

EWDA Conference

# Healthy wildlife, healthy people

Abstractbook



13<sup>th</sup> to 16<sup>th</sup> of September 2010  
Vlieland, The Netherlands



# Healthy wildlife, healthy people

Abstract book

# Colofon

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# “Healthy wildlife, healthy people”

9<sup>th</sup> Biennial Conference of the European Wildlife Disease Association  
Vlieland, The Netherlands, 13 to 16 September 2010

Dear colleague,

Welcome to the 9<sup>th</sup> biennial conference of the European Wildlife Disease Association on the island of Vlieland, The Netherlands. This conference brings together scientists from a broad range of disciplines who are connected by their common interest in the health of wild animals. As you will notice from the program, we made a conscious effort to strengthen the public health angle to the meeting, reflected in the conference theme, *Healthy wildlife, healthy people*. We are pleased to have a larger than usual contingent of participants with a background in human medicine, and expect to see lively discussions by bringing this perspective into the meeting. In this way, we hope to contribute to the “One Health” concept that promotes the unity of approach to the health of wildlife, domestic animals, and humans.

It truly is an international conference, with over 230 participants from 25 countries, most of them from Europe—with 18 countries represented—but also from North America, the Middle East, and Africa. Not only the countries from which the participants originate is