatia, Tyva and Altai. Four RABV isolates from the central part of Mongolia were sequenced and compared with available isolates from Russia, Kazakhstan and China. The isolates from Mongolia all belonged to the “steppe” phylogenetic clade, which includes viruses circulating in vast steppe territories ranging from south-eastern Europe to Tyva, West Siberia and Kazakhstan. In contrast, RABV isolates from Mongolian-type steppes in Chita region of Russia (East Siberia) belong to the eastern group of arctic-like viruses. Further research on characterisation of RABV from Mongolia is needed to confirm these findings.
EPIDEMIOLOGY AND CONTROL OF RABIES IN IRAN

A.R. Janani & A. Fayaz
W.H.O Collaborating Centre for Reference and Research on Rabies, Pasteur Institute of Iran

Rabies is endemic in Iran. It is the most important zoonotic disease in the country. Based on studies conducted during the past decades there is evidence that the main reservoir for rabies are wolves.

Rabies incidence in man and animals is increasing each year. In 2006, more than 130,000 people received post-exposure prophylaxis. Similar figures apply for preventive vaccination in animals.

Official data suggest that the majority of human exposures are due to biting episodes caused by dogs. However, our investigations showed that mainly wolves are the responsible animal for rabies transmission to humans. Because of the potential feasibility of oral rabies vaccination, we proposed a pilot study to evaluate the use of an oral rabies vaccine for vaccination of wildlife to control rabies in Iran.

Aims and objectives: To evaluate the possibility to control rabies in Iran using oral vaccination of wolves and to evaluate the immune response to oral rabies vaccine among wolves.

Materials and Methods: Twenty wolves were experimentally vaccinated using an oral rabies virus vaccine. Prior to vaccination the health status of each animal was examined by veterinarians. For the application of the oral rabies vaccine the animals were anaesthetised using tranquillizers. Also, a blood sample was taken to show the animals to be sero-negative for rabies prior to vaccination. One month after vaccination a second blood sample will be collected and tested for the presence of virus-neutralising antibodies using the rapid fluorescent focus inhibition tests (RFFIT).

This results obtained in this pilot study will be presented and discussed. Also, conclusions concerning the use of ORV under Iranian conditions will be drawn.
RABIES EPIDEMIOLOGY IN RACCOON DOGS AND FOXES

A. Singer¹, K. Kauhala², K. Holmala³ & G.C. Smith¹

¹ Central Science Laboratory (Sand Hutton), York YO41 1LZ, United Kingdom
² Finnish Game and Fisheries Research Institute, Turku Game and Fisheries Research, Itäinen Pitkäkatu 3 A, FI-20520 Turku, Finland
³ Kunnaantie 18 C 12, FI-01370 Vantaa, Finland

Aims/Objectives: Raccoon dogs are seen as a new host for fox rabies in Europe. Disease spread in a community of species can change the epidemiology and require new disease control strategies. This study assesses the risk and strength of a rabies outbreak, introduced to a community of foxes and raccoon dogs in Southern Finland.

Materials and Methods: Epidemiology is simulated with a two-species model, based on approaches for rabies in foxes and parameterised from recently published data on raccoon dog and fox ecology in North-east Europe. Risk of rabies establishment, strength of disease and temporal dynamics of the epidemics were investigated.

Results / Discussion / Conclusions: Results show that rabies would not spread in a single species, when population densities are low, as in Finland. However persistent epidemics are very likely in the species’ community. Threshold density for the system of combined species reduces non-linearly, compared to thresholds of each of the species. Strength of a persistent disease depends on population density of both species; at high territory density raccoon dogs become the driving force. Even at low densities, the model predicts about twice as many infected raccoon dogs as foxes. Another behavioural factor that influences rabies epidemiology is raccoon dog hibernation, which may alter with climate change. Invasive raccoon dogs increase risk of rabies epizootics, especially in association with red foxes. The changed dynamics of epidemics in the community might require modification of control strategies. Assessing potential strategies should be the next step towards managing this new risk.
DOWNSIDE RISK OF WILDLIFE TRANSLOCATION

R. Chipman¹, D. Slate², C.E. Rupprecht³ & M. Mendoza⁴

¹ United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Castleton, NY 12033 USA
² United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Concord, NH 03301 USA
³ Poxvirus and Rabies Section, Centers for Disease Control and Prevention, Atlanta, GA 30333 USA
⁴ United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, Washington, DC 20250 USA

Translocation has been successfully used by wildlife professionals to: enhance or reintroduce populations of rare or locally extirpated wildlife, provide hunting or wildlife viewing opportunities, farm wild game, and to reduce local human-wildlife conflicts. Translocations of wildlife in the U.S. are estimated in the hundreds of thousands of animals each year. Accidental and intentional translocations may have multiple unintended negative consequences, including increased stress and mortality of relocated animals, as well as negative impacts on resident animals at release sites; increased conflicts with human interests; and the spread of diseases. Many wildlife professionals now question the need for translocation in light of the need to contain or eliminate high profile, economically important wildlife diseases such as chronic wasting disease, bovine tuberculosis and rabies. Moreover, translocation may jeopardize international wildlife disease management initiatives to control rabies in raccoons, coyotes and foxes in North America. Incidents where specific variants (Texas gray fox, canine variant in coyotes, and raccoon) have been moved well beyond their current range, including emergence of raccoon rabies from translocations, will be compared for their relative socioeconomic and ecologic impacts. The pervasiveness of translocation in the U.S. is reflected in part by the sale of nearly 1 million live traps annually. We review the substantial challenge (legal and social dimensions) of attempting to curtail translocation, while recognizing that it logically deserves serious attention in conjunction with ORV as the movement of infected animals could jeopardize sizeable commitments of resources and personnel, ORV programs, and the future of ORV in the U.S.
SYLVATIC RABIES IN RUSSIA WITH SPECIAL ATTENTION TO PERSPECTIVES OF ORAL VACCINATION OF WILD CARNIVORES

A.D. Botvinkin1, D.V. Bankovsky2 & G.A. Safonov2

1 Irkutsk State Medical University, 664003, Irkutsk, Russia
2 Pokrov Plan of Biologics, 601125, Volginsky, Vladimir region, Russia

An overview of rabies control strategies in wildlife is presented. In Russia, there exist several fragmented rabies-enzootic territories. Based on epidemiological and phylogenetic data 4 different areas can be distinguished: (1) central and northwestern European part of Russia with the red fox (Vulpes vulpes) and the raccoon dog (Nyctereutes procyonoides) as the main virus reservoirs; (2) the southeastern European part of Russia and southwestern Siberia where rabies is maintained predominantly by the red and corsac fox; (3) the southern Russian part of the Far East and (4) the arctic and subarctic region of Russia with the raccoon dog and the arctic fox (Alopex lagopus) as the main reservoir species, respectively. Natural barriers within those areas and between areas (1) and (2) are not reliable, particularly during winter. Areas (3) and (4) are separated by wide zones of conifer forests, where the population density of the reservoir species is extremely low. These territories are huge and go far beyond the size of vaccination areas in other European countries. Therefore oral rabies vaccination (ORV) can only be implemented using a step-by-step approach. Within territory (1) ORV can be implemented along the borders with Baltic states, Belarusia and Ukraine with the establishment of vaccination cordons around the metropolises of Moscow and St. Petersburg. ORV campaigns could also be conducted along the northern border of area (2), where the rabies incidence is low to sporadic. Implementation of ORV in area (3) would be suggestive as it is surrounded by broad rabies-free territories in the north and probably represents the northernmost spread of a common rabies enzootic area with China. Area (4) with circumpolar circulation of arctic rabies virus is not recommended for ORV because of low economic and public health significance, the vast territory and unfavourable climatic conditions.
FINNISH-RUSSIAN COLLABORATION PROGRAMME ON RABIES CONTROL IN WILDLIFE: THE OUTCOME OF FIVE YEARS AND THE FUTURE PROSPECTS

A.E. Metlin¹23, S.S. Rybakov¹, K.N. Gruzdev¹, V.V. Mikhališhin¹, A. Huovilainen² & E. Neuvonen²

¹ Federal Governmental Institution “Federal Centre for Animal Health”, 600901, Yur’evets, Vladimir, Russia
² Finnish Food Safety Authority, Evira, Mustialankatu 3, 00790 Helsinki, Finland
³ Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine P.O. Box 66, Fi-00014, University of Helsinki, Finland

Rabies is a serious veterinary and public health problem in Russia and in bordering countries, except Finland. In Finland, the last rabies outbreak occurred in 1988-1989. Thereafter re-infection of rabies from neighbouring regions has been prevented by oral rabies vaccination of wildlife (ORV) along the common border with Russia.

A Finnish-Russian Collaboration Programme on Rabies Control in Wildlife was launched in 2000. The main foci of this programme are (i) ORV of wildlife in the Leningrad oblast and Karelia in Russia, (ii) rabies surveillance and monitoring of the immune status of wild animals in vaccination areas and (iii) molecular characterisation of field and attenuated rabies viruses.

In the framework of the programme, so far rabies surveillance has been conducted annually and the efficacy and safety of two attenuated rabies vaccines, e.g. Sinrab (Russia) and Fuchsoral (Germany), has been tested in caged silver foxes in a comparative study. In the first vaccination campaign in Russian vaccination areas Fuchsoral vaccine (SAD B19) was used, whereas in subsequent years the Sinrab vaccine (RV-97) was applied annually. Since then, no rabies case has been detected in wildlife from vaccination areas.

Also, more than 200 field rabies viruses (RABV) were collected in different regions of the Russian Federation and typed together with archived RABV from Finland and Estonia using anti-nucleocapsid monoclonal antibodies. Virus typing revealed five antigenic variants circulating in those regions. Subsequent genetic characterization of RABV revealed two distant phylogenetic groups; e.g. a Pan-Eurasian and a Caucasian group. Furthermore, the entire genome of the Russian vaccine strain RV-97 was sequenced and its genetic characteristics analysed.

Finnish-Russian collaboration was considered valuable and successful by Finnish, Russian and international veterinary authorities and therefore, will be continued. A plan for the next 5 years has been worked out.
Rabies Eradication Programme in Estonia by Means of Oral Vaccination of Wildlife: First Results

E. Niin, M. Laine & A. Pärtel
Veterinary and Food Board, Tallinn, Estonia

The first case of sylvatic rabies in Estonia was recorded in 1968. Since then rabies is maintained in raccoon dogs and red foxes as well. Until 2005, the main prophylactic measure in the fight against rabies in animals was a compulsory vaccination of dogs and cats. In October 2005, the first large scale oral vaccination campaign of wildlife was carried out in the northern part of the country ranging from the western to the Eastern border comprising about 25,800 km². In 2006, two vaccination campaigns were conducted covering the whole territory.

Thorough rabies surveillance and monitoring of the vaccination efficiency (detection of tetracycline marker) was conducted to assess the success of the vaccination campaigns. As a result the number of rabies cases drastically decreased from 266 in 2005 to 114 in 2006. The results will be discussed in the context of rabies surveillance in Estonia and Europe as well.
RABIES SURVEILLANCE IN POLAND BETWEEN 1992 - 2006

M. Smreczak, P. Trębas, A. Orłowska & J.F. Żmudziński
National Veterinary Research Institute, Virology Department, 24-100 Pulawy, Poland

Aims/Objectives: To study the epidemiological situation of rabies in Poland between 1992 - 2006.

Materials and Methods: Analysis of rabies data from monthly reports of the regional veterinary laboratories.

Results / Discussion / Conclusions: After World War II the dog still was the main reservoir for rabies in Poland. Due to compulsory vaccination of dogs rabies could be successfully eliminated in domestic animals. Between 1949 and 1956 only single cases of rabies were diagnosed in wildlife. Unexpectedly, a high rabies incidence in wildlife, especially in the red fox, was reported in 1957. Since then the number of wildlife rabies cases steadily increased with every year. The rabies epizootics were characterized by seasonal and periodic fluctuations. During 1992 and 2006 a total of 22,591 rabies cases were detected of which 18,566 (82.2%) 3,968 (17.6%) were recorded in wildlife and domestic animals, respectively. 52 (0.2%) bats were found to positive for EBLVs. In domestic animals, the highest number of rabies cases was diagnosed in cattle – 2,414 cases – 10.7% in relation to the total number of rabies cases and 60.8% in relation to the number of rabies cases found in domestic animals. With 1,459 positive animals cats were ranked second in rabies prevalence during the past 15 years – 6.4% in relation to the total number of rabies and 36.8% in relation to the rabies in domestic animals. Dogs accounted for 955 of the rabies cases representing 4.2% of the total number of rabies cases and 24.1% of the rabies cases found in domestic animals. The highest number of rabies cases in wildlife was found in red foxes. With a total of 15,583 rabies cases which equals 69% in relation to the total number of rabies cases and 84% in relation to the number found in wildlife, the red fox is the main reservoir of rabies in Poland. Rabies in raccoon dogs is an increasing problem; 1,661 cases were detected in raccoon dog (7.3% of total and 8.9% in relation to the number of rabies cases in wildlife). The peak of the rabies epizootic in wildlife was observed in 1992 resulting in 3,084 cases nationwide of which 2,547 cases originated from wildlife. Eighty two percent of the wildlife rabies cases were foxes. In 1993, for the first time oral vaccination of foxes was implemented as a new method of rabies control. The ORV program was started in the western part of Poland along the border with Germany. In subsequent years was continuously extended eastward to cover the whole territory of Poland in 2002. Oral vaccination campaigns are conducted twice a year (spring, autumn). After 13 years of ORV the rabies incidence drastically decreased by 97% from 3,084 cases in 1992 to 82 in 2006. Provided ORV will be continued there is hope that rabies in Poland can be eliminated in the near future.
EFFICACY OF A SQUARE V-RG VACCINE BAITS IN RED FOX, DOMESTIC DOG AND RACCOON DOG

F. Cliquet¹, A.L. Guiot², C. Schumacher³, J. Maki³ & J. Barrat¹

¹ WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory for Rabies, Community Reference Laboratory for Rabies Serology, AFSSA Malzeville, France
² Conseils en Pharmacie et Biologie Santé, Lyon, France
³ Mérial, Lyon (France) and Athens (USA)

In response to a request of the Commission of the European Union (EU) the European Food Safety Agency (EFSA) has carried out a quantitative assessment of the risk of rabies introduction into the UK, Ireland, Sweden, and Malta as a possible result of movements of pets incubating rabies at the time of movement.

The risk that a pet is incubating rabies at the time of first vaccination is equal to the prevalence of rabies-incubating pets in the population of origin. Despite induction of immunity by vaccination animals already incubating rabies will still develop clinical disease as a function of time after vaccination (termed type A risk). Waiting time reduces this risk.

A small percentage of animals may not be protected after single-shot primary vaccination and hence, may become infected after vaccination. The risk of becoming infected after the first vaccination (termed type B risk) depends on the prevalence and efficiency of vaccination. Serological testing can be used to identify non-immune pets and will therefore reduce this risk accordingly. Type A and B risk were modelled as a function of waiting time after vaccination and fitted to a non-linear model incorporating test specificity and vaccination efficiency.

Within the EU, the prevalence and hence the type A risk is so low, that for most countries the risk is negligible. However, for some countries the risk is non-negligible. In this case inclusion of a waiting time is necessary to reduce this risk. Here, serological testing is appropriate, because type B risk becomes relatively more important as type A risk is reduced with extended waiting times (over 100 days).
SCENARIO-ANALYSIS EVALUATING EMERGENCY STRATEGIES AFTER RABIES RE-INTRODUCTION

H.-H. Thulke¹, D. Eisinger², T. Selhorst² & T. Müller²
¹ Helmholtz Centre for Environmental Research – UFZ, Leipzig, Germany
² FLI-Friedrich Löffler Institut, Wusterhausen, Germany

Aims/Objectives: After the fairly complete elimination of rabies in Western Europe using the rabies control strategies, e.g. ORV undertaken during the last 25 years, research is required to prepare a sound control strategy for emergency situations which could be a result of re-introduction of rabies from still infected areas. Such strategies should aim at minimizing the risk of falling back to a large-scale vaccination in a cost efficient manner.

Materials and Methods: We adopted an approved spatially-explicit model on the spread and control of rabies to provide solutions to new problems associated with a possible re-introduction of rabies into free areas. We explain the logic of the model and the different options for a local emergency vaccination around the first detected rabies case in an area; for example ring-vaccination vs. compact area treatment; heavily concentrated vs. thin extended; or adaptive extension of the vaccination area.

Results / Discussion / Conclusions: Based on systematic simulation experiments we assessed the performance of strategic options. Key factors such as the public health risk (i.e. number of rabies cases), the error risk (i.e. disease breakout from the area under control), and the budgetary risk (i.e. planned costs vs. potential excesses) were simultaneously considered. The results obtained often resulted in efficiency relations that contradict a-priori fixed intuitive management suggestions. We explain the causes and formulate appropriate recommendations.
THE FINANCIAL CHALLENGE TO KEEP A LARGE REGION FREE OF RABIES – THE EU EXAMPLE

C. Freuling, T. Selhorst & T. Müller
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, D-16868 Wusterhausen, Germany

Aims/Objectives: Oral vaccination of foxes has been developed into the preferred method of rabies control. Since its implementation a continuous decrease in rabies incidence has been reported and eventually rabies was eliminated in Western Europe. Once fox rabies is eradicated in a given area, re-infection from neighbouring infected countries is a permanent threat. In a theoretical approach we took the actual EU member states as a prime example, assuming these countries as being free from rabies but neighbouring countries still infected. The area of the vaccination belt to be established and also annual costs for the prevention of re-infection were calculated.

Materials and Methods: Using GIS a 50km deep vaccination belt beyond the front of the rabies endemic zone, as recommended by the EU, was installed in countries bordering those regions. It was assumed to vaccinate twice a year, using aerial and complementary hand distribution, with a bait density of 25 baits per sqkm on average. Minimum and maximum prices for commercial available vaccine baits, aircrafts and rabies surveillance were considered for the calculation of costs.

Results / Discussion / Conclusions: The total vaccination area to be established is about 263,000 sqkm. With fixed-wing aircraft used, the annual cost for vaccination including rabies surveillance varies between a minimum of 8.2 Mio and a maximum of 16 Mio € depending on the vaccine bait cost. If helicopters are used exclusively, the maximum costs increase to about 24.5 Mio. Depending on the length of the border to infected regions, countries could pay up to 25% of the total cost. Countries which need to install a vaccination belt will never get a rabies free status because of the likely occurrence of rabies cases in border zones.
WHAT IS THE FUTURE OF RABIES CONTROL IN EUROPE?


¹ Central Science Laboratory, Sand Hutton, York YO41 1LZ, United Kingdom
² Helmholtz Centre For Environmental Research –UFZ, Leipzig, Germany
³ Veterinary Laboratory Agency, New Haw, Addlestone, Surrey, KT15 3NB, UK
⁴ École nationale vétérinaire de Lyon, 1, av. Bourgelat, 69280 Marcy l’Etoile, France
⁵ WildCRU, University of Oxford, Tubney House, Abingdon Road, Tubney, OX13 5QL, UK
⁶ Friedrich Loeffler Institute, Wusterhausen, Germany

Over the last fifteen years or so, large areas of Western Europe have been cleared of classical rabies in terrestrial wildlife. Over the next few years, terrestrial rabies will only occur east of a line from the Baltic Sea to the Adriatic Sea, with an overall aim to eliminate terrestrial rabies from the whole European Union. Elimination of rabies from the less rich countries of Eastern Europe and in the longer term the protection of Europe requires a new strategy. Here we discuss the options available to eliminate rabies when cost becomes an important issue, and how to maintain a ‘cordon sanitaire’ along the eastern boundary of the EU. This is complicated by the growing presence of the Raccoon Dog, a significant host for fox rabies. Minimising cost for countries is necessary where there are more important economic and health issues. This could be achieved either by the EU increasing the proportion of the cost it funds, or by changing the currently agreed vaccination strategy to reduce costs, but without significantly reducing the chances of rabies elimination. The cordon sanitaire may be placed outside the economic area of the EU, to protect the whole of the EU, or it may be placed within the easternmost countries to ensure logistical consistency of vaccination. However, these are the countries for which we must consider cost minimisation. We will suggest how models could help to determine these strategies, by minimising bait density, bait frequency or area.
THE STRENGTH OF 70% - REVISING A GIVEN THRESHOLD OF RABIES CONTROL

H.-H. Thulke & D. Eisinger
Helmholtz Centre for Environmental Research –UFZ, Leipzig, Germany

Aims/Objectives: Oral vaccination of foxes (ORV) requires a minimum percentage of immunized foxes to interrupt the infectious chain of rabies among susceptible animals and hence, successfully eliminate rabies. A famous estimation of this benchmark was provided by Anderson et al. as published in Nature in 1981. Using a population model the authors at the time raised the hope that ORV would be a feasible strategy for wildlife rabies control, in line with promising field trials. By preparing standard applications they deduced minimum immunisation coverage of 70% required to achieve rabies elimination in a given area. Since then the success of ORV campaigns is measured against the 70% immunisation rate. Twenty five years later we were forced to re-evaluate this threshold due to noticeable problems with respect to increased fox densities, increasing bait densities, observed field data, theoretic predictions and cost-benefit analysis.

Methods: We developed a spatially explicit rabies for the control of rabies in foxes that is keen to replay the dynamics of the classic population model but in the same fashion can consider the important peculiarities in the vaccination process, i.e. spatial distribution of infected hosts, irregular home-range use, heterogeneous bait coverage etc. We demonstrate that both model representations of the fox-rabies system equally well reproduce field data from the last 40 years.

Results / Discussion / Conclusions: Interestingly, the more explicit model proofed that a 10 percentage-point lower herd immunity (i.e. 60% instead of 70%) is necessary to eliminate rabies under all reasonable fox densities tested. The economic benefit gained from such a reduced target value of herd immunity was estimated in terms of baiting density. In general, 30% less resources were required to successfully eliminate rabies. Thus, adapting the benchmark would save an enormous amount of resources in future control programs.
A RANDOM GRID BASED MOLECULAR EPIDEMIOLOGICAL STUDY ON EBLV ISOLATES FROM GERMANY

C. Freuling¹, T. Selhorst¹, L. Geue¹, D. Marston², N. Johnson², A.R. Fooks², N. Tordo³ & T. Müller¹

¹ Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, D-16868 Wusterhausen, Germany
² Veterinary Laboratories Agency (Weybridge), Surrey, United Kingdom
³ Unité des Stratégies Antivirales, Institut Pasteur, Paris, France

Aims / Objectives: Bat rabies in Europe is overwhelmingly caused by European bat lyssavirus (EBLV) types 1 and 2 (genotypes 5 and 6). Germany has reported one of the highest numbers (n = 197) of EBLV cases in bats in Europe during the past 50 years. All German isolates were identified as EBLV-1 using monoclonal antibodies and a preliminary epidemiological study has indicated that there is a distinct geographical distribution of EBLV-1 in Germany. To further investigate the spatial and temporal distribution of EBLV-1 variants within Germany, and its impact on molecular epidemiology, we have selected 50 isolates from a total number of 12, using a random grid sampling procedure based on GIS.

Materials and Methods: Applying a grid layer of 30 km length over the entire area of Germany to the geo-referenced isolates, at least one isolate of each grid cell containing EBLV isolates was randomly chosen. Once selected, the nucleoprotein (N) plus parts of the phosphoprotein (P) gene of each isolate were sequenced using direct cycle sequencing.

Results / Discussion / Conclusions: Results of the subsequent phylogenetic analysis confirmed previous studies on European EBLVs showing a high sequence homology between the German EBLV-1 isolates. Almost identical sequence homologies within certain geographical regions indicate genomic stability during the transmission cycle of EBLV with little geographic spread or intermixing. Interestingly, a 6bp insertion, detected in the N-P intergenic region, has been repeatedly isolated from EBLV1b isolates from south-western Germany.
ASYMPTOMATIC RHABDOVIRUS INFECTION IN MERIDIONAL SEROTINE BATS (EPTESICUS ISABELLINUS) FROM SPAIN

J.E. Echevarría¹, S. Vázquez-morón¹, C. Ibáñez², J.C. Aznar¹, E. Ruiz³ & J. Juste²

¹ Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain
² Estación Biológica de Doñana, Sevilla, Spain
³ Laboratorio Central Veterinario, Santa Fe, Granada, Spain

The serotine bat (Eptesicus serotinus) accounts for more than 95% of episodes resulting in human exposure to European Bat Lyssavirus type 1 (EBLV-1). Recent studies showed that southern Iberian populations of this bat species belong in fact to a sibling species named Eptesicus isabellinus. Previous results showed EBLV-1 RNA in the oral cavity of free-living serotine bats from southern Spain captured during flight activities, suggesting asymptomatic excretion of this virus. The aim of this work was to evaluate the health condition of individuals harboring EBLV-1 RNA, as well as the search for other rhabdoviruses. A total of 1033 animals from 19 colonies were captured between 1998 and 2003 using mist-nets, subsequently ringed, weighted, sized, sexed, aged, and sampled for oro-pharyngeal swabs and blood, and finally released into nature. A body condition index was calculated with data for each individual. Serum samples were tested for EBLV-specific antibodies by fluorescent focus inhibition test and Lyssavirus-specific RNA in saliva swabs was detected by nested RT-PCR. Another group of 71 saliva swabs was also analysed using another nested RT-PCR specific for the whole Dhimirhabdovirus complex. The results showed no temporal patterns, but might indicate an independent circulation of EBLV-1 among different colonies due to lack of exchange of individuals. The body condition index of the RT-PCR positive animals was not different from the negative ones. RNA from two apparently new Dhimirhabdoviruses was amplified from two bats.

These results suggest that survival of bats after EBLV-1 infection might be possible and that other Rhabdovirus infections could also be a common event in this bat species.
PUBLIC HEALTH HAZARD OF EUROPEAN BAT LYSSAVIRUS, THE NETHERLANDS

W.H.M. Van Der Poel¹⁴, K. Takumi¹, E.R.A.M. Verstraten², P.H.C. Lina³, J. Van Der Giessen² & J.A. Kramps²
¹ Microbiological Laboratory for Health Protection, National Institute for Public Health and the Environment
² Central Institute for Animal Disease Control (CIDC– Lelystad), Lelystad, the Netherlands
³ National Museum of Natural History “Naturalis” Leiden, the Netherlands
⁴ Animal Sciences Group, Wageningen University Research

To study the European bat lyssavirus (EBLV) epidemiology in bat reservoirs in the Netherlands, there is a bat lyssavirus surveillance in place since 1984. Since 1997, all detected EBLV RNAs encoding the nucleoprotein have been sequenced and analysed phylogenetically. To investigate how often bats come into contact with other terrestrial species, the nature of the contacts was determined for serotine bats (Eptesicus serotinus) submitted for rabies diagnosis between 2000 and 2004. PCR positive brain tissues do not necessarily mean that the bats can transmit the disease by biting. Therefore 30 EBLV-1a positive bats were additionally tested for the presence of virus RNA in other body parts.

From 1984 to 2003, a total of 1,219 serotine bats were tested and 251 (21%) were found lyssavirus antigen positive. All lyssavirus sequences obtained from serotine bats originating from the years 1997 to 2003 clustered with genotype 5 (EBLV-1) sequences. Between 2000 and 2004, thirteen people were bitten by bats. Five of the bats involved were identified as belonging to the subgroup EBLV-1a (38%). Overall prevalence of EBLV-1a in serotine bats that came into close contact with humans was 35% (8/23). The most important finding concerning the risk of bat rabies transmission by biting was the presence of EBLV-1a in the salivary glands of 22 bats (73%).

Our findings indicate that EBLVs of genotype 5 are endemic in the serotine bat in the Netherlands and biting incidences with humans regularly take place. Therefore, a public health risk of bat acquired rabies cannot be ignored. As EBLVs can cause fatal infections in humans, bats involved in contact incidents with humans should be tested to determine if the victim was exposed to EBLVs. For this purpose, in the Netherlands a nation-wide passive surveillance program is put in place and an active surveillance program will be started in 2007.
SEROLOGICAL INVESTIGATION OF BATS INFECTED WITH RABIES VIRUS IN CHINA

L. Wang¹, Y. Jiang¹, Z. Lu³, K. Sun⁴, H. Xuan², A.R. Fooks⁵ & C. Tu¹

¹ Academy of Military Medical Sciences, Changchun, 130062, China
² Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
³ Foshan University, Foshang, 528000, China
⁴ Northeast Normal University · Changchun, 130062, China
⁵ Veterinary Laboratories Agency (Weybridge), Surrey, United Kingdom

In order to investigate rabies virus infection of bats in China the indirect fluorescent antibody test (IFA) using a FITC-conjugated Protein A/G mixture was established for detection of rabies specific antibodies. A total of 685 serum samples from frugivorous and insectivorous bats belonging to 8 different species of 4 bat families originating from 10 different areas of 4 provinces in China were tested using the IFA and a modified fluorescent antibody virus neutralization test (mFAVN). The results showed that 2.2% (15/685) of the bats tested were RABV antibody positive representing 3 different species, e.g. Rosettus leschenaulti, Rhioophus blythi and Rhinolophus ferrumequinum. Bats of the species Rosettus leschenaulti were found positive in 3 of 4 provinces suggesting a wide geographic range of infection for this species. Moreover, the IFA results were confirmed by mFAVN, which was used to test samples with sufficient amount of serum; 3 IFA positive sera were also positive in mFAVN although exhibiting very low titers (0.15, 0.08, and 0.33 IU/mL), while 15 IFA negative serum samples were also negative in mFAVN, indicating a 100% concordance of the two methods. The low antibody titers in all positive serums imply that natural infection of RABV in bats most likely induce low levels of antibody responses. This is the first report about natural infection of RABV in bats in China. The role of bats in rabies transmission amongst human and dogs requires further investigation with respect to severe epidemics of human rabies in China.
EPIDEMIOLOGY AND PATHOGENICITY OF AFRICAN BAT LYSSAVIRUSES

W. Markotter, C. Wilsenach, L.H. Nel et al.
Department of Microbiology, Faculty of Natural and Agricultural Sciences, University of Pretoria, 0001 South Africa

Viruses belonging to all four known African lyssavirus genotypes (gt’s) have been reported and isolated almost exclusively from South Africa over the past few decades. These are: (1) Duvenhage virus (gt 4), isolated in 2006 from a human fatality for the first time in 36 years; (2) Mokola virus (gt 3), isolated irregularly, mostly from cats; (3) Lagos bat virus (gt 2) continually isolated from *Epomophorus* fruit bats and from incidental terrestrial animals during the past 3 years; (4) Rabies virus (gt 1) – with two virus biotypes being endemic in mongooses and in canines (domestic and wildlife), respectively.

Only two of these are associated with bats in southern Africa, viz. Duvenhage virus and Lagos bat virus (gt’s 4 and 2). We are interested in the evolutionary history and likely future of these unique viruses. Therefore, for both these genotypes we have embarked on a programme of comparative study of the molecular epidemiology and pathogenicity of all the virus isolates in existence. For Duvenhage virus, full-length genome sequences indicated a pattern of evolution strikingly similar to that observed for European bat lyssavirus 1. In the case of Lagos bat virus, individual isolates were found to differ dramatically with respect to molecular genetic and pathogenicity profiles.
EXPERIMENTAL INFECTION OF BIG BROWN BATS (EPTESICUS FUSCUS) WITH WEST CAUCASIAN BAT VIRUS

I.V. Kuzmin, R. Franka & C.E. Rupprecht
Centers for Disease Control and Prevention, Atlanta, GA, USA

The aim of the study was to assess the susceptibility and general pathogenesis patterns of West Caucasian Bat virus (WCBV) in big brown bats. The bats, either recently captured and survivors from the previous experiments with other lyssaviruses were inoculated intramuscularly into masseter (7), neck (8) or orally (6). They were bled prior to inoculation and bi-weekly thereafter. Oral swabs were taken weekly during the first 3 months and bi-weekly during the next 3 months. Several tissues of bats that died during 6 months of observation, as well as brains and salivary glands of survivors, all oral swabs and blood pellets were subjected to nested RT-PCR. The PCR-positive samples were additionally subjected to virus isolation. Serum samples were studied for the presence of virus-neutralizing antibodies.

As a result, 3 bats developed rabies and died on days 10, 10 and 18. All 3 were inoculated into neck. Viral RNA was detected in a number of tissues but isolation was successful only from the brain. An oral swab of one of these bats was also PCR-positive, however the isolation attempt failed. Brains, salivary glands and swabs from survivors were negative, as well as all blood pellets collected. WCBV-neutralizing antibodies were identified in 4 bats, all inoculated into masseter. They appeared 14-80 days post challenge, were highest up to 5th months, and decreased by the end of observation. The bats that previously survived challenge with Irkut virus still maintained remnant Irkut-neutralizing antibodies; however, challenge with WCBV did not boost their titers.

________________________
SUSCEPTIBILITY OF FOXES (VULPES VULPES) TO EUROPEAN BAT LYSSAVIRUSES TYPES-1 AND -2

F. Cliquet1, E. Picard1, J. Barrat1, S.M. Brookes2, D.M. Healy2, A.R. Fooks2

1 WHO Collaborating Centre for Research and Management in Zoonoses Control, OIE Reference Laboratory for Rabies, Community Reference Laboratory for Rabies Serology, AFSSA Malzeville, France
2 WHO Collaborating Centre for the Characterization of Rabies and Related Rabies Viruses, OIE Reference Laboratory for Rabies, Rabies and Wildlife Zoonoses Group, Veterinary Laboratories Agency, Weybridge, United Kingdom

The objective of the AFSSA/VLA collaborative study was to assess the susceptibility of silver-haired Norwegian foxes to European Bat Lyssavirus types 1 and 2 (EBLV-1 and EBLV-2). Both EBLV-1 and EBLV-2 have been isolated in European bats since 1954, mainly in Eptesicus serotinus and Myotis species. Since 2000, the number of reported cases has increased due to enhanced in epidemiological surveillance of bat rabies throughout Europe. Although more than eight hundred EBLV cases have been reported in bats in Europe, EBLV-1 and -2 viruses have been rarely reported to infect humans and terrestrial animals.

Silver foxes were susceptible to EBLV infection (3-4 log dosis) following direct intracranial inoculations, 100% mortality during 8-282 days post-infection. However, the susceptibility of the foxes was low following peripheral (intramuscular) inoculation with a similar dose; EBLV-1 infection resulted in 19% (n=4/21) mortality with an incubation period of 14-24 days. None of the animals challenged with EBLV-2 succumbed to disease. Further analysis is in progress to investigate whether the infected foxes excreted infectious virus in saliva.

These data suggest that bat to fox spillover of EBLVs is possible, with a greater probability of spillover for EBLV-1 than EBLV-2.
The United Kingdom has performed passive surveillance for European bat lyssaviruses (EBLVs) since 1985, and active surveillance since 2003. A critical component of these studies is the accurate identification of bats either submitted for testing or sampled in the field. Identification is currently dependent on a number of morphological characteristics. Whilst this is an effective means of bat identification a number of problems remain with this approach. It relies on the experience of bat handlers and can lead to problems in differentiating members of the *Myotis* genus, particularly between *Myotis mystacinus* (Whiskered bat) and *Myotis brandtii* (Brandt's bat). Furthermore degradation of bats submitted for testing can also lead to problems in making an accurate species identification. Comparison of genetic sequence data could offer an alternative approach to differentiating bat species when morphological characterisation is not possible. Using tissue samples from UK resident bat species, sequence analysis of the mitochondrial DNA Cytochrome *b* gene, and the β-actin gene allowed identification of many of the most commonly found bat species in the UK, and genetic separation of two morphologically cryptic species. Application of this approach identified the species of a bat infected with EBLV-2 found in Surrey as *Myotis daubentoni* (Daubenton's bat). Furthermore, haplotyping of the Cytochrome *b* gene enabled further characterisation of native bat species. This could provide a useful tool to assist in conservation strategies.
HUMAN RABIES INCLUDES EARLY GENERALIZED VASOSPASM OF CRANIAL ARTERIES THAT Responds TO TETRAHYDROBIOPTERIN, L-ARGININE OR NITROPRUSSIDE


1 Medical College of Wisconsin, Milwaukee, Wisconsin, United States of America
2 Children’s Hospital of Oakland, Oakland, California, United States of America
3 Riley Hospital for Children, Indianapolis, Indiana, United States of America
4 Viral and Rickettsial Disease Laboratory, Richmond, California, United States of America
5 Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America
6 Horizon Molecular Medicine, Atlanta, Georgia, United States of America

Aims / Objectives: In 2004, a teenager survived bat-associated rabies through the Milwaukee protocol. This survivor and a patient with dog-associated rabies developed deficiencies in tetrahydrobiopterin (BH4) and associated neurotransmitters. BH4 is essential for neuronal nitric oxide synthase (nNOS), so rabies is predicted to cause constriction of cerebral arteries. We sought medically significant spasm of the middle cerebral arteries (MCA) by transcranial doppler (TCD).

Materials and Methods: Case-report methodology. Flow velocities and resistive indices (RI) of MCA were obtained daily by TCD.

Results / Discussion / Conclusions: One child with bat-associated rabies developed severe spasm of MCA on day-8 of hospitalization that responded to 0.2 mcg/kg/min nitroprusside. Onset of systemic immunity resulted in clearance of virus from saliva by PCR. The child died of cerebral edema on day-25, associated with thrombosis of cerebral venous sinuses. One child with dog-associated rabies developed spasm of MCA on day-7 of hospitalization that responded to 6 mg/kg/day BH4. A second spasm with high RI (without cerebral edema) responded to 20 mg/kg/day BH4 and 0.5 g/kg/dose L-arginine. Onset of systemic immunity resulted in partial clearance of virus from saliva by PCR. In retrospect, the second spasm caused brainstem ischemia. The child died of cerebral edema on day-27, without sinus thrombosis. Cerebral artery vasospasm occurred on day-7-8 of hospitalization for 2 children with rabies, but was clinically silent by standard monitoring. Spasm responded to drugs directed at the NOS pathway. Animal models for treatment of rabies are sorely needed to evaluate therapeutic options.
THERAPY OF HUMAN RABIES: LESSONS FROM EXPERIMENTAL STUDIES IN A MOUSE MODEL

A.C. Jackson, C.A. Scott, J. Owen, S.C. Weli & J.P. Rossiter

Departments of Medicine (Neurology), Microbiology and Immunology, and Pathology and Molecular Medicine and Centre for Neuroscience Studies, Queen’s University, Kingston, Ontario, Canada K7L 3N6

Human rabies is almost always fatal. Ketamine was one of the therapeutic agents used on a survivor who did not receive vaccine. The therapy was based on previous experimental work performed in primary neuron cultures and in a rat model of rabies. We have reexamined ketamine therapy in infected mouse primary neuron cultures and in adult ICR mice using the CVS strain with both intracerebral and peripheral routes of inoculation with ketamine (120 mg/kg/day) vs. vehicle given intraperitoneally. We did not observe any significant beneficial therapeutic effect of ketamine in the cultures or mouse models. We do not recommend further widespread clinical use of ketamine on human rabies patients until further experimental work confirms therapeutic efficacy.

Because of the potential neuroprotective and anti-apoptotic properties of minocycline, we also assessed minocycline therapy in infected primary neuron cultures and in neonatal ICR mice infected by peripheral inoculation with a highly attenuated rabies virus strain (D29). No beneficial effect of minocycline was observed in the primary neuron cultures. In the mouse model, minocycline therapy (50 mg/kg/day for 18 days) aggravated the clinical neurological disease and resulted in higher mortality. An anti-apoptotic effect of minocycline was noted in brains of infected mice, which may have very mildly increased viral spread. An anti-inflammatory effect of minocycline was also noted in the brain using a CD3 T cell marker. These effects likely aggravated the disease. Consequently, we recommend that empirical therapy with minocycline be avoided in the management of rabies and viral encephalitis in humans until the results of more experimental work becomes available.
RABIES CONTROL AND PREVENTION IN GEORGIA: CURRENT STATUS AND PERSPECTIVES

P. Imnadze, V. Surguladze, T. Tushishvili, L. Baidoshvili
National Center for Disease Control, 9, M. Asatiani str., 0177, Tbilisi, Georgia

Objectives: In 2006, there was a relatively high incidence of rabies post-exposure prophylaxis (PEP) of about 876 / 100,000 persons annually in Georgia. The aim of this study was to improve post-exposure policy and practice, as well as to determine priorities for a rabies surveillance and control at a national level.

Method: Analysis of data from the National Center for Disease Control on rabies surveillance in the years 2004, 2005 and 2006.

Results: Between 1986-1995 a total of 40 rabies cases in humans were reported in Georgia. In 2004, 2005 and 2006 a total of 12, 10 and 17 rabies cases were officially reported, respectively, representing a significant increase in overall incidence compared to a decade ago. About 93% of the rabies cases were caused by dog bites. A high population of stray and/or ownerless dogs and a high percentage of unvaccinated pets were identified as major risk factors.

In 2006, there were 38,569 patients having received PEP following exposure to suspect rabid animals compared to 23,712 and 30,254 in 2004 and 2005, respectively, representing an increase of about one third. From the total of PEPs initiated in 2004, 2005 and 2006, 70 % (16,568), 76% (23,106) and 78.4% (30,254) were discontinued, respectively, because the biting animals remained healthy during the required observation period. Safety concerns concerning available immunoglobulins, uncertainty in usage of commercially available vaccines and immunoglobulins and inconsistency of treatment guidelines with international recommendations are considered obstacles for better administration of PEP.

Conclusions: For an effective prevention of rabies in humans strikt implementation of international guidelines on the use of rabies vaccines and immunoglobulins in PEP at a national level is needed. National rabies eradication programs as well as attempts to control stray dogs must be implemented to reduce the risk of transmission of rabies to humans.
A SIMPLIFIED ECONOMICAL 4-SITE INTRADERMAL POST-EXPOSURE RABIES VACCINE REGIMEN IS AS IMMUNOGENIC AS THE CURRENT INTRAMUSCULAR AND INTRADERMAL METHODS

M.J. Warrell¹, A. Riddell¹, L.-M. Yu¹, J. Phipps¹, L. Diggle¹, J. Deeks¹, H. Bourhy², L. Audry², A.R. Fooks³, S. Brookes³, F.-X. Meslin⁴, R. Moxon¹, A.J. Pollard¹ & D.A. Warrell¹

¹ Oxford Vaccine Group, Centre for Clinical Vaccinology & Tropical Medicine, University of Oxford UK
² UPRE Lyssavirus Dynamics and Host Adaptation, Institut Pasteur, Paris, France
³ Rabies Unit, Veterinary Laboratories Agency, New Haw, Surrey, UK
⁴ WHO, Geneva, Switzerland

Economical intradermal (ID) rabies post-exposure prophylaxis (PEP) regimens use only 40% of the amount of vaccine required for the familiar intramuscular (IM) regimen, yet they are used for only 3% of 5 million annual tissue culture vaccine treatments in Asia, and are not used in Africa. This is because of confusion over regimens, lack of confidence in ID injection technique, and fear of reducing the vaccine dosage. Their use has been restricted to large immunisation clinics.

The immunogenicity of a simplified 4-site intradermal regimen was compared with the two current ID PEP regimens, and the standard IM regimen in a randomised single blind controlled trial, using purified vero cell rabies vaccine. The regimen is adapted from the current 8-site method: four ID sites on day 0, two sites on day 7 and single sites on days 28 and 90. Rabies antibody was measured on days 0, 7, 14, 90 and 1 year after immunisation.

All the ID regimens proved equally immunogenic and were not inferior to the IM treatment. The 4-site regimen has advantages over current regimens including one less visit than the 2-site ID method and it can be used economically with all WHO recommended vaccines. Injecting a whole ampoule of vaccine divided between ID sites on day 0 is more practicable for widespread use and is safer, especially in inexperienced hands.

An increased uptake of ID PEP methods and reduction of cost could be achieved by introducing this regimen, which proved at least as immunogenic as the more expensive IM regimen.

_______________
SINGLE DOSE FOR RABIES PRE-IMMUNIZATION

P. Khawplod¹, H. Wilde², T. Kamolthum³, Tantawichien¹,² & V. Sitprija¹
¹ Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand
² Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
³ Provincial Public Health Office of Pitsanulok, Thailand

Background: Pre-exposure rabies vaccination is recommended for travelers to endemic countries. It consists of three injections administered over 2-4 weeks. Travelers often do not have enough time to receive a complete course prior to departure and leave with only one or two injections.

Objective: To evaluate the secondary antibody response in volunteers who received one or two injections of PVRV or PCEC compared to the recommended 3 injections regimen when administered booster one year later.

Result: All volunteers developed an accelerated antibody response within 7 days when given boosters.

Conclusion: One injection pre-exposure rabies vaccination is enough to prime the host immune memory for at least one year.
IMMUNE MEMORY AFTER REMOTE PRE- AND POST-EXPOSURE RABIES VACCINATION: A PROSPECTIVE STUDY IN THAILAND

H. Wilde
Division of Research Affairs and Infectious Diseases, Faculty of Medicine, Chulalongkorn University, 10330 Bangkok, Thailand

Objective: To determine whether volunteers who had received a rabies vaccination 5-10 years or 10-21 years previously would still respond with an accelerated immune response to booster injections with tissue and avian culture rabies vaccines.

Method: Records of patients from the Queen Saovabha Memorial Institute who had pre- or post-exposure rabies prophylaxis with a tissue culture vaccine were identified randomly and were asked to provide blood samples. They then received two 0.1 mL intradermal booster injections of purified Vero cell rabies vaccine on days 0 and 3 and were followed by determination of neutralizing antibody on days 5, 7 and 14. A total of 118 volunteers were recruited. Sixty-five had been vaccinated 5-10 years previously and 53 had been vaccinated 10-21 years earlier.

Results: One healthy female 75 years of age had a detectable antibody level on day 0 (0.08 IU/mL) which increased to 0.11 on day 5 and to 0.48 IU on day 7. It rose to 6.25 on day 14. All remaining subjects had detectable antibody titers on day 0 and titres above 0.5 IU/mL on day 7. This indicated an accelerated immune response. Statistical analysis showed no significant differences between subjects who had received pre- or post-exposure vaccination, intradermal or intramuscular injections, additional immunoglobulin, or sex and age.

Conclusion: Tissue and avian culture rabies vaccination confers long lasting immune memory in normal hosts.
EPIDEMIOLOGY AND PROPHYLAXIS OF RABIES IN HUMANS IN FRANCE. EVALUATION AND PERSPECTIVES OF A TWENTY FIVE YEAR SURVEILLANCE PROGRAM

Y. Rotivel¹, M. Goudal¹, A. Simons De Fanti², D. Van Der Vliet² & French Rabies Treatment Centres³

¹ Rabies Treatment Centre, Medical Centre, Institut Pasteur, Paris, France
² National Reference Centre for Rabies, Institut Pasteur, Paris, France
³ List of French Rabies Treatment Centres on: www.pasteur.fr/sante/

In 1968, an epizooty of fox rabies reached the French territory. In the following years, the National Reference Centre for Rabies was founded at the Pasteur Institute, Paris. Among others, its main tasks included the surveillance of rabies cases in humans as well as the consultation and application of rabies post-exposure prophylaxis (PEP). Surveillance has been effective since 1982. A Bulletin on the Epidemiology and the Prophylaxis of Rabies in Humans in France is being published every year. This Bulletin is now available on the Internet for both national and local human health authorities as well as veterinary authorities. Since 2005, data are collected with the new software Voozanoo® directly via the Internet.

Since 1970, twenty cases of rabies in humans have been reported in France; however, none of the rabies cases was acquired in the country. The number of PEP was highest in 1990, when the number of rabies cases in wildlife peaked out. Due to the decline of rabies cases in the wildlife in subsequent years, the number of PEP decreased by 60%. Nevertheless, about 4,000 people had to receive PET. Those patients either were persons with a work-related predisposition or persons who had been exposed to bats or rabid animals illegally introduced to French territory or during a stay in countries where rabies is still enzootic.

Based on the information of this database the following conclusions can be drawn:

- Human rabies cases are mainly due to (i) canine rabies virus variants acquired directly in countries with enzootic canine rabies, (ii) exposure to rabid animals illegally translocated to France, or (iii) through iatrogenic exposure

- In recent years bats appear to become an increasing source of exposure;

- PEP surveillance is of utmost importance to monitor and to improve the quality of case management.
IMMUNE EVASION, A CRITICAL STRATEGY FOR RABIES VIRUS

M. Lafon
Neuroimmunologie Virale, Institut Pasteur, 75724 Paris cedex 15, France

Rabies virus (RABV) is a pathogen well-adapted to the nervous system, where it infects the neurons. RABV is transmitted by the bite of an infected animal. It enters the nervous system via a motor neuron through the neuromuscular junction, or via a sensory nerve through nerve spindles. It then travels from one neuron to the next, along the spinal cord to the brain and the salivary glands. The virions are then excreted in the saliva of the animal and can be transmitted to another host by bite. It is intriguing to note that RABV progression is interrupted neither by destruction of the infected neuron nor by the immune response. Thus, it is likely that RABV has developed a subversive strategy to avoid functional neuron impairment which compromises the infectious cycle. We showed that rabies virus neuroinvasiveness results of two factors: 1) not only neurotropic rabies virus avoids to induce neuron cell death, but also 2) “protective” T cells that migrate into the infected nervous system are killed by apoptosis, as a result of the overexpression of immunosubversive molecules such as Fasl, HLA-G or B7-H1 in the infected nervous system. Our data suggest that the preservation of the neuronal network and the destruction of T cells that invade the nervous system in response to the infection are crucial events for rabies virus neuroinvasion and for transmission of rabies virus to another animal. This may shed new lights for improvement of post-exposure treatments of rabies.
BHK-21 CELL CULTURE RABIES VACCINE- A CANDIDATE VACCINE FOR HUMANS

D. Lalosevic¹, V. Lalosevic²
¹ Faculty of Medicine and Pasteur Institute, Novi Sad, Serbia
² Department of Veterinary Medicine, Faculty of Agriculture, Novi Sad, Serbia

Aims: Rabies vaccines produced on BHK-21 (C-13) cell culture are well-known for veterinary use. Research initiated by WHO raised great expectations on the usefulness of such vaccines for humans. Based on experimental results the designed product is safe due to virus inactivation by beta-propiolactone destroying DNA contamination from cell culture.

Materials and Methods: We propagated the Pasteur virus (PV) strain of rabies virus in BHK-21 (C-13) cells and produced a beta-propiolactone inactivated and aluminium-phosphate adsorbed rabies vaccine. In a clinical trial, a total of 300 adult subjects were vaccinated. Three doses of vaccine were administered intramuscularly in the deltoid region following the 0-7-21 pre-exposure vaccination regimen. At day 30 post vaccination blood samples were drawn and virus neutralising antibodies (VNA) were measured by RFFIT.

Results: Local reactions in few percents and no systemic adverse reactions were registered. All volunteers had titer over 0.5 IU/ml.

Discussion: Rabies vaccine produced on BHK-21 (C-13) cells has been used for animals for a long time. Those rabies vaccines have been shown to be safe. Contamination with cellular DNA is not dangerous because the treatment with beta-propiolactone during virus inactivation completely inactivates the biological activity of DNA. This finding, which was accepted also by WHO, encourages the use of BHK cells as substrate for human vaccine production.

Conclusions: We conclude that this low cost vaccine is safe and effective for humans. The preliminary results with BHK/21 vaccine from clinical trials confirmed its good tolerance and immunogenicity.
FACTORS INFLUENCING THE ANTIBODY RESPONSE TO RABIES VACCINATION

V. Jakel¹, K. Cussler², M. König¹, H.-J. Thiel¹
¹ Institute of Virology, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
² Paul-Ehrlich-Institut, D-63207 Langen, Germany

Pre-expositional vaccination against rabies virus is a highly effective method to prevent rabies in humans and animals. For travel purposes vaccination of domestic carnivores is obligatory. In addition some countries demand testing on virus neutralizing antibodies (VNA) against rabies. The minimal threshold level accepted by WHO/OIE is 0.5 IU/ml. Despite orderly vaccination some animals do not reach the threshold.

Aims / Objectives: Identification of specific risk factors in dogs and cats with respect to VNA titres < 0.5 IU/ml after vaccination as measured by the FAVN-Test. Review of rabies vaccination protocols and recommendations with regard to travel regulations.

Materials and Methods: To collect comprehensive data on vaccination history from animals tested for rabies VNA a standardised questionnaire was developed and sent to veterinarians who submitted sera for testing. Data on species, age, sex, breed, vaccine used, date of last vaccination and blood sampling, vaccination history and further medical treatment at the time of vaccination were collected. Data sets of about 1200 animals were analysed.

Results / Discussion / Conclusions: Most animals older than one year already received more than one rabies vaccination. The influence of breed and sex on the VNA titre seems to be insignificant. Young dogs after their first vaccination have a high risk of developing VNA titres < 0.5 IU/ml. The risk can be minimised by application of a second vaccination and blood sampling according to the recommendations of the manufacturer. An important factor for the test outcome in seroneutralisation assays (FAVN) might be the virus strain used in the vaccine.

Joint OIE/WHO/EU International Conference: “Towards the Elimination of Rabies in Eurasia”
Paris (France), 27-30 May 2007
ATTAINING RACCOON RABIES MANAGEMENT GOALS: HISTORY AND CHALLENGES

D. Slate\(^1\), C.E. Rupprecht\(^2\), D. Donovan\(^3\), J. Badcock\(^4\), A. Messier\(^5\), R. Chipman\(^6\) & M. Mendoza\(^7\)

\(^1\) United States Department of Agriculture, Animal and Plant Heath Inspection Service, Wildlife Services, Concord, NH 03301 USA
\(^2\) Poxvirus and Rabies Section, Centers for Disease Control and Prevention, Atlanta, GA 30333 USA
\(^3\) Rabies Unit, Ontario Ministry of Natural Resources, Peterborough, Ontario K9J8N8 Canada
\(^4\) New Brunswick Department of Health and Wellness, Fredericton, New Brunswick, E3B 5G8 Canada
\(^5\) Agency of Health and Social Services of Montérégie, Longueuil, Québec, J4K 2M3 Canada
\(^6\) United States Department of Agriculture, Animal and Plant Heath Inspection Service, Wildlife Services, Castleton, NY 12033 USA
\(^7\) United States Department of Agriculture, Animal and Plant Heath Inspection Service, Wildlife Services, Washington, DC 20250 USA

Prior to 1977, raccoon rabies was confined to the southeastern United States. Raccoon translocations from Florida to western Virginia-West Virginia can be attributed to emergence of this rabies virus variant in the mid-Atlantic region, which has spread to the north, with incursions into Ontario, Quebec, and New Brunswick, Canada, and as far west as northeast Ohio. Raccoon rabies also spread southward, with its current distribution contiguous from southwest Alabama to southeastern Canada. Since 1998, a growing coalition of state, federal, county and municipal as well as Canadian and Mexican expertise has collaborated on rabies management goals and strategies to prevent raccoon rabies from gaining an increasing foot-hold in North America. Coordinated programs have been established from Maine to Alabama to contain raccoon rabies, while exploring elimination strategies. Successes in containing raccoon rabies have been realized through strategies that rely predominantly on oral vaccination. International coordination of programs targeting raccoon rabies continues in eastern Canada, where contingency actions have led to elimination or near elimination in Ontario and New Brunswick. However, increasingly, focus in the United States has been directed toward contingency actions to “hold-the-line” on raccoon rabies spread beyond existing ORV zones (e.g. Vermont, Ohio) or untreated areas (Alabama, Tennessee) where it threatens to spread, rather than increasing emphasis on broader raccoon rabies elimination. We report on these management challenges; progress on initiatives toward new or improved baits, biologicals, and strategies; and a shift toward increasing continental collaboration on rabies control to better assure long-term success in raccoon rabies management.
EVALUATION OF THE STABILITY OF RABIES VACCINE BAITS – FIELD TRIAL

P. Mačiulskis, K. Lukauskas, E. Jacevičius, V. Kiudulas, J. Jokimas, A. Pockevičius
Lithuanian Veterinary Academy, Kaunas, Lithuania

Objective: To analyse the stability of oral rabies virus vaccine baits in terms of virus titre and physical stability of the bait casing over a two week observation period in relation to air temperature, sunlight and rainfall.

Materials and Methods: Two batches of Lysvulpen vaccine produced by Bioveta (Czech Republic) were tested. The vaccine titre indicated by the producer was not less than 107,0 TCID50/dose. A 100 vaccine baits each were distributed by hand in the forest and in the outskirts of the forest in spring and autumn. Air temperature, precipitation and sunlight were daily recorded and the shape and consistency of the vaccine bait casing was documented. Vaccine titration was conducted at the Lithuanian National Veterinary Laboratory, Kaunas.

Results: The mean air temperature during the spring test period was 10,0 °C (10,0 °C ±1,1 °C) and 18,9 °C (18,9 °C ±3,5 °C) during night and day, respectively. It was sunny, no rainfall. The baits, distributed in the forest, were totally covered by the casing for 12 days of the two observation period. On day 3 and 6 the bait casing of 23% and 19% of the tested baits became soft and sticky. On day 6 and 9 one and two baits were disintegrated, respectively, and the vaccine capsule was visible. The vaccine titre on day 9 was 105.6 TCID50/dose. The casing of baits distributed in the outskirts of the forest became soft and sticky on day 3. On day 9 all tested vaccine baits were deformed and 17 out of 27 baits were disintegrated with the vaccine capsules visible. The vaccine titre on day 9 was 103.75 TCID50/dose. The mean air temperature during the autumn test period was 1,5 °C (1,5 °C ± 0,8 °C) and 2,5 °C (2,5 °C ± 1,0 °C) during night and day, respectively. It was heavy rainfall. On day 1 the casing of the baits became soft and sticky and on day 3 the vaccine capsule was visible. The vaccine titre on day 3 decreased to 104.4 TCID50/dose.

Discussion and conclusions: Stability of rabies vaccine baits in the field is very important for the success of oral vaccination against rabies. Some experiments showed that field efficacy of oral rabies virus vaccines depend on the dose and stability of the vaccine virus titre and of the bait casing. In our experiment we demonstrated that the bait casing was more stable in shadow than in sunlight and bait casing became soft and melted in rainfall. The vaccine virus titre was enough stable in dry conditions and vaccine virus titre decreased rapidly in wet conditions. Distribution of baits in rainfall can compromise the efficacy of the oral vaccination program if the baits are not rapidly consumed by wildlife.
IMMUNOGENICITY OF ERA G 333 STRAIN IN FOXES AND RACCOON DOGS

D. Bankovskiy1, 2, G. Safonov1 & Y. Kurilchuk1

1 Pokrov Plant of Biologics, Volginsk, Russia
2 All-Russian Research Institute of Veterinary Virology and Microbiology, Pokrov, Russia

Objectives: The immunogenic properties of the attenuated rabies virus strain ERA G 333, a proposed candidate for oral rabies vaccine in Russia, in foxes and raccoon dogs after oral administration were examined. As demonstrated previously, ERA G 333 (provided by the Centers for Disease Control and Prevention in the framework of the Biotechnology Engagement Program) is apathogenic for 3-week old and adult mice, for other target and non-target species, even when administered intracerebrally.

Materials and Methods: Ten foxes aged 7 to 8 month and 8 wild captured raccoon dogs were orally vaccinated with 2.0 ml of ERA G 333 (titer 107.5 FFU) by direct administration on the tongue. Another 3 foxes and 3 raccoon dogs were used as controls. Blood samples were collected prior to immunization and 60 days post vaccination. Virus neutralising antibody (VNA) titers were determined by the fluorescent antibody virus neutralization (FAVN) test.

Results: Eight foxes and four raccoon dogs showed seroconversion on 60 day post vaccination. In six foxes and three raccoon dogs rabies VNA- titers exceeded 0.5 IU/ml.

This study demonstrated the principle possibility of the use of ERA G 333 for oral vaccination of red foxes and raccoon dogs. However, further experiments are needed to determine the effective doze, virus stability, strength and duration of the immunity.
Adenovirus based vectors are very attractive candidates for vaccination purposes as they induce in mammalian hosts potent humoral, mucosal and cellular immune responses to antigens encoded by the inserted foreign genes. We have generated E1-deleted and replication-competent recombinant canine adenoviruses type-2 expressing the rabies virus glycoprotein (G). The effectiveness of both vectors to express a native G protein have been characterized in vitro in permissive cell lines. Following intramuscular (im) or oral inoculation in mice, we have compared the humoral and cellular immune responses offered by the canine vectors with an Ad5 E1-deleted virus. Humoral responses specific of both adenovirus and the rabies glycoprotein antigens were studied. An influence of the mouse strain was observed using replication-competent canine adenovirus. High levels of rabies-neutralizing antibody were observed upon im inoculation, lower but significant levels upon oral immunization. 100% and 80 % of mice inoculated by the muscular and the oral route, respectively, resisted to a lethal peripheral challenge. These results are very promising in the perspective of oral vaccine for dog rabies control.
DEVELOPMENT OF AN EDIBLE RABIES VACCINE IN MAIZE, USING VNUKOVO STRAIN

E. Loza-Rubio\textsuperscript{1}, E. Rojas-Anaya\textsuperscript{1}, L. Gómez Nieto\textsuperscript{1}, M.T.J. Olivera Flores\textsuperscript{2}, M.A. Gómez\textsuperscript{3}

\textsuperscript{1} National Center of Veterinary Microbiology (CENID-Microbiología), INIFAP, Mexico
\textsuperscript{2} Faculty of Chemistry-UNAM
\textsuperscript{3} CINVESTAV-IPN

The availability of plant-based rabies vaccines for veterinary use would facilitate distribution, administration and long-term storage. The aim of this work was to obtain transgenic maize expressing the rabies virus G protein of Vnukovo strain and to evaluate immunogenicity in mice, by oral route. We employed the ubiquitin maize promoter fused to the whole coding region of rabies virus G gene, and a constitutive promoter from cauliflower mosaic virus (CaMV). Maize embryogenic calli were transformed with the above construct by biolistics. Regenerated maize plants were recovered and grown in a greenhouse. The presence of G gene and its product was detected by PCR and Western blot, respectively. Co-integration percentage in analyzed plants was of 93.3%. The amount of G protein detected in the grains was approximately 1% of the total soluble plant protein. Transformed kernels containing 50 µg of G protein were given once by oral route in adult mice (BALB-C strain). Challenge was done at 90 days post-vaccination using a lethal dose of a vampire bat rabies virus (100 LD50% in mice). The edible vaccine was able to induce neutralizing antibodies which were able to protect mice in 100%, against challenge with a vampire bat-strain, which is one of the main reservoirs in Latin America. Control group did not survive. G protein of Vnukovo strain expressed in transgenic maize may work as oral immunogen against rabies, provoking cross protection.
GENETICALLY ENGINEERED SINGLE-CHAIN ANTIBODY FUSION PROTEINS FOR DETECTION OF RABIES VIRUS ANTIGEN

M. Mousli¹, I. Turki¹, H. Kharmachi² & K. Dellagi¹
Laboratoire Immuno-Biotechnologie
¹ Unité Spécialisée Rage
² Institut Pasteur de Tunis, 13, Place Pasteur BP74 1002, Tunis-Blevedere, Tunisia

Aims / Objectives: The most widely used test for rabies diagnostics is the Fluorescent Antibody Test, which is recommended by both WHO and OIE. This test may be used directly on a smear, and can also be used to confirm the presence of rabies antigen in cell culture or in brain tissue of animal that have been inoculated for diagnosis. The colorimetric enzymes are usually coupled to an antibody by chemical means using cross-linking reagents. However, such non-specific procedures lead to heterogeneous conjugates, sometimes with reduced activity and specificity. To by-pass these problems, genetic engineering has provided a way to create chimeric bifunctional molecules in which the variable domains of an antibody are genetically linked to unrelated proteic tracers. In this study, our objective is to design a novel class of “artificial antibodies” based on a alkaline phosphatase protein-labeled recombinant antibody to develop a simple colorimetric immunoassay method for detecting in vitro the rabies virus glycoprotein in an environmental sample.

Methods: We report the genetic construction and expression of a recombinant enzyme-linked antigen binding protein. A 50AD1 is monoclonal antibody raised against the rabies virus glycoprotein. The genes corresponding to antibody 50AD1 VH and VL fragments were cloned and assembled in synthetic gene coding for a single-chain antibody variable fragment (scFv). Then, the scFv gene was fused in frame with the gene of the bacterial alkaline phosphatase in a prokaryotic expression vector.

Results: The scFv-alkaline phosphatase fusion protein was expressed in periplasm of recombinant bacteria and even released in culture medium. The soluble fusion protein preserved both IgG binding and alkaline phosphatase enzymatic activities as shown by native PAGE-immunoblotting, dot blot, ELISA and immunohistochemistry.

Conclusion: This technology simplifies the production of immunotracers and has other advantages such as better fusion due the possibility of rapid single-step immunodetection. This new approach to the expression of an colorimetric antibody conjugate could lead to generate new reagent that be useful in the intensification of the follow-up of the actions of rabies control programs, such as epidemiological monitoring, or for the online monitoring of vaccine production.
USE OF RABIES VIRUS AS A TRANSNEURONAL TRACER OF NEURONAL CONNECTIONS: IMPLICATIONS FOR THE UNDERSTANDING OF RABIES PATHOGENESIS

G. Ugolini
Lab. Neurobiologie Cellulaire et Moléculaire, NBCM, UPR9040, CNRS, 91198 Gif-sur-Yvette, France

In neurosciences, rabies virus (CVS strain) has become a very powerful tool for studying multisynaptic neuronal connections, due its ability to function as a self-replicating marker and to propagate exclusively between connected neurons by transneuronal transfer, which is strictly time-dependent. Our transneuronal tracing studies of rabies virus propagation in primates and rodents models (asymptomatic period) have provided valuable information on rabies pathogenesis. We have shown that rabies virus propagates by fast axonal transport at similar speeds in primates and rodents, after inoculation into the peripheral or central nervous system (CNS). Intracellular transport of rabies virus is preferentially addressed to neuronal dendrites rather then axons, since transneuronal transfer occurs only retrogradely, i.e., from dendrites of first infected neurons to presynaptic terminals of connected neurons. Rabies virus propagation occurs at chemical synapses, but not via gap junctions or local spread. Our results show that rabies virus receptors have a ubiquitous distribution on neurons within the CNS. Conversely, in the peripheral nervous system, rabies virus receptors are present only on motor endings, since uptake is restricted to motor endplates and axons of fast and slow motoneurons, whereas sensory and autonomic endings are not infected. Thus, after peripheral inoculations, motoneurons are the only gateway for rabies virus propagation to the CNS. Infection of sensory and autonomic neurons requires longer incubation times, since it reflects centrifugal propagation of rabies virus from the CNS to the periphery, i.e., it is the result of retrograde transneuronal transfer to sensory and autonomic terminals within the CNS.
INHIBITION OF RABIES VIRUS REPLICATION BY MICRO-RNA

N.Israsena¹, N. Ratanasetyuth², P. Supavonwong², P. Virojanapirom² & T. Hemachudha³

¹ Department of Pharmacology, Chulalongkorn University Hospital
² Queen Saovabha Memorial Institute, Thai Red Cross Society, Bangkok, Thailand
³ Department of Medicine, Chulalongkorn University Hospital

Objective: To determine whether MicroRNA (miRNA) technology can be used to modulate rabies proteins synthesis and rabies virus replication.

Methods: Neuro-2a cells were transfected with plasmid expressing miRNA designed against multiple location of rabies N, G mRNA, viral genome, and negative controls for 8 hours before challenged with rabies virus (CVS strain). Total RNA from the transfected neuro-2a cells and supernatant were collected at 8, 24, and 48 hours after infection and quantified for rabies virus N,G,P mRNA, and viral genome using real-time PCR technique. Viral N and G proteins in the infected cell were analyzed by direct immunofluorescence assay (DFA) and western blot.

Results: It was found that the miRNA against rabies virus N-protein mRNA(mi-N) effectively reduced amount of viral N,G mRNA in the cell and viral genome both in the cell and in the supernatant. Led to more than 95 % reduction in rabies genomic RNA compared to neuro-2a transfected with control miRNA vector. miRNA against rabies virus G-protein mRNA and viral genome were less effective in inhibiting viral replication. A marked decrease in virus titers from mi-N transfected neuro-2a cells compared to the control group was observed.

Conclusions: These results indicate that miRNA against rabies N-protein mRNA have strong ability to inhibit rabies virus infection in cell culture and may have the potential to be used for therapy in clinical rabies.
RABIES AT THE DAWN OF THE 21ST CENTURY

H. Koprowski
Thomas Jefferson University, Philadelphia, PA – USA

Reference to an ancient Hindu picture of a snarling dog may be convincing enough proof to consider the fact that rabies has been known in the world for the past 50 centuries. Prior to the monumental observation about rabies of Fracastoro in the 16th century, facts and fantasies were intermingled in the study of rabies. In the realm of fantasy, consider the statement of Aristotle, (otherwise a great philosopher), that only animals and not man die of rabies. It took 19 centuries before Fracastoro finally established that infection with rabies is lethal for all warm-blooded beings including humans. The new era of rabies dates from the time of Galtier who isolated the virus and Pasteur who was able to create a somewhat attenuated strain of virus fixe which became the tool of laboratory studies for many decades after Pasteur.

During the last 50 years of the past century, the knowledge of rabies was increased by leaps and bounds. First of all, using molecular biology as a tool it was possible to “take the virus apart” so to speak and describe and analyze all of its components. Establishment of multivariability among viruses as “de la rue” permitted not only a construct of a genetic linkage among lyssa family but also solved some puzzles of pathogenesis of rabies which defied solution when all work was concentrated on one laboratory strain of the virus. As an example, we know much more now about the genetic background regulating virulence of the virus. Also, it is now possible to use rabies as a vector of biological materials such as vaccines or sera.

There is no progress in the treatment of the uniformly lethal disease. Perhaps, optimistically speaking, the 21st Century will bring us a glimmer of hope for the successful treatment of human rabies.
OIE GUIDELINES ON THE CONTROL OF DOG POPULATIONS

S. Kahn¹, L. Stuardo¹ & S.A. Rahman²
¹ OIE Central Bureau, 12 rue de Prony, 75017, Paris, France
² Retd. Dean Bangalore Veterinary College, No 123, 7th B Main Road 4th Block(West) Jayanagar, Bangalore 560 011 INDIA

At the 73rd General Session the OIE decided to develop guidance for Member Countries on humane methods for the control of stray dog populations. In 2006 an ad hoc Group was convened under the auspices of the OIE Permanent Animal Welfare Working Group. With valuable assistance from the OIE Collaborating Centre on Animal Welfare, a Questionnaire was developed and sent to OIE Member Countries, of which 81 countries submitted responses. In light of this information, the ad hoc Group prepared a first draft report, which notes the importance of controlling stray dog populations to help prevent zoonotic diseases and non-disease related nuisances to society and the environment. In choosing the preferred method of control, the risks to operators must be taken into account, as well as religious, cultural and economic contexts of the country. Depending on the situation, methods requiring individual animal restraint or methods for use at a distance may be recommended. While activities that aim to physically reduce the numbers of stray dogs are important, achievement of the long term goals of population control and avoidance of risks to human health depends on education of dog owners and the general public as to their responsibilities. The draft report notes that sub-national jurisdictions are often those responsible for the control of stray dog populations on the ground. The key role played by non-governmental organisations in stray dog management in many countries is acknowledged. The draft report emphasises that the close involvement of veterinarians and of official Veterinary Services, working in collaboration with public health authorities, is necessary to realise long term goals.
Looking backward, we continue to emphasize the fact that rabies is a very neglected totally preventable disease with the highest case fatality rate of any infectious disease known to man and 45 to 60% of all human deaths occur in children. Unfortunately, rabies continues to receive very little attention on a global scale. Even the progress made to reduce, and in some cases eliminate, canine rabies from countries in Latin America has not received the international attention that it should have received. The reasons for the lack of international attention have always focused on the fact that the responsibility for rabies control is split between human health professionals and agricultural entities and the failure of governments in developing countries to recognize and support rabies prevention programs, both of which have lead to a lack of support and funds from international organizations. Although these are valid points, looking forward, it is time for all of us in the rabies community to join together globally and devise a different strategy for increasing global awareness of rabies focusing on the fact that rabies can be totally eliminated from the animal population. Rabies has many unique features that make its elimination a challenge but by using these challenges as a focal point for awareness we can dramatically increase global attention and consequently increase funding support for its elimination. It is only by working together that we will be successful. It is clear that we have all of the tools necessary to eliminate canine rabies. Together we can accomplish this goal by utilizing a four pronged approach which includes the use canine vaccination and control programs, increasing the availability of human rabies biologicals, increasing educational awareness for rabies prevention and Lastly by involving governmental officials to assist in the implementation of programs.
DONT’ FORGET RABIES WHEN YOU SETTLE ABROAD OR TRAVEL WITH CHILDREN

M. Goudal\textsuperscript{1} & Y. Rotivel\textsuperscript{2}
\textsuperscript{1} Rabies Treatment Center, Medical Center, Institut Pasteur, Paris, France
\textsuperscript{2} National Reference Center for Rabies, Institut Pasteur, Paris, France

Human mortality from rabies is estimated to be about 55,000 deaths per year. Half of these deaths concern children who live in developing countries where rabies is enzootic. In France, among 20 cases of rabies in humans that occurred between 1970 and 2006, 10 were children less than 10 years old. All rabies cases were imported from rabies enzootic areas. A retrospective study was carried out in the population of children who received a Post-Exposure Prophylaxis (PEP) at the Pasteur Institute of Paris, after having been bitten or scratched by an animal while travelling in rabies enzootic countries. 204 children from 6 months to 15 years old received PET:

- 41% were exposed to dogs, 30% to cats and 16% to monkeys
- 35% were less than 6 years old
- 60% were boys.

The majority of exposures happened to occur in North Africa (42%) and in Asia (27%).

Medical recommendations and guidelines for preventive vaccinations of travellers visiting high endemic rabies areas and staying there a long time are necessary, especially for families with young children coming back to their native country. Primary pre-exposure immunization consists in 3 boosters (Day0, D7, D21 or D28). In case of exposure to rabies suspect animals, previously immunized subjects, especially children, only need 2 booster doses without administration of rabies immunoglobulin. This is very important for young children (around 3 years old) who often play with animals and who may escape the close surveillance of responsible adults.
DETERMINATION OF THERMOSTABILITY AND MELTING POINT OF THE BAIT CASING FOR THE VACCINE LYSVULPEN

V. Vrzal
Bioveta, a.s., Komenského 212, 683 23, Ivanovice na Hané, Czech Republic

Modified live virus vaccines for oral vaccination of foxes against rabies shall both meet requirements of the European Pharmacopoeia monographs and should also take WHO and EU recommendations into account. The oral rabies virus vaccine LYSVULPEN produced by Bioveta is based on the attenuated rabies virus strain SAD Bern. One vaccination dose contains 1.8 $\times$ 10^7 TCID50 of vaccine virus.

Objectives: The work was aimed at determining both the thermostability of the vaccine virus and the melting point of the bait casing according to EU recommendations. These factors directly influence the potency of vaccines under field conditions.

Materials and Methods: Stability testing was performed under the following environmental conditions: The vaccine titre was determined at day 0 (initial titre) and day 7 after storage at 25°C. Stability of the bait casing was tested at 40 °C for 7 days.

Results: The vaccine titre decreased by 0.4 log10 TCID50 after 7 days at 25°C. The bait casing remained intact under the test conditions. The melting point of bait casing is above 40°C.

Discussion and Conclusions: The results obtained in relation with the 100% protective dose (1.8 $\times$ 10^5.0 TCID50) as determined for the vaccine LYSVULPEN fulfil EU recommendations on thermostability. The vaccine titre at batch release should correspond to at least ten times the dose found to completely protect an experimental group (indicative 100% protective dose). The vaccine titre should not fall below the indicative 100% protective dose following exposure to 25 °C for 7 days. The stability of the bait casing also fulfils EU recommendations: the melting point of the bait casing is above 40°C.

The LYSVULPEN vaccine has been successfully used for rabies control in a number of countries. The use of this vaccine resulted in a drastical decrease in rabies cases in Slovakia and elimination of rabies in the Czech Republic.
TEN-DAY OBSERVATION OF LIVE-RABID DOGS

V. Tepsumethanon, H. Wilde & V. Sitprija
Queen Saovabha Memorial Institute, Bangkok 10330, Thailand
(WHO Collaborating Centre for Research on Rabies Pathogenesis and Prevention)

Objectives: To analyze the ten-day observation and six criteria of live-rabid dogs.

Materials and Methods: The Queen Saovabha Memorial Institute in Bangkok, Thailand, is an institution where owners bring rabies suspected dogs for observation in case the animals had either bitten a person, other animals or in case the animals show abnormal behavior or symptoms of unusual illness. If animals die, detection of rabies antigen in brain smears or impressions using fluorescent antibody testing (FAT) is routinely performed. If the animals survive the 10-day observation period, they are either released to owners or transferred to the municipal animal shelters.

Retrospective and prospective data during the 10-day observations period were collected from 1,222 dogs and 303 cats taken to our institute between 1985 and 2005. Six criteria were analyzed prospectively for 208 dogs developing rabies between 1997 and 2005: age; general condition (sick or healthy); day of sickness until admission; onset of disease; progressiveness of illness during the last 3-5 days and special clinical symptoms such as “circling” in the cage with hitting the head on the wall as if blind.

Results: The experience with the implemented 10-day observation period confirms WHO recommendation on identifying suspected rabid dogs or cats under veterinary supervision after exposure to humans. All dogs and cats found rabid died within 10 days of observation. None of the dogs dying of rabies was younger than 1 month of age. All animals were considered healthy at the first day of observation, had a history of >10 days of illness before the first day of observation, and showed an acute onset of illness. During the last 3-5 days of illness (without treatment) clinical symptoms were stable or the disease was progressive. A striking clinical sign was “circling” as if the animals were blind.
A SIMPLE SANDWICH ELISA (WELYSSA) FOR THE DETECTION OF LYSSAVIRUS NUCLEOCAPSID IN RABIES SUSPECTED SPECIMENS USING MOUSE MONOCLONAL ANTIBODIES

G. Xu1, P. Weber2, Q. Hu1, H. Xue1, L. Audry2, C. Li1, J. Wu1 & H. Bourhy2

1 Wuhan Institute of Biological Products, Wuhan, Hubei Province, China, 430060
2 UPRE Lyssavirus Dynamics and Host Adaptation, World Health Organization Collaborating Centre for Reference and Research on Rabies, National Reference Centre for Rabies, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

Aims: To test a monoclonal antibody (MAb)-based capture enzyme-linked immunosorbent assays (WELYSSA) for the detection of lyssavirus nucleocapsid antigen on its sensitivity and specificity.

Methods: Brain samples of different animal species were obtained from the UPRE Lyssavirus Dynamics and Host Adaptation department at Institut Pasteur, France. Among the tissue samples, 67 were positive and 963 were negative for rabies virus. All positive samples were characterized by partial or complete sequencing of the N gene and were selected that way to represent the genetic diversity of lyssaviruses circulating in Europe, Africa and Asia. The nucleocapsid of the PV strain was purified. A combination of four mouse monoclonal antibodies (MAb) directed against the rabies virus nucleocapsid was selected and used for the detection in ELISA.

Results: Using prototype viruses from different genotypes of lyssaviruses and from various geographic origins and phylogenetic lineages, the threshold of the WELYSSA ELISA for the detection of lyssavirus nucleocapsids is low (0.8 ng/ml). With a panel of 1030 specimens received for rabies diagnostic testing, this test was found to be highly specific (0.999) and sensitive (0.970) when compared to other recommended rabies diagnostic methods.

Conclusions: The test was optimized and standardized so that maximum concordance could be maintained with the standard procedures of rabies diagnosis recommended by the WHO expert committee.
RABIES RESEARCH TRENDS IN THE FGI “FEDERAL CENTRE FOR ANIMAL HEALTH”

Federal Governmental Institution “Federal Centre for Animal Health”, 600901, Yur’evets, Vladimir, Russia

The FGI “Federal Centre for Animal Health” (FGI “ARRIAH”) is the All-Russia Research Centre for Rabies. The scope of activities comprises (i) animal rabies surveillance and diagnosis, (ii) improvement of rabies diagnostic tools and methods, (iii) production of anti-rabies vaccines and evaluation of their potency and safety, and (iv) molecular and biological characterisation of rabies viruses and vaccine strains.

The following techniques are established for laboratory diagnosis of rabies: the fluorescent antibody test (FAT), mice inoculation test (MIT), the rabies tissue culture infection test (RTCIT), ELISA, latex agglutination, histological and immunohistochemical methods. Monoclonal antibodies and reverse transcriptase polymerase chain reaction (RT-PCR) followed by sequencing are used for the molecular and antigenic characterisation of rabies viruses.

As a result of our work a commercially FAT rabies diagnostic kit has been produced. This kit contains all reagents needed to conduct FAT including buffer ingredients as well as positive and negative lyophilized control samples. Also, we have developed a direct liquid-phase FAT method for rabies diagnosis in brain samples conserved in formalin, which was patented in the Russian Federation. This method allows testing of samples conserved in 10% formalin for at least 3 months. The FGI “ARRIAH” is an active partner in the Russian-Finnish Collaboration Program for the Control of Rabies in Wildlife.
RFFIT-MODIFIED ANTIBODY-BINDING TEST (ABTMR) AS A FAST AND REPRODUCIBLE TEST FOR DETERMINATION OF POTENCY OF INACTIVATED PURIFIED NON-ADJUVANTED RABIES VACCINES FOR HUMAN USE

S. Stankov, V. Simin, D. Vujin, U. Ungurović, N. Vranješ & J. Desnica
Pasteur Institute, Hajduki Veljkova 1, 21000 Novi Sad, Serbia

Aims/Objectives: Despite its serious drawbacks such as a low reproducibility, the considerable duration and the required number of about 100 mice per test sample, the NIH test is still the only internationally recognized potency test for inactivated rabies vaccines is. Promising results had previously been obtained with antibody-binding tests (ABT) as a possible replacement for NIH test. However, current versions of ABT are not suitable yet for vaccine batch release by national control laboratories due to specific adaptations to PCEC vaccine production systems. Here we present a modification of the ABT called RFFIT-modified ABT (ABTmR), which differs from current ABT in use of CVS strain and mouse neuroblastoma (MNA) cells in the second and third phase of the test, respectively.

Materials and Methods: Test sensitivity is determined by the antigenic value of the test vaccine sufficient to yield fluorescent foci in the lowest dilution used. Reproducibility was asessed by comparing results for a single sample in eight consecutive tests. Furthermore, comparison of results obtained in ABTmR and NIH tests was done using four different samples of the Verorab and Rabipur vaccines.

Results / Discussion / Conclusions: The total duration of ABTmR is 26-28 hours, confidence limits range from 78% to 129% of the estimated vaccine potency in IU/ml, and sensitivity is about 0.5 IU/ml. In conclusion, the ABTmR is both a promising rapid and reproducible in vitro test for batch release of purified inactivated rabies vaccines and an in process control test for manufacturers of rabies vaccines. Therefore, it is a candidate for the replacement of the NIH test.
We report the first full-length genomic sequences for European bat lyssavirus type-1 (EBLV-1) and type-2 (EBLV-2). The EBLV-1 genomic sequence was derived from a virus isolated from a Serotine bat in Hamburg, Germany in 1968 and the EBLV-2 sequence derived from a virus isolate from a human case of rabies that occurred in Scotland in 2002. A long distance PCR strategy was used to amplify the open-reading frames (ORFs), followed by standard and modified RACE (rapid amplification of cDNA ends) techniques to amplify the 3’ and 5’ ends. The lengths of each complete viral genome for EBLV-1 and EBLV-2 were 11,966 and 11,930 base-pairs respectively and follow the standard rhabdovirus genome organisation of five viral proteins. Comparison with other lyssavirus sequences demonstrates variation in degrees of homology with the genomic termini showing a high degree of complementarity. The N-protein was the most conserved both intra- and intergenotypically, followed by the L-, M- and G- with the P-protein being the most variable. In addition, we have shown that both EBLVs utilise a conserved transcription termination and polyadenylation motif, approximately 50 nucleotides upstream of the L gene start methionine. All available lyssavirus sequences to date, including the EBLVs, use the second TTP site with the exception being Pasteur Virus (PV) and PV-derived isolates. This observation may explain differences in pathogenicity between lyssavirus strains, dependent on the length of the untranslated region, which might affect transcriptional activity and RNA stability.
OVERVIEW OF RABIES IN GREECE FROM 1966 TO 2006

O. Mangana¹, K. Nomikou¹, P. Lliadou¹ & G. Anastasiadis²
¹ Virology Department, Centre of Athens Veterinary Institutes, 25 Neapoleos Str. Athens 15310 Greece
² Virology Department, Centre of Thessaloniqui Veterinary Institutes, 80 26th Octovriou Str. Thessaloniqui 54627 Greece

The history and epidemiology of rabies in Greece from 1966 to 2006 is described. During the post – World War II period an increase of rabies cases was noticed in Greece with a peak in 1954 resulting in 1,153 reported rabies cases. After this year, rabies cases drastically decreased until the detection of the last rabies case in year 1987.

During the decades 1966-1975 and 1976-1985, a total of 1,036 and 39 rabies cases were reported, respectively. The majority of rabies cases in Greece were due to dog-mediated rabies; the last rabies case in wildlife was reported in a fox in 1974. In 1970, the last human case was reported and in 1987 the last animal rabies case- a hunting dog- was reported from the prefecture of Evros, near the Turkish border. Since then, no human or animal rabies cases have been reported.

All suspected domestic or wild animals submitted for rabies diagnosis during the period 1988-2006 were negative. The fluorescent antibody test (FAT), mouse inoculation test (MIT) and the rabies tissue culture infection test (RTCIT) are standard techniques used for rabies diagnosis. Antigen detection ELISA was also applied when test kits were available. Compulsory vaccination campaigns of dogs and the low prevalence of rabies among wild animals resulted in rabies elimination from the country. As rabies is still present in neighboring countries Greek authorities are very precautious in the future.
BAYESIAN ESTIMATION OF RABIES AB-ELISA PERFORMANCES IN ABSENCE OF A GOLD STANDARD

S. Guillossou\(^1,3\), M. Rabilloud\(^1\), S. Leterme\(^2\) & R. Ecochard\(^1\)

\(^1\) Biostatistics Department, Hospices Civils de Lyon, Lyon, France
\(^2\) Synbiotics Europe, Lyon, France
\(^3\) Synbiotics Corporation, San Diego, CA, USA

**Background:** Today, international rabies control is based on antibody measurement after vaccination and relies partially on diagnostic tests interpretation. Unfortunately, it is admitted that there is no perfect reference test.

**Objectives:** Bayesian approach for latent class analysis has been one of several statistical methods proposed to estimate tests parameters such as sensitivity and specificity.

**Material and Methods:** Two European populations of vaccinated dogs and cats were used to determine the performances of two tests FAVN and ELISA. Estimation of prevalence, sensitivities and specificities of the two tests are obtained by combining data observed from populations to information from previous studies named informative priors.

**Results:** Estimates of the sensitivities and prevalence were high (prevalence: 96.1%; FAVN sensitivity: 98.9%; ELISA sensitivity: 93.8%) and are in agreement with previously published data. Estimations of the specificities were close to prior information and did not differ significantly between the two tests.

**Discussion:** The results obtained underline the need for adequate informative priors in Bayesian estimations as well as the need for adequate sample size. Data suggest also that FAVN and ELISA specificities are often imperfectly determined.

**Conclusions:** This innovative approach may be useful in designing improved tools in the Pet Travel Scheme.
THE FIRST CASE OF EUROPEAN BAT LYSSAVIRUS TYPE 1B INFECTION IN E. SEROTINUS IN POLAND

M. Smreczak, A. Orłowska, P. Trebas & J.F. Zmudzinski
National Veterinary Research Institute, Virology Department, 24-100 Pulawy, Poland

Aims/Objectives: For centuries, rabies has been present in many different species of terrestrial animals. In Poland in recent years, rabies virus was found in bats representing a risk for public health. The aim of the study was to describe the first isolation of EBLV-1b virus from bats in Poland and to compare its nucleoprotein sequence with sequences available in GenBank.

Materials and Methods: In this study FAT positive bat brains submitted for rabies diagnosis from regional laboratories were analysed. All positive samples from the field were confirmed by mouse inoculation test (MIT) at the national reference laboratory for rabies at the National Veterinary Research Institute. EBLV-positive bat brains or first mouse passage brains were used for RNA extraction and subsequent hemi-nested RT-PCR (hnRT-PCR) amplification. Amplicons were subjected to sequencing in sense and anti-sense directions. The first 400 bp of nucleoprotein sequences were aligned using Clustal W programme with 22 N sequences available in the GenBank. Phylogenetic analysis was conducted with the DNA maximum likelihood method using BioEdit software (7.0.5 version).

Results / Discussion / Conclusions: The hnRT-PCR amplified 410 bp products of the n-gene of EBLV-1. The phylogenetic analysis revealed that all bat rabies virus isolates belonged to EBLV-1 (genotype 5). The maximum likelihood method demonstrated that the EBLV-1 isolates could be divided into two phylogenetic groups. One out of four isolates was similar to the subgroup EBLV-1b and formed a cluster with other known EBLV-1b isolates from Europe. This is the first case of isolation and identification of EBLV-1b type from serotine bats (Eptesicus serotinus) from Poland.
PRELIMINARY RESULTS OF AN ACTIVE SURVEY OF BAT RABIES IN BELGIUM

L. Audry², F. Klein¹ & H. Bourhy²
¹ Institut de Santé Publique / Département Pasteur, Engelandstraat 642, B-1180 Bruxelles
² Institut Pasteur 25-28 rue du Docteur Roux F-75724 Paris CEDEX 15, France

For Belgium only scarce data about bats were available before 2005. Conventional testing of dead found bats did not yield significant findings because of poor conservation conditions. During a 15 year period, only about 30 brains samples from bats were tested by IFI or RT-PCR; none were positive.

Since 2005, active rabies surveillance in bats has been established to gain better information on the presence of bat rabies in Belgium. In order to cause minimal disturbances to the reproductive and social behaviour of the protected bat species, captures were carried out after the weaning of offsprings and before the hibernation from mid august to the first cold weather period, in front of the entrance of natural caves not used as nursery. In 2005, free-living bats were captured in 6 caves from Thierache and Haute Ardenne belonging to 6 different species (Myotis myotis, Myotis mystacinus brandti, Myotis nattererii, Myotis bechsteinii, Myotis daubentonii and Plecotus spp). A total of 24 sera from 40 bats could be analysed for the presence of EBLV-1 antibodies. High EBLV-1 VNA (>1:100) were found in 1 out of 8 Myotis myotis and 1 out of 8 Plecotus spp. Virus neutralising antibodies were also found at lower levels in 1 Myotis myotis and in 2 Myotis nattererii.

Those preliminary results underlined that serology is more sensitive than virology to control bat exposition to rabies virus and to have an index of virus circulation among bats. A better epidemiological description of the viral circulation(s) is necessary to assess the risk exposure of humans and domestic animal to bat rabies virus.
EXPERIMENTAL IMMUNIZATION OF CATS WITH A RECOMBINANT RABIES-CANINE ADENOVIRUS VACCINE

R.L. Hu, Y. Liu, S.F. Zhang & F. Zhang
Laboratory of Epidemiology, Veterinary Institute, Academy of Military Medical Sciences, 1068 Qinglong Road, Changchun 130062, P.R. China

During the past decade, human rabies caused by cats has ranked the second highest in China. Several recombinant rabies vaccines have been developed for dogs. However, seldom have these vaccines been assessed or used in cats. In this trial, we report the experimental immunization of a recombinant canine adenovirus-rabies vaccine, CAV-2-E3Δ-RGP, in cats. Thirty cats were inoculated with the recombinant vaccine intramuscularly, orally and intranasally. Safety and efficacy studies were undertaken using the fluorescent antibody virus neutralization (FAVN) test and evaluated. Results showed that this recombinant vaccine is safe for cats as demonstrated by the three different routes of administration. The vaccine stimulated an efficient humoral response in the vaccinated cats when 108.5 PFU/ml of the recombinant vaccine was injected intramuscularly in a single dose. The neutralizing antibody level increased above 0.5 IU/ml at 4 weeks after the vaccination. The mean antibody level ranged from 0.96±0.26 to 4.47±1.57 IU/ml among individuals, and the antibody levels were elicited for at least 12 months. After this period, the immunized cats survived the challenge of CVS-24 and an obvious anemnestic and protective immune response was stimulated after the challenge. The immune response occurred later than the inactivated vaccine and the overall antibody level in the vaccinated cats was lower, but it was sufficient to confer protection of cats against infection. This demonstrated that a single, intramuscular dose of CAV-2-E3Δ-RGP stimulated a long-lasting protective immune response in cats and suggested that CAV-2-E3Δ-RGP could be considered as a potential rabies vaccine candidate for cats.

F.G. Bastida-González¹, J.M. Jiménez-Estrada¹, M.D. Hernández-Ramírez¹, G. Arteaga-Troncoso², F. Guerra-Infante², A. Meléndez-Felix³, J.L. Rubí-Salazar¹ & M.E. Barrera-Tapia¹

¹ Laboratorio Estatal de Salud Pública del Estado de México, ISEM
² Instituto Nacional de Perinatología, SSA
³ Instituto de Diagnostico de Referencia Epidemiológico, SSA

Background: A concise description of the epidemiology of rabies virus in the State of Mexico, Mexico, and the characterisation of the rabies virus variants.

Objective: To determine the seasonal incidence of human and animal rabies cases in the State of Mexico, Mexico, and to identify rabies virus variants circulating in the region.

Materials and Methods: Brains samples from animals originating from the State of Mexico, Mexico, were forwarded to the State Laboratory of Public Health (LESP). Initially, brain specimens from different animal species were examined with the IFD. Specific identification of rabies virus variants (RABV) was performed using IFA. Kruskal-Wallis test was used to compare differences in the incidence of rabies cases between seasons of the year.

Results: Between 1996 and 2006, a total of 14,588 brains samples were tested for the presence of rabies virus antigen, of which 190 samples (1.3%) were positive for rabies in IFD. Out of the 190 rabies positive animals, 146 (76.8%) positive samples originated from dogs, 32 (16.8%) from cows, 2 (1.05%) from bats, 3 (1.6%) from humans, 2 (1.05%) from cats, 2 (1.05%) from pigs, 2 (1.05%) from sheeps, and 1 (0.5%) from mustelids. The characterization RABV identified 3 variants circulating in the State of Mexico with the majority of rabies cases (N=76; 74.29%) caused by variant V1, followed by variants V8 (22.86%) and V3 (2.86%). An analysis of the seasonal frequency of rabies cases during the year showed that 2.83%, 1.96% and 0.06% of the specimens submitted for rabies diagnosis to LESP in winter, spring and summer-autumn seasons, were rabies virus positive respectively.

Conclusions: Rabies infections of terrestrial species occur in the State of Mexico, and disease transmission can persist at endemic levels for decades.
Bats are important reservoir hosts of RNA viruses, including lyssaviruses, which can cross the species barrier to infect humans and other domestic and wild non-flying mammals. Bats are primary lyssavirus reservoirs on all inhabited continents. Six of the seven Lyssavirus genotypes described to date infect bats. In Europe, two genotypes of Lyssavirus, European bat lyssavirus types 1 and 2 (EBLV-1 and EBLV-2), circulate among several bat species and numerous bats are found infected every year. To provide epidemiologists and public health officials with data to effectively implement public health measures, we have undertaken field studies to identify the temporal dynamics of virus infection in bat colonies by combining multidisciplinary approaches.

We have focused our work on a long-term longitudinal survey of different bat colonies in the Balearic Islands. The prevalence of virus RNA and neutralizing antibodies was analysed in captured free-living bats. The bats were ringed to allow individual monitoring of infection and movements between colonies. Modelling was used to calculate the survival of bats during infection.

The results show different lyssavirus infection episodes across a twelve year study period and no mortality of bats after infection. These findings have important implications on the monitoring of colonies where positive bats have been identified.
In two separated studies we compared the immune responses of goats and lambs vaccinated with an inactivated antirabies vaccines produced in Serbia.

Twenty adult goats and 9 lambs were vaccinated. The lambs were born to vaccinated goats and were tested in intervals of 26-52 days. Virus neutralising antibody (VNA) titers were determined for the period of 8 months after vaccination and/or 21 months after the second vaccination (booster) as well as 10.5 months after third vaccination using RFFIT.

Inactivated antirabies vaccine (RABIVET, Veterinarski Zavod Zemun) is based on PV strain of rabies virus (PARIS/BHK/ purified / 3 passages). The virus was propagated in BHK-21 C13 cells using roller bottles, subsequently inactivated using beta-propiolactone (0.04%) adsorbed on AlPO4 (1.5 mg/ml) and conserved with thiomerosal (0.01%). Three batches of RABIVET vaccine had AV of 1.3, 2.2 and 10.4 IU/ml. An imported commercial vaccine had an AV of 7.4 IU/ml (NIH test). The AV of second lot of commercial vaccine was unknown. We concluded that immunological responses after first vaccination is poor with all vaccines used. Immune response was much better after the 2nd (21 months) and 3rd vaccination. In a second study, satisfactory level of VNA was achieved after the 1st vaccination, which lasted up to 6 months.

Maternally transferred rabies antibodies were detected in 4 (44.4%) of 9 lambs. There was an impartially impaired immune response until day 52 post partum.
Rabies, one of the major zoonotic diseases, remains an important but under-reported health problem in many countries, in particular developing countries. Current rabies vaccines or rabies infection induce the formation of antibodies directed to different viral proteins. Although virus-neutralising antibodies are not the only factor responsible for protection against rabies, their presence (mainly against the envelope glycoprotein) in serum is used as a relevant indicator of the degree of immunity in case of human pre-exposure rabies vaccination or during post-exposure treatment.

Virus neutralisation tests (RFFIT / FAVN) are the current prescribed reference methods. But these methods are time-consuming, expensive, need very well-trained technicians and laboratory facilities in accordance with the need of handling live rabies virus.

The PLATELIA™ RABIES II is an ELISA assay based on titration of anti-glycoprotein antibodies in sera. This test may be the method of choice because it is simple (no need of specialised laboratory containment), safe (no use of life virus), and rapid (less than 4 hours) and can easily be automated.

Sensitivity, specificity, linearity, accuracy of the test was evaluated using human serum samples from a total of 1348 vaccinated or non-vaccinated people. The results obtained with the ELISA were compared to results obtained with the RFFIT. The data generated indicate a linear relationship across the range of titration between the two methods and show that this new ELISA test is as sensitive and specific as the current standardized reference methods. The method is simple, safe, rapid and can be considered as a useful alternative to seroneutralisation assays.
Yearly vaccination of dogs and cats as well as oral vaccination in foxes has been a major element for many countries to eradicate rabies from their territory.

To assess the seroconversion after vaccination, measurement of the level of rabies anti-glycoprotein G antibodies is a relevant indicator.

A new microplate assay, PLATELIA™ RABIES II, for the detection and titration of these particular antibodies has been developed by Bio-Rad Company for a use on dogs, cats and foxes.

An evaluation of PLATELIA™ RABIES II has been performed on 1778 animals from different populations of dogs, cats and foxes in collaboration with the WHO/OIE/CRI rabies reference laboratory of AFSSA Nancy France.

The results of this evaluation and the correlation with reference methods have been presented to the OIE scientific experts in 2006.

As a result, PLATELIA™ RABIES II is the first ELISA assay to have passed the Certification of Diagnostic Assay in regard to the OIE procedure.
The European bat lyssaviruses (EBLV) type-1 and -2 (genotypes 5 and 6) are emerging zoonoses and have been known to infect not only their primary hosts (insectivorous bats) but on rare occasions other incidental animal hosts. EBLVs are bat variants of rabies virus that have evolved and adapted to bats between 5000 – 9000 years before rabies virus was identified in terrestrial mammals suggesting that spillover of rabies virus strains from Chiroptera to Carnivora has occurred. Molecular typing, particularly using the N- (nucleoprotein) and G- (glycoprotein) gene, provides epidemiological information regarding the geographical and host origins of the viruses analysed. The ability to derive useful information from virus sequence data is dependent upon the availability of an extensive database. Sequence and clinical information derived to date has been collated into a central European molecular epidemiological database [http://www.medvetnet.org/cms], which is freely accessible online to all researchers throughout Europe. This database will facilitate the interpretation and publication of sequence data to enable rapid genotyping of new viruses and provide a greater understanding of the geographical and host specific evolution of the European bat lyssaviruses. Additionally, genotyping viral isolates from European bats will act as an ‘early warning system’ to the emergence of new lyssavirus strains throughout Eurasia.
AN OUTBREAK OF PIG RABIES IN HUNAN PROVINCE OF CHINA

Y. Jiang¹, X. Yu², L. Wang¹, H. Xuan³, Z. Hu¹ & C. Tu¹
¹ Academy of Military Medical Sciences, Changchun, 130062, China
² Veterinary College of Hunan Agricultural University, Changsha, 410128, China
³ Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China

In 19 December of 2005 a rabies outbreak in pigs emerged in Yongzhou city of Hunan province where severe dog-associated human rabies is prevalent. The affected pig farm raised 56 fattening pigs in 4 pens linked together in a line. The outbreak was initiated by a dog which was from the farmer’s neighbor and found to be acting strangely, roaming to pen 2 and leaving a bleeding bite on the snout of a pig through the pen wall bars. This injured pig developed a furious disease 20 days post exposure, then started to bite other pigs in the same herd. In following days the cases increased in pen 2 with a few furiously rabid pigs jumping the pen walls to bite pigs in the two flanking pens, triggering the rabies outbreak in 3 pens. The clinical manifestations included hyperexcitation, roaring and attack on other pigs within the herd. Some affected pigs exhibited spasms in response to tactile, auditory and visual stimuli, and some showed hydrophobia and fear of people. This outbreak resulted in 20 pig deaths which occurred 2 to 3 days following the appearance of symptoms.

The cases were subjected to laboratory diagnoses and all tests showed positive results in the detection of rabies virus by fluorescent antibody test, mouse inoculation test and RT-PCR. The N gene-based phylogenetic analysis showed that the pig isolate was genetically close to the viruses circulating in dogs, indicating that the outbreak in swine was caused by that rabid dog. This was the first laboratory confirmed pig rabies in China.
ISOLATION OF EUROPEAN BAT LYSSAVIRUSES (EBLVS) ON NEUROBLASTOMA CELLS IN COMPARISON WITH MOUSE INOCULATION TEST

J.A. Kramps¹, E. Van Weezep¹, E.R.A.M. Verstraten¹, W.H.M. Van Der Poel² & E.A. Kooi¹
¹ Central Institute for Animal Disease Control (CIDC-Lelystad), Wageningen University and Research Centre, Lelystad, The Netherlands
² Animal Sciences Group, Wageningen University Research Centre, Lelystad, The Netherlands

Aims/Objectives: In rabies diagnosis a questionable Fluorescent Antibody Test (FAT) result or a negative FAT result with evidence of exposure of the animal to humans often needs to be confirmed by a Mouse Inoculation Test (MIT). To avoid animal experiments, the expensive and laborious MIT should be replaced by virus isolation in cell culture. Previously different groups described that virus isolation using murine neuroblastoma (MNA) cell culture for isolation of rabies street virus (genotype 1) is at least as sensitive as the MIT. The aim of the study was to investigate the sensitivity of MNA cells for EBLV isolation in comparison to MIT.

Materials and Methods: EBLV was isolated from the brain of an Eptesicus serotinus (E.s.) bat. Ten-fold serial dilutions of EBLV of a virus stock were prepared in 10% brain suspensions from 11 non-infected E.s. bats. MIT was performed by intercerebral injection of 30 µl suspension in 5 mice for each virus dilution. Virus isolation was performed by mixing 30 µl suspension with 2.25x10⁴ MNA cells in 150 µl DMEM/1%strep/1%fungizone/10%FCS/0.005 % (w/v) DEAE-dextran. After incubation for 4 days at 35ºC and 5% CO₂ cells were fixed in 80% acetone and stained with anti-rabies FITC-conjugate. MID50 (mouse infective dose) and TCID50 were calculated according to Spearman & Kärber.

Results: The table summarizes the results of the experiments:

<table>
<thead>
<tr>
<th>Virus dilution:</th>
<th>Experiment 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIT (10 wks old mice)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁰</td>
<td>3† of 3</td>
<td>5† of 5</td>
<td>3† of 4</td>
<td>0† of 4</td>
<td>0† of 4</td>
<td>0† of 4</td>
<td>0† of 5</td>
<td>0† of 5</td>
<td>0† of 5</td>
</tr>
<tr>
<td>cell culture</td>
<td>5+ of 5</td>
<td>5+ of 5</td>
<td>5+ of 5</td>
<td>1+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
</tr>
<tr>
<td>MIT (baby mice)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁰</td>
<td>5† of 5</td>
<td>5† of 5</td>
<td>5† of 5</td>
<td>0† of 5</td>
<td>1† of 5</td>
<td>1† of 5</td>
<td>0† of 5</td>
<td>0† of 5</td>
<td>0† of 5</td>
</tr>
<tr>
<td>cell culture</td>
<td>5+ of 5</td>
<td>5+ of 5</td>
<td>5+ of 5</td>
<td>4+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
<td>0+ of 5</td>
</tr>
</tbody>
</table>

Based on the results as presented in the table, the following infective doses were calculated:

Experiment 1: MID50/ml = 5.6 and TICD50/ml = 5.8
Experiment 2: MID50/ml = 6.0 and TICD50/ml = 6.6

Conclusion: Based on the results obtained it can be concluded that virus isolation on MNA cells is at least as sensitive as MIT in demonstrating the presence of European Bat Lyssavirus (EBLV) in brain tissue of E.s. bats.
RABIES SURVEILLANCE, DIAGNOSIS AND ERADICATION IN AUSTRIA

E. Vanek¹, Z. Bagó¹, S. Revilla-Fernández¹, E. Wodak¹, J. Weikel¹ & A. Höflechner²

¹ Austrian Agency for Health and Food Safety (AGES), Institute for Veterinary Disease Control Moedling
² Federal Ministry for Health and Women 1030 Vienna

This report gives a graphic description of the ongoing and successful rabies eradication program in Austria which is performed by the National Reference Laboratory at the AGES Institute for Veterinary Disease Control at Moedling and supported by the BMGF (Federal Ministry for Health and Women). This rabies eradication program is based on oral vaccination of foxes, monitoring of vaccination campaigns (bait uptake and seroconversion) and rabies surveillance.

Interruption of rabies transmission from terrestrial animals to other terrestrial animals and to humans using oral vaccination of foxes is now a successful tool in rabies control. The results of intensive rabies surveillance in wildlife and domestic animals in Austria of the last years are presented. For this purpose different diagnostic tools such as the Fluorescence Antibody Test (FAT), Immunohistochemistry (IHC), Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Virus Isolation in Cell Culture are applied. This report gives an overview of the eradication as well as of preventive measures, their efficiency and side effects, and the tools to check the efficiency of these measures in terms of Fluorescence Antibody Virus Neutralisation Test (FAVNT), Enzyme Linked Immunosorbent Assay (ELISA) and FAT.

We share our experiences with the use of a life attenuated rabies virus vaccine and the difficulties of establishing blood sampling in foxes. Results obtained with an ELISA technique to evaluate the efficiency (seroconversion) of the vaccination programme in foxes are shown. Finally, the costs spent for vaccination, surveillance, diagnosis, and eradication measurements per year are presented.
A RABIES CELL PHONE ADVISORY SYSTEM: A CULTURALLY TARGETED TOOL DESIGNED TO REDUCE HUMAN RABIES SUFFERING IN THE PHILIPPINES

E. Adriano1, M. Vinluan2 & S.J. Scholand3
1 ARF World Philippines, Quezon City, Philippines
2 Department of Health, Manila, Philippines
3 A Rabies Free World, Philadelphia, United States of America

Morbidity and mortality from human rabies in the Philippines remains high. Major factors responsible for the continued problem include lack of knowledge regarding aspects of disease prevention, perceived high cost of treatments, cultural inhibitions to legitimate medical care, as well as finite resources. In resource constrained areas where rabies is endemic, low cost technologies developed to address these issues could reduce suffering by providing information to make more intelligent decisions.

We sought to develop a cell phone tool to disseminate information about rabies to the people of the Philippines. We envisioned users of the system to increase their understanding of rabies so as to more appropriately access existing health resources.

We contracted a private company to develop a cell phone accessible information system designed to provide basic information about rabies. Through “text messaging”, users can download to their cell phone information on rabies including: rabies prevention, rabies prevalence, disease basics, appropriate treatment for potential rabies exposures, and animal and dog information. Additionally, the system provides the location of Department of Health (DOH) approved Animal Bite Treatment Centers and Bureau of Animal Industry recognized veterinary diagnostic laboratories.

The three major telephone carriers in the Philippines now support this tool, with the potential to reach up to 20 million cell phone users. Promotional efforts to make the tool more widely known are ongoing. Future studies to assess the impact of the rabies cell phone advisory system in terms of health access and utilization are planned.
In Poland, for decades the predominant rabies reservoir has been the red fox (Vulpes vulpes). However, since the last decade rabies in the raccoon dog (Nyctereutes procyonoides) has been of increasing concern. Therefore, it is of utmost importance to determine the role of this species in epidemiology of rabies in certain parts of the country. The implementation of the oral rabies vaccination (ORV) of foxes significantly influenced the epidemiological situation of rabies. ORV was implemented at the western border of Poland in 1993 and successively expanded eastwards in subsequent years to eventually cover the entire territory of Poland in 2002.

The purpose of this study is to assess possible key factors contributing to the maintenance of rabies in raccoon dogs in Poland. We investigated the following variables predictive of rabies incidence in raccoon dogs on a district level: (i) rabies incidence in foxes, (ii) raccoon dog population density, (iii) time for initiation of ORV in years, (iv) calendar year and (v) variant of genotype 1 rabies virus circulating in a given area. During the years 2003-2006 incidence of raccoon dog and fox rabies ranged from 0 - 4.25 and 0 – 6.4 cases per 10,000, respectively. Results of a multivariate analysis indicate that the time from initiation of ORV is the most influential factor causing a 34% decrease in raccoon dog rabies incidence per each additional year of vaccination. Independently, an increase of 1 case per 10,000 in fox rabies incidence resulted in an approximately 4% increase in raccoon dog incidence. The virus variant seems to play less significant role.
IMPLEMENTATION OF ULTRA-LIGHT AIRCRAFTS IN BAITS DISTRIBUTION FOR PER-ORAL VACCINATION OF VENISON AGAINST RABIES

M. Sinkovic¹, D. Bosiljka² & D. Lalosevic³
¹ Dr. Sinkovic Mirko, Zoo graden - Palic, Republic of Serbia
² Prof. Dr. Bosiljka Djuricic, Faculty of Veterinary Medicine, Belgrade, Republic of Serbia
³ Dr. Dusan Lalosevic, Medical Faculty, Novi Sad, Republic of Serbia

For many years rabies has been present in the northern part of the Backa region, Serbia. The number of rabies positive animals ranged between 1 – 16 per year with the majority of rabies cases found in foxes followed by cats and dogs. Based on its topography, the North Backa region is considered an ideal habitat for foxes with an estimated population of 1400 to 2200. Considering the fact that the fox is the main rabies reservoir in Serbia oral rabies vaccination (ORV) of foxes was launched by distributing vaccine baits using ultra-light aircrafts.

Materials and Methods: A total of 20,000 vaccine baits were used for ORV (donation of Pharmaceutical Company “Virbac”, SAG 1 oral, in ampoules with 1,75 ml suspension content, with concentration 10 7.5 GKD 50/ml). The vaccine baits were stored at -20°C prior to distribution in the field. The vaccination area comprised two lakes (Palicko lake and Ludosko lake) and one nature conservation area (Selevenjska forest) considered ideal habitats for foxes, birds and other wild animals. Vaccine baits were distributed during five days in February 2002 using ultra-light aircrafts of the local aero-club in Subotica. The temperatures were around + 4°C without snow. Bait density was 18 – 24 vaccine baits per km².

Results and Discussion: In the following 16 months, in the territory of Subotica, only one case of fox with rabies was reported. During the next two years, the number of foxes increased resulting in an increase in the number of fox rabies cases in regions outside of the vaccinated territory. Based on our experience using ultra-light aircrafts the distribution of vaccine baits and screening of foxes habitats is more exact and easier simply because the speed of the aircraft is lower and maneuver abilities are better. Minimal logistic support, simple and easy maintaining and easy movement (if necessary) make ultra-light aircrafts an alternative choice for aerial distribution of baits. Logistic and media support is needed and proved helpful.

Conclusion: ORV using aerial distribution of vaccine baits was implemented for the first time in Serbia, Subotica community. ORV is the method of choice for use in the whole Republic taking experiences of other countries into account. The use of ultra-light aircrafts is cost-effective and easy to perform under field conditions.
RETROSPECTIVE ANALYSIS OF 174 HUMANS WHO RECEIVED P.E.P. (RABIES IMMUNOGLOBULIN (RIG) AND VACCINE)

M. Goudal¹, Y. Rotivel¹, A. Simmons De Fantis² & D. Van Der Vliet²

¹ National Reference Center for Rabies, Institut Pasteur, Paris, France
² Rabies Treatment Center, Medical Center, Institut Pasteur, Paris, France

WHO recommends the use of RIG and vaccine for severely exposed (category III) subjects. The possibility of an interference of RIG and antibodies elicited by inactivated vaccine has been reported. To evaluate this effect the Rabies Treatment Center of the Pasteur Institute in Paris decided to prescribe antibody titration to patients who received RIG and vaccine simultaneously. Detection of rabies specific antibodies was conducted using the Platelia rabies II test on Evolis Automat during routine testing.

During a two years period (2005 and 2006) 174 persons were given both RIG and vaccine against rabies. A total of 154 antibody titrations (88.5%) were performed on the last day of treatment. Antibody titters as determined by ELISA were greater than 1 UE/ml for 133 patients and correspondent treatments were finished. For 13 patients the antibody titers ranged between 0.5 and 1 UE/ml resulting in an additional vaccine shot for 6 patients. Four of the 6 developed an antibody titer between 2.1 and 4.9 UE/ml after the second antibody titration. The antibody titters were less than 0.5UE/ml for 8 patients. Six persons received an additional vaccine shot developed antibody titters between 0.9 and 5.5 UE/ml.

In conclusion:

Rabies serology seems to be unnecessary for most of the patients who received PEP including RIG and vaccine. Therefore, we recommend antibody titration only in special cases:

- patients more than 50 years old,
- immunocompromised patients,
- patients who received low vaccine potency or unvalidated regimen,
- patients who received high dose of immunoglobulin.

Serology should be preferably performed one week after the end of the treatment.
E.P.D. 1

POSTERS

EPIDEMILOGY OF RABIES IN THE KYRGYZ REPUBLIC

E.K. Akmatova, R.Z. Nurgaziev & N.T. Dzhaparaliev
Kyrgyz Research Institute of Livestock, Veterinary and Pastures, Bishkek, Kyrgyz Republic

Of all officially registered cases of rabies at agricultural animals revealed in 2005 makes 23.7% and in 2006 33.3% of cases, of dogs is 63.2% and 58.7% correspondingly, and of other wild animal (wolves, foxes, rats, cats, etc.) to 13.2% and 8%.

Rabies among wild animals is widespread mainly in southern areas of republic. Here the greater density of wild animals which is essential above than in other areas is observed.

The epidemiological data of Department of State veterinary of the Ministry of Agriculture admits that rabies is registered both of domestic and of wild animals. As the analysis shows - dogs are prevailing source of danger.

The purposes of carrying out of our researches is organization of referent laboratory on rabies, equipped by the modern labware; epidemiological monitoring; training of experts to modern methods of diagnostics; development of the scientifically-proved program of control of rabies in the Kyrgyz Republic. One of the primary goals is equipment by the modern equipment for diagnostics of rabies of the Osh regional veterinary laboratory and training of its personnel as the largest on the population in southern region of republic with purpose to provide control of this infection in this region.

Therefore for further epidemiological research of rabies especially in wild and domestic carnivores' animals is necessary. The knowledge of an area of distribution of rabies then can be used to advance adequate measures of preventive maintenance for the people and domestic animals.
To prevent the introduction of rabies into free areas, the European Union Commission set out the health rules for trade in dogs and cats between EU Member States. In particular, pet owners should have their dogs identified by microchip implantation and protected against rabies by vaccination with inactivated vaccines. The test recommended by the World Organization for Animal Health (OIE) to detect rabies antibodies in carnivores is the Fluorescent Antibody Virus Neutralization (FAVN). Only officially authorized laboratories are allowed to monitor the sera for determining the antibody titers for rabies before moving the animals to foreign Countries. The Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise has been authorised and routinely perform such a test since 2000.

A two year period study on the immune response in pets following rabies vaccination was realized. The serum titer was determined according to the FAVN test procedure and results analyzed to evaluate the antibody dynamic following vaccination. Four hundreds twenty six dogs were included in the study and four groups were formed based on the time of serum collection.

Four hundreds eleven animals had detectable level (0.5 U.I./ml) of neutralising antibodies against rabies and no differences (P>0.05) were found between groups. Conversely, when the mean values of the antibody titres found in the four groups were compared, animals of the group A showed higher (P<0.05) titers related to those found in other three groups.
LIST OF PARTICIPANTS

Chairpersons/Rapporteurs/Speakers

Alvarez Lucas Carlos H.
Prevention Programs General Direction, 132 of Benjamin Franklin Street, México MEXICO
zoonosis@podernet.com.mx

Bankovskiy Denis
Pokrov Plant of Biologics Volginsky, Vladimir Region, 601125 RUSSIA
den_bankovskiy@rambler.ru

Belev Nikola
Regional Coordinator O.I.E. Representation for Eastern Europe Bld Wasil Lewski 110 1527 Sofia BULGARIA
rr.easteurope@oie.int

Blancou Jean
11 rue Descombes 75017, Paris FRANCE
jblancou@noos.fr

Botvinkin Alexandr
Irkutsk State Medical University, 664003, Irkutsk, RUSSIA
botvinkin_ismu@mail.ru

Briggs Deborah
College of Veterinary Medicine Kansas State University 1600 Denison Avenue Manhattan Kansas 66506 USA
briggs@vet.k-state.edu

Brückner Gideon
O.I.E. 12 rue de Prony 75017, Paris FRANCE
g.bruckner@oie.int

Caporale Vincenzo
Istituto Zooprofilattico Sperimentale dell’Abruzzo e del Molise “G. Caporale” Via Campo Boario 64100 Teramo ITALIA
caporale@izs.it

Chipman Richard
USDA, APHIS, Wildlife Services 1930, Route 9 Castleton, New York, 12033 USA
r.chipman@aphis.usda.gov

Cliquet Florence
AFSSA Lerpas Laboratoire d’études sur la rage et la pathologie des animaux sauvages Domaine de Pixérécourt BP 9 54220 Malzéville FRANCE
f.cliquet@afssa.fr

David Dan
Kimron Veterinary Institute Derech Hamcabiim street Bet Dagan, 50250 ISRAEL
davidd@int.gov.il

Dietzschold Bernhard
Thomas Jefferson University 10th and Locust Street Philadelphia, 19107 USA
bernhard.dietzschold@jefferson.edu

Dodet Betty
IABs - C/o Dodet Bioscience 66 cours Charlemagne 69002, Lyon FRANCE
betty.dodet@dodetbioscience.com

Echevarria Juan
Centro Nacional de Microbiología, Instituto de Salud Carlos III Ctra. Majadahonda-Pozuelo s/n Majadahonda/Madrid, 28220 SPAIN
jeecheva@isci.sii.es

Fooks Anthony
Rabies and Wildlife Zoonoses Group, Virology Department Veterinary Laboratories Agency New Haw Addlestone, Surrey, KT15 3NB UNITED KINGDOM t.fooks@vla.defra.gsi.gov.uk

Freuling Conrad Martin
Friedrich-Loeffler-Institute, Wusterhausen, Seestrasse 55 Wusterhausen, 16868 GERMANY
Conrad.Freuling@fli.bund.de

Fu Zhen
University of Georgia Department of Veterinary Pathology 108 Veterinary Medicine Athens, GA 30602 USA
zhenfu@vet.uga.edu

Gruzdev Konstantin
FGI ARRIAH 600901 Jur’evets, Vladimir, RUSSIA
mail@arriah.ru

Harris Sarah
Rabies and Wildlife Zoonoses Group, Virology Department Veterinary Laboratories Agency Weybridge, New Haw, Addlestone Surrey, KT15 3NB UNITED KINGDOM s.harris@vla.defra.gsi.gov.uk

Have Per
E.F.S.A. (European Food Safety Authority) Radkildevej 23 Stege 4780 DENMARK perhave@gmail.com

Hemachudha Thiravat
Department of Medicine, Neurology Chulalongkorn University Hospital Rama 4 Road Bangkok, 10330 THAILAND th-cu@usa.net fmedthm@gmail.com

Hu Rongliang
Veterinary Institute, Academy of Military Medical Science 1068 Qinglong Road Changchun, Jilin, 130062 P.R.CHINA hurongliang@hotmail.com

Imnadze Paata
National Centre for Disiese Control 9 M.Asatiani st. Tbilisi, 0177 GEORGIA pimnadze@ncdc.ge
<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Address</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Israsena Nipan</td>
<td>Chulalongkorn University</td>
<td>Bangkok, 10330 Thailand</td>
<td><a href="mailto:inipan@gmail.com">inipan@gmail.com</a></td>
</tr>
<tr>
<td>Jackson Alan</td>
<td>Queen's University</td>
<td>Kingston General Hospital Connell</td>
<td><a href="mailto:ajackson2@hsc.mb.ca">ajackson2@hsc.mb.ca</a></td>
</tr>
<tr>
<td>Jakel Verena</td>
<td>Universität Giessen Institut für Virologie</td>
<td>Giessen, 35392 Germany</td>
<td><a href="mailto:uta.v.jakel@vetmed.uni-giessen.de">uta.v.jakel@vetmed.uni-giessen.de</a></td>
</tr>
<tr>
<td>Janani Ali Reza</td>
<td>W.H.O. Collaborating Centre for Reference &amp; Research on Rabies, Pasteur Institute of Iran</td>
<td><a href="mailto:Arjan@pasteur.ac.ir">Arjan@pasteur.ac.ir</a></td>
<td></td>
</tr>
<tr>
<td>Johnson Nicholas</td>
<td>Veterinary Laboratories Agency</td>
<td>Woodham Lane, Addlestone KT15 3NB</td>
<td><a href="mailto:n.johnson2@vla.defra.gsi.gov.uk">n.johnson2@vla.defra.gsi.gov.uk</a></td>
</tr>
<tr>
<td>Kahn Sarah</td>
<td>O.I.E.</td>
<td>12 rue de Prony, Paris, France</td>
<td><a href="mailto:s.kahn@ioe.int">s.kahn@ioe.int</a></td>
</tr>
<tr>
<td>Kasempimolporn Songsri</td>
<td>Queen Saovabha Memorial Institute, Thai Red Cross Society</td>
<td>Bangkok, 10330 Thailand</td>
<td><a href="mailto:songsri_ks@hotmail.com">songsri_ks@hotmail.com</a></td>
</tr>
<tr>
<td>Khawplod Pakamatz</td>
<td>Queen Saovabha Memorial Institute (WHO Collaborating Centre for Research on Rabies Pathogenesis and Prevention)</td>
<td>Bangkok, 10330 Thailand</td>
<td><a href="mailto:pakamatz@yahoo.com">pakamatz@yahoo.com</a></td>
</tr>
<tr>
<td>Koprowski Hilary</td>
<td>Thomas Jefferson University</td>
<td>Philadelphia, PA, 19107 USA</td>
<td><a href="mailto:hilary.koprowski@jefferson.edu">hilary.koprowski@jefferson.edu</a></td>
</tr>
<tr>
<td>Kuzmin Ivan</td>
<td>Rabies Unit</td>
<td>Mail Stop G33 1600 Clifton Rd NE Atlanta, GA 30329</td>
<td><a href="mailto:lbk3@cdc.gov">lbk3@cdc.gov</a></td>
</tr>
<tr>
<td>Lafon Monique</td>
<td>Unité de Neuroimmunologie Virale Institut Pasteur</td>
<td>25 rue du Dr Roux, Paris Cedex 15</td>
<td><a href="mailto:mlafon@pasteur.fr">mlafon@pasteur.fr</a></td>
</tr>
<tr>
<td>Laine Marjana</td>
<td>Animal Health Office</td>
<td>Veterinary and Food Board</td>
<td><a href="mailto:marjana.laine@vet.agri.ee">marjana.laine@vet.agri.ee</a></td>
</tr>
<tr>
<td>Lalosevic Dusan</td>
<td>Faculty of Medicine</td>
<td>Hajduk Veljikova 3, Novi Sad, 21000</td>
<td><a href="mailto:pasteuri@eunet.yu">pasteuri@eunet.yu</a></td>
</tr>
<tr>
<td>Loza-Rubio Elizabeth</td>
<td>Animal Viral Diseases Project</td>
<td>Carretera México Toluca Km. 15.5</td>
<td><a href="mailto:loza.elizabeth@inifap.gob.mx">loza.elizabeth@inifap.gob.mx</a></td>
</tr>
<tr>
<td>Maciulskis Petras</td>
<td>Lithuanian State Inspection on Veterinary Preparations</td>
<td>J. Naujalis g. 21b, Kaunas, LT-48332</td>
<td><a href="mailto:pmaciulskis@vet.lt">pmaciulskis@vet.lt</a></td>
</tr>
<tr>
<td>Matouch Oldrich</td>
<td>State Veterinary Institute, National Reference Laboratory for Rabies - U Sila 1139</td>
<td>LITHUANIA</td>
<td><a href="mailto:matouch@volny.cz">matouch@volny.cz</a></td>
</tr>
<tr>
<td>McElhinney Lorraine</td>
<td>Veterinary Laboratories Agency</td>
<td>New Ham, Surrey, KT15 3NB</td>
<td><a href="mailto:l.mcelhinney@vla.defra.gsi.gov.uk">l.mcelhinney@vla.defra.gsi.gov.uk</a></td>
</tr>
<tr>
<td>Meslin François-Xavier</td>
<td>Zoonoses and VPH, W.H.O.</td>
<td>CH-1211 Genève 27 SWITZERLAND</td>
<td><a href="mailto:meslinf@who.int">meslinf@who.int</a></td>
</tr>
<tr>
<td>Mettin Artem</td>
<td>Institut Pasteur de Tunis, Laboratoire Immuno-Biotechnologie</td>
<td>13, Place Pasteur BP74 Tunis-Belvedere, 1002 TUNISIA</td>
<td><a href="mailto:artem.mettin@inbox.ru">artem.mettin@inbox.ru</a></td>
</tr>
<tr>
<td>Mousli Mohamed</td>
<td>Institut Pasteur de Tunis, Laboratoire Immuno-Biotechnologie</td>
<td>13, Place Pasteur BP74 Tunis-Belvedere, 1002 TUNISIA</td>
<td><a href="mailto:mohamed.mousli@pasteur.rns.tn">mohamed.mousli@pasteur.rns.tn</a></td>
</tr>
<tr>
<td>Müller Thomas</td>
<td>Federal Research Institute for Animal Health</td>
<td>Friedrich-Loeffler Institut</td>
<td><a href="mailto:thomas.mueller@wus.bfav.de">thomas.mueller@wus.bfav.de</a></td>
</tr>
<tr>
<td>Nel Louis</td>
<td>University of Pretoria</td>
<td>Dept of Microbiology Main Campus</td>
<td><a href="mailto:louis.nel@up.ac.za">louis.nel@up.ac.za</a></td>
</tr>
<tr>
<td>Picard Meyer Evelyn</td>
<td>AFSSA Nancy</td>
<td>Technopole Agricole et Vétérinaire</td>
<td><a href="mailto:e.picard@afssa.fr">e.picard@afssa.fr</a></td>
</tr>
<tr>
<td>Pradhan Hare Krishna</td>
<td>High Security Animal Disease Laboratory</td>
<td>Indian veterinary Research Institute</td>
<td><a href="mailto:hkrpadhan45@rediffmail.com">hkrpadhan45@rediffmail.com</a></td>
</tr>
</tbody>
</table>
LIST OF PARTICIPANTS

Rotivel Yolande  
Instutd Pasteur  
28 rue du Docteur Roux  
Paris 75724  
FRANCE  
yrotivel@pasteur.fr

Rupprecht Charles  
Division of Viral and Rickettsial  
Diseases Centers for Disease  
Control  
Rabies Unit  
1600 Clifton Road, N.E., Mail  
Stop G 33  
Bldg 15, 55B6 11  
Atlanta, Georgia 30333  
USA  
cy5@cdc.gov

Seimenis Aristarco  
Inter-country Programme Co-ordinator  
Director, WHO/MZCC  
24, Stournari str.  
GR-10682 Athens  
GREECE  
mzcc@ath.forthnet.gr

Singer Alexander  
Central Science Laboratory  
Sand Hutton York  
North Yorkshire, YO41 1LZ  
UNITED KINGDOM  
a.singer@csl.gov.uk

Singh C.K.  
Rabies Research-cum-Diagnostic  
Laboratory, Punjab  
INDIA  
rabiesck@gmail.com

Slate Dennis  
USDA, APHIS Wildlife Services  
59 Chenell Drive, Suite 2  
Concord, NH, 03301  
USA  
dennis.slate@aphis.usda.gov

Smith Graham  
Central Science Laboratory  
Sand Hutton York  
North Yorkshire, YO41 1LZ  
UNITED KINGDOM  
g.smith@csl.gov.uk

Smreczak Marcin  
National Veterinary Research  
Institute  
Virology Department  
u. Partzantom 57  
24-100 PULAWY  
POLAND  
smreczak@piwet.pulawy.pl

Thulke Hans-Hermann  
Helmholtz Centre for  
Environmental Research - UFZ  
Dept. Ecological Modelling,  
Project Group Ecological  
Epidemiology  
Permoserstr. 15 Leipzig D -  
04318  
GERMANY  
hans.thulke@ufz.de

Tordo Noël  
Laboratoire des Lyssavirus Institut  
Pasteur  
25-28 rue du Docteur Roux  
75015 PARIS  
France  
ntordo@pasteur.fr

Tu Changchun  
Ministry of Agriculture of CHINA  
The Institute of Veterinary  
Sciences  
Academy of Military Medical  
1068 Qinglong Road  
Changchun, Jilin Province,  
130062  
P.R. CHINA  
changchun_tu@hotmail.com

Ugolini Gabriella  
Lab Neurobiologie Cellulaire et  
Moléculaire  
1 av de la Terrasse  
Gif-sur-Yvette, 91198  
FRANCE  
gabriella.ugolini@nbcm.cnrs-gif.fr

Van der Poel Wim  
Division of Infectious Diseases,  
Infection Biology Virology  
Animal Sciences Group,  
Wageningen University Research,  
P.O. Box 65, 8200 AB Leystad,  
NETHERLANDS  
wim.vanderpoel@wur.nl

Wandeler Alex  
Centre of Expertise for Rabies,  
Animal Diseases Research  
Institute  
3851 Fallowfield Road, P.O. Box  
11300, Station H, Nepean,  
Ontario K2H 8P9  
CANADA  
wandelera@inspection.gc.ca

Warrell Mary  
Oxford Vaccine Group Centre for  
Clinical Vaccinology & Tropical  
Medicine, Churchill Hospital  
Old Road Headington  
Oxford OX3 7JL  
UNITED KINGDOM  
may.warrell@ndm.ox.ac.uk

Weihe Eberhard  
Institute of Anatomy and Cell  
Biology  
Philips University Marburg  
Robert-Koch-Strasse 8  
Marburg, 35032  
GERMANY  
weihe@staff.uni-marburg.de

Wilde Henry  
Chulalongkorn University Hospital  
Infectious Diseases  
Rama IV Road  
Bangkok, 10330  
THAILAND  
wildehenry@yahoo.com

Willoughby Rodney  
Medical College of Wisconsin  
Suite C450, Pediatric Infectious  
Diseases  
P.O. Box 1997  
Milwaukee, Wisconsin, 53201- 
1997  
USA  
rewillou@mcw.edu

Yakobson Boris  
Kimron Veterinary Institute  
Bet Dagan P.O.B 12  
ISRAEL 50250  
dir-kimron@moag.gov.il

Joint OIE/WHO/EU International Conference: “Towards the Elimination of Rabies in Eurasia”  
Paris (France), 27-30 May 2007
Provisional list of participants

Abdurahman Omar
Swedish Board of Agriculture
Vallgatan 8 Jönköping, 551 82
SWEDEN
omar.abdurahman@sjv.se

Akmatova Elmira
Kyrgyz Research Institute of Livestock Veterinary and Pastures named after A.Duysheev
60, Togolok Moldo str.Bishkek 720033
KYRGYZ REPUBLIC
akmatova_elmira@mail.ru

Aksenov Alexandr
15 Kirov street
Minsk, 220030
BELARUS
prvet@mshp.minsk.by

Al-Eryani Ghalib Fadl
P.O. Box 13449
Sana’a
YEMEN
g_eriani@yahoo.com

ALYousef Mohammed
Ministry of agriculture
Riyadh King Abdulaziz road
11672 P.O BOX 88709 Riyadh
SAUDI ARABIA
m.alyosef@hotmail.com

Amador Rita
Direcção Geral de Veterinária
Largo da academia nacional de belas artes, 2
1249-105, Lisboa
PORTUGAL
ramador@dgv.min-agricultura.pt

Amengual Blanca
Universitat de Barcelona
Avenida. Diagonal, 645
Barcelona, 08028
SPAIN
amengual@areambiental.com

Anders Gerlind
Novartis Vaccines and Diagnostics
GmbH & Co. KG
Emil-von-Behring-Str. 76
Marburg, 35041
GERMANY
gerlind.anders@novartis.com

Attlan Michael
Sanofi Pasteur
2 avenue du pont Pasteur
69007, Lyon
FRANCE
attlan@sanofipasteur.com

Aylan Orhan
Ministry of Agri. and Rural Affairs
Central Veterinary Control and Research Institute
A.S. kolayli cad. no.23, etlik-kecioren
Ankara, 06020
TURKEY
orhan@aylan.name.tr

Bachir Harif
Biopharma Km 2 route de casablanca
B.P. 4569 rabat akkari
MAROC
bachirh@hotmail.com

Bakker Alexander
Crucell Holland BV
Archimedesweg 4 PO BOX 2048
Leiden, 2301 CA
THE NETHERLANDS
l.bakker@crucell.com

Barrat Jacques
AFSSA Nancy
Technopole Agricole et Vétérinaire
BP 40009
malizérieur, 54220
FRANCE
n.stroucken@afssa.fr

Barret Janine
Queensland Government
Department of Primary Industries and Fisheries
80 Ann St Brisbane
Qld Australia, Brisbane, 4068
AUSTRALIA
janine.barrett@dpi.qld.gov.au

Bastida Fernando Guadalupe
Laboratorio Estatal De Salud Publica
Instituto De Salud Del Estado De
Av. Paseo Tollocan s/n colonia moderna de la cruz
Toluca, Estado De Mexico, 500130
MEXICO
mijomeil@hotmail.com

Battilocchi Bruno
EU Commission
DG SANCO - Food & Veterinary Office Grange / Dunsany, Co. Meath
IRELAND
Bruno.Battilocchi@ec.europa.eu

Bellenzoni Rodolfo
Biogenesis Bago S.A.
Ruta Panamericana km 38,5 Garin
B1619IEA
ARGENTINA
rodolfo.bellenzoni@biogenesisbago.com

Benes Lubomir
USKVBL Brno Hudcova 56a
Brno, 621 00
CZECH REPUBLIC
benes@uskvbl.cz

Berndtsson Louise
SVA, National Veterinary Institute
Ulvs väg 2B
Uppsala SE-755 89
SWEDEN
Louise.T.Berndtsson@sva.se

Biddle Robert
Department of Agriculture, Fisheries and Forestry
GPO Box 858, Canberra City 2601
AUSTRALIA
bob.biddle@daff.gov.au

Blanchard Irène
Bio-Rad
43 boulevard Raymond Poincaré
92430, Marnes la Coquette
FRANCE
irene.blanchard@bio-rad.com

Bosiljka Djuricic
Faculty of veterinary medicine
Bul oslobođenja 18
Belgrade 11 000
SERBIA
bosa@beotel.net

Bourhy Hervé
Institut Pasteur
National Reference Centre for Rabies, WHO Collaborating Centre for Reference and Research on Rabies
25-28 rue du Dr Roux
75015, Paris
FRANCE
hbourhy@pasteur.fr

Joint OIE/WHO/EU International Conference: “Towards the Elimination of Rabies in Eurasia”
Paris (France), 27-30 May 2007
111
<table>
<thead>
<tr>
<th>Name</th>
<th>Organization</th>
<th>Address</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brackman Christopher</td>
<td>Agriculture, Fisheries and Conservation Department</td>
<td>SAR 5/F, Cheung Sha Wan Government Offices 303 Cheung Sha Wan Rd Cheung Sha Wan, Kowloon, Hong Kong P.R. CHINA</td>
<td><a href="mailto:ozvet@netvigator.com">ozvet@netvigator.com</a></td>
</tr>
<tr>
<td>D Hooghe Wilem</td>
<td>Public Service Public Health, Safety of the Food Chain</td>
<td>Place Victor Horta 40 bte 10 Brussels, 1060 BELGIUM</td>
<td><a href="mailto:willem.dhooghe@health.fgov.be">willem.dhooghe@health.fgov.be</a></td>
</tr>
<tr>
<td>Dacheux Laurent</td>
<td>Institut Pasteur National Reference Centre for Rabies</td>
<td>25 rue du Dr. Roux Paris, 75015 FRANCE</td>
<td><a href="mailto:ldacheux@pasteur.fr">ldacheux@pasteur.fr</a></td>
</tr>
<tr>
<td>Chang Shiow-Lian</td>
<td>National Chung Hsing University</td>
<td>250 Kuo Kuang Road Taichung city 402 TAIPEI CHINA</td>
<td><a href="mailto:tjiang@dragon.nchu.edu.tw">tjiang@dragon.nchu.edu.tw</a>, <a href="mailto:sjleng@mail.baphiq.gov.tw">sjleng@mail.baphiq.gov.tw</a></td>
</tr>
<tr>
<td>Danes Doina</td>
<td>Faculty of Veterinary Medicine</td>
<td>Splaiul Independentei, No.105, sector V Bucarest, 005036 ROMANIA</td>
<td><a href="mailto:doinadanes@yahoo.com">doinadanes@yahoo.com</a></td>
</tr>
<tr>
<td>De Benedictis Paola</td>
<td>Istituto Zoodomenticando Sperimentale delle Venezie Research &amp; Development Department</td>
<td>Viale dell’Università 10 35020 Legnaro, Padova ITALY</td>
<td><a href="mailto:pdebenedictis@izsvenezie.it">pdebenedictis@izsvenezie.it</a></td>
</tr>
<tr>
<td>Diop Bernard Marcel</td>
<td>Service des maladies infectieuses CHNU Fann</td>
<td>Université Cheick Anta Diop de Dakar 31 avenue Pasteur Dakar 6394 SENEGAL</td>
<td><a href="mailto:bmdiopmi@yahoo.fr">bmdiopmi@yahoo.fr</a>, <a href="mailto:bmdiopmi@hotmail.com">bmdiopmi@hotmail.com</a></td>
</tr>
<tr>
<td>Dirbakova Zuzana</td>
<td>State Veterinary Institute</td>
<td>Pod drahami 918 Zvolen, 960 86 SLOVAKIA</td>
<td><a href="mailto:molbiol@suzuv.sk">molbiol@suzuv.sk</a></td>
</tr>
<tr>
<td>Djuricic Bosiljka</td>
<td>Faculti of veterinary medicine</td>
<td>Bul.oslobodjenja 18 Belgrade 11000 SERBIA</td>
<td><a href="mailto:bosa@beotel.net">bosa@beotel.net</a></td>
</tr>
<tr>
<td>Dumitrescu Florina</td>
<td>Institute for Diagnosis and Animal Health</td>
<td>Dr. Stacicovici Street no.63 , sect. 5 Bucharest 050557 ROMANIA</td>
<td><a href="mailto:dumitrescu.florina@idah.ro">dumitrescu.florina@idah.ro</a></td>
</tr>
<tr>
<td>Durga Datt Joshi</td>
<td>National Zoonoses and Food Hygiene Research Centre</td>
<td>Chagal, Jeenan Smriti marg house no. 468/32, G.P.O.Box 1885, Kathamandu NEPAL</td>
<td><a href="mailto:ddjoshi@healthnet.org.jp">ddjoshi@healthnet.org.jp</a></td>
</tr>
<tr>
<td>Edwards Steve</td>
<td>Veterinary Laboratories Agency</td>
<td>New Haw Addleston Surrey, KT15 3NB UNITED KINGDOM <a href="mailto:s.edwards@vla.defra.gsi.gov.uk">s.edwards@vla.defra.gsi.gov.uk</a></td>
<td></td>
</tr>
<tr>
<td>Ferrari Giancarlo</td>
<td>F.A.O.</td>
<td>Via delle Terme di Caracalla Rome, 00100 ITALY</td>
<td><a href="mailto:Giancarlo.Ferrari@fao.org">Giancarlo.Ferrari@fao.org</a></td>
</tr>
<tr>
<td>Feyssaguet Muriel</td>
<td>Bio-Rad 3, bd Raymond Poincaré</td>
<td>92430, Marnes-La-Coquette FRANCE</td>
<td><a href="mailto:muriel_feyssaguet@bio-rad.com">muriel_feyssaguet@bio-rad.com</a></td>
</tr>
<tr>
<td>Ghvinjilia Gia</td>
<td>Food Safety, Veterinary and Plant Protection National Service</td>
<td>15a Tamarashvili Ave 4th Floor Tbilisi 0177 GEORGIA</td>
<td><a href="mailto:dr_gia_vet@hotmail.com">dr_gia_vet@hotmail.com</a></td>
</tr>
<tr>
<td>Gilbernair Elia</td>
<td>Sanofi Pasteur</td>
<td>Direction Régional Afrique Occidentale et Centrale 1 bd de l’indénié 28 BP 832 Abidjan 28 COTE D’IVOIRE <a href="mailto:elia.gilbernair@sanofipasteur.com">elia.gilbernair@sanofipasteur.com</a></td>
<td></td>
</tr>
<tr>
<td>Goudal Maryvonne</td>
<td>Institut Pasteur</td>
<td>Centre national de reference de la rage, 28 rue du Dr Roux Paris, 75015 FRANCE</td>
<td><a href="mailto:mgoudal@pasteur.fr">mgoudal@pasteur.fr</a></td>
</tr>
</tbody>
</table>
LIST OF PARTICIPANTS

Griere Geneviève
Centre antirabique Pau  Centre hospitalier François Mitterrand
4 bld Hauterive BP 1156
PAU 64046
FRANCE
genevieve.griere@ch-pau.fr

Jimenez Juan Manuel
Laboratorio Estatal De Salud Publica, Instituto De Salud Del
Estado de Mexico
Paseo Tollocan S/N Colonia Moderna de la Cruz, Toluca,
Estado de Mexico, 500130
MEXICO
jesjm@hotmail.com

Krogmann Vincent
MERIAL
29 avenue Tony Garnier
69007, Lyon
FRANCE
brigitte.accary@merial.com

Le Roux Kevin
Veterinary Services of RSA
Private Bag X2 Cascades
KwaZulu natal RSA
Pietermaritzburg 3200
SOUTH AFRICA
klroux@allerton.kzntl.gov.za

Hammarin Anne-Lea
Nobels väg 18, Solna 171 82
SWEDEN
anna-lena.hammarin@smi.ki.se

Hoffman Anna
General Veterinary Inspectorate
30 Wspolna, Warsaw, 00-930
POLAND
anna.hoffman@wetgiw.gov.pl

Huether Sven
Planet ID GmbH -
Schmachtenbergstr. 20
Essen, 45219
GERMANY
sven.huether@planet-id.com

Kouame Kanga
Cité Administrative, Tour C,
11ème étage
B.P. V 84 Abidjan
COTE D’IVOIRE
kcem1@yahoo.fr

Jerg Slavomir
State Veterinary Institute
Pod drahami 918
Zvolen, 960 86
SLOVAKIA
rabies@svuzv.sk

Kouame Kanga
Cité Administrative, Tour C,
11ème étage
B.P. V 84 Abidjan
COTE D’IVOIRE
kcem1@yahoo.fr

Kramps Hans
CIDC Lelystad
Houtriweg 39 Lelystad
Flevoland, 8221 RA
THE NETHERLANDS
hans.kramps@wur.nl
**List of Participants**

**Mainali Prustom Prasad**  
Department of Livestock Services  
Ministry of Agriculture and cooperatives Department of Livestock Services Hariharbhanw Lalitpur, Kathmandu  
NEPAL  
ahd@healthnet.org.np

**Mangana - Vougiouka Olga**  
Ministry of rural development & food, 25 Neapoleos Str. Agia Paraskevi, 15310  
Greece  
viruslab@ath.forthnet.gr,  
olga_mangana@yahoo.gr

**Marissen Wilfred**  
Crucell Holland BV  
Archimedesweg 4  
Leiden 2333 CN  
THE NETHERLANDS  
wilfred.marissen@crucell.com

**Maurer Wernig Jedrt**  
Veterinary Administration of the Republic of Slovenia  
Parmova 53 Ljubljana, 1000  
SLOVENIA  
jedrt.maurer@gov.si

**Meincke Maylin**  
WHO  
Avenue Appia 20  
1211 Genève 27  
SWITZERLAND

**Mendoza Martin**  
USDA, APHIS, Wildlife  
1400 Independence Avenue  
SW Room 1624 South Building  
Washington, DC, 20250  
USA  
sheila.d.miller@aphis.usda.gov

**Milius Jonas**  
National Veterinary Laboratory of the Republic of Lithuania  
Siesisku str. 19, Vilnius, 07170  
LITHUANIA  
jmilius@nvl.lt

**Miranda Mary Elisabeth**  
Veterinary Public Health Specialist  
113 Champaña St., Sta. Rosa Village  
Sta. Rosa, Laguna, 4026  
PHILIPPINES  
rhysmiranda@yahoo.com

**Mojzis Miroslav**  
State Veterinary Institute  
Pod drahi 918  
Zvolen, 960 86  
SLOVAKIA  
mojzis@svuзв.sk

**Molina Miguel Angel**  
Laboratorio Estatal de Salud Publica, Instituto de Salud del Estado de Mexico  
Paseo Tollocan s/n Colonia moderna de la Cruz  
Toluca, Estado de Mexico 50180  
MEXICO  
lespedomex@hotmail.com

**Moore Susan**  
Kansas State University College of Veterinary Medicine  
Mosier Hall Rm O-242 1800 Denison Avenue  
Manhattan, KS, 66506  
USA  
smoore@vet.ksu.edu

**Mortier Jill**  
Australian Department of Agriculture, Fisheries and Forestry  
GPO Box 858  
Canberra, ACT 2600  
AUSTRALIA  
jill.mortier@daff.gov.au

**Moset Sagrario**  
Ministerio de Agricultura, Pesca y Alimentación, Alfonso XII, 62 28071, Madrid  
SPAIN  
smostem@mapya.es

**Motsisi Thabo**  
Department of Agriculture Private Bag X138 Pretoria  
0001 Pretoria 0001  
SOUTH AFRICA  
thabostra@nda.agric.za

**Nzunguent Etienne**  
Ministère de la Santé / Institut épidemiologique et des épidémies  
BP 50 Libreville  
Gabon  
nzenguette@gmail.com

**Panzer Evelyn**  
Hipoosoft, Hertzog 36 st.  
Gyataym, 53587  
ISRAEL  
evelyn.panzer@gmail.com

**Patz Marie-Claire**  
Ministry of Health  
14 avenue Duquesne  
75350 Paris SP 07  
FRANCE  
marie-claire.paty@sante.gouv.fr

**Perrin - Vidoz Laurent**  
MERIAL  
29 avenue Tony Garnier  
69007, Lyon  
FRANCE  
brigitte.accary@merial.com

**Petrovic Nenad**  
Ministry of Agriculture Forstry and Water Management  
Veterinary Directorate  
St. Omladinskih 1, SIV3  
New Belgrade, Belgrade 11070  
SERBIA  
nenad.petrovic@minpolj.sr.gov.yu

**Rahkonen Riitta**  
Ministry of Agriculture and Foresty, Food and Veterinary department  
PO Box 30 Helsinki, FI-00023 Government  
FINLAND  
riitta.rahkonen@mmm.fi

**Ramishvili Levan**  
9, Tarkhnishvili St., Office 6  
0179 Tbilisi  
GEORGIA  
levanramishvili@yahoo.com

**Raouf Gritli**  
Centre Militaire de Transfusion Sanguine  
BP N°479 - Le Bardo, Tunis 2000  
TUNISIA  
a.gritlidvm@yahoo.fr

**Rassouli Anvar**  
Sanofi Pasteur  
2 avenue du Pont Pasteur  
69367, Lyon CEDEX 07  
TUNISIA  
anvar.rassouli@sanofipasteur.com

**Regnault André**  
Virbac  
13eme rue Lid - BP 27  
06511, Carros CEDEX  
FRANCE  
aregnault@virbac.fr

**Ronsyn Rudi**  
Alfonslaan 85  
Geraardsbergen 9500  
BELGIUM  
r.ronsyn@synbiotics.fr
LIST OF PARTICIPANTS

Roumiantzeff Micha
International Association for Biologicals (IABs)
1, rue Dangon
Lyon, 69004
FRANCE
micharou@numericable.fr

Royle Paul
The Binding Site P.O. Box 11712
Birmingham, B14 4Z
UNITED KINGDOM
paul.royle@bindingsite.co.uk

Roumiantzeff Micha
International Association for Biologicals (IABs)
1, rue Dangon
Lyon, 69004
FRANCE
micharou@numericable.fr

Shahzada Sana-Ul-Haq
Committee for Rehabilitation Aid to Afghanistan
House# 133, Street# 6,N-4, Phase IV, Hayatabad Peshawar,
NWFP
PAKISTAN
sanashefa@yahoo.com; sancraa@brain.net.pk

Sihvonen Liisa
Evira Mustialankatu 3
Helsinki 00790
FINLAND
liisa.sihvonen@evira.fi

Sinkovic Mirko
Zoo garden Palic
Krfiska 4 Palic
24413
SERBIA
zoovet@EUnet.yu

Skalka Premysl
State Veterinary Institute
Jakoubka ze Stribra 1
Olomouc, 779 00
CZECH REPUBLIC
pskalka@svuol.cz

Smit Jaco
Sanofi Pasteur
2 avenue du Pont Pasteur
69367, Lyon CEDEX 07
FRANCE
jaco.smit@sanofipasteur.com

Soavi Marie-Josèphe
APHM Hôpital Nord Centre Antirabique
Chemin des Bourrely
13006, Marseille
FRANCE
mariejo.soavi@ap-hm.fr

Stankov Srđan
Pasteur Institute
Hajduk Veljkova 1
Novi Sad, 21000
SERBIA
stankov.paster@ptt.yu

Stanojevic Slavoljub
Ministry of Agriculture Forstry and Water Management
Veterinary Directorate
St. Omladinški brigada 1, SIV III,
New Belgrade Belgrade, 11070
SERBIA
slavavet@yahoo.com

Steijn Klaas
Food and Consumer Product Safety Authority
Prinses Beatrixlaan 2
The Hague 2595 AL
THE NETHERLANDS
klas.steijn@vwa.nl

Strady Alain
Service des Maladies Infectieuses Centre Antirabique
Hôpital Robert DEBRE
Avenue du général Koenig
51100, REIMS
FRANCE
astrady@chu-reims.fr

Rybakov Sergey
FGI ARRIAHI
600901, Jur’evets, Vladimir
RUSSIA
mail@arriah.ru

Sadikowska-Todys Malgorzata
National Institute of Hygiene
Chocimska 24, Warsaw, 00-791
POLAND
mtodys@pzh.gov.pl

Santrač Violeta
Veterinarski Institut RS
Branka Radicevica 18
Banja Luka 78000
BOSNIA AND HERZEGOVINA
santracv@veterinarskiinstitutrs.com

Sedlaric Rudi
Rudolf Virk Institute
Alderferova 3
Slavonski Brod 31000
CROATIA
sedlaric@vrk.si

Serebrennikov Yuri
Rabid Center
Peschanoe,
Saratov oblast,
RUSSIA
serebr@rambler.ru

Svpov Rudi
Rudolf Virk Institute
Alderferova 3
Slavonski Brod 31000
CROATIA
sedlaric@vrk.si

Svoboda Elisabeth
Agri-Food and Veterinary Authority
of Singapore
5 Maxwell Road, #18-00 MND
Tower, Singapore, 069110
SINGAPORE
tay_choon_nghee@ava.gov.sg

Surguladze Vakhtang
State United Social Insurance Fond
of Georgia, 51 Javakhishvili st.
Tbilisi, 0102
GEORGIA
info@susif.ge

Swart Michael
North West Province, Private Bag
XB2087 Rustenburg 0300
SOUTH AFRICA
mswart@nwpg.gov.za

Swoboda Elisabeth
Federal Ministry of Health and
Women Radetzkystrasse 2
Vienna, A-1030
AUSTRIA
elisabeth.swoboda@bmgfj.gv.at

Takahashi Sachiko
Tuberculosis and Infectious Disease Control Division
Health Service Bureau
Ministry of Health Labour and
Welfare, 1-2-2, Kasumigaseki
Chiyoda-ku, Tokyo 100-8916
JAPAN
takahashi-sachiko@mhlw.go.jp

Tay Choon Nghee
Agri-Food and Veterinary Authority of Singapore
5 Maxwell Road, #18-00 MND
Tower, Singapore, 069110
SINGAPORE
tay_choon_nghee@ava.gov.sg

Joint OIE/WHO/EU International Conference: “Towards the Elimination of Rabies in Eurasia”
Paris (France), 27-30 May 2007

115
LIST OF PARTICIPANTS

**Tepsumethanon Veera**  
Queen Saovabha Memorial Institute (WHO Collaborating Centre for Research on Rabies Pathogenesis and Prevention)  
1871 Rama 4 Rd.  
Bangkok 10330  
THAILAND  
tepsumethanonv@yahoo.com

**Threaenhart Olaf**  
Eurovir Hygiene-Institut  
Alte Nussdorferstr. 18  
Ueberlingen D-88662  
GERMANY  
thraenhart@eurovirhygiene.freeyellow.com

**Un Hikmet**  
Ministry of Agri., and Rural Affairs Central Veterinary Control and Research Institute  
a.s.kolayli cad. no 23 etlik-kecioren, Ankara, 06020  
TURKEY  
hikmetun@gmail.com

**Vanek Elisabeth**  
AGES Institute for veterinary disease control Moedling  
Kochgasse 17, Moedling, 2340  
AUSTRIA  
elisabeth.vanek@ages.at

**Vermeersch Jean-Pierre**  
European Commission  
Rue Béliard 232 (B 232 B/59)  
Brussels 1049  
BELGIUM  
Jean-Pierre.Vermeersch@ec.europa.eu

**Visser Nico**  
Intervet Int.  
PO Box 31 5830  
THE NETHERLANDS  
nico.visser@intervet.com

**Vlasich Clemens**  
Sanofi Pasteur  
Lemboeckgasse 49/2  
Vienna A-1230  
AUSTRIA  
clemens.vlasich@sanofipasteur.com

**Vos Ad**  
IDT GmbH  
Streetsweg 15a  
Rodleben, 06862  
GERMANY  
ad.vos@idt-direct.de

**Vrzal Vladimir**  
Bioveta, a.s.  
Komenskeho 212  
Ivanovice na Hane 68323  
CZECH REPUBLIC  
vrzal.vladimir@bioveta.cz

**Vuta Vlad**  
Institute for Diagnosis and Animal Health, Dr. Staicovici Street no.63 sect. 5.Bucharest 050557  
ROMANIA  
vuta.vlad@idah.ro

**Xu Gelin**  
Institute of Biological Products  
9# Linjiang Ave, 430060, Wuchang, Wuhan  
P.R. CHINA  
xugelin@yahoo.com.cn

**Zienius Dainius**  
Veterinary Institute of Lithuanian Veterinary Academy Instituto  
2 Kasiadorys lt - 56115  
LITHUANIA  
dainzien@yahoo.com

**Zietara Magdalena**  
European Commission  
Rue de la Loi 200  
1049, Brussels  
BELGIUM  
Magdalena.Zietara@ec.europa.eu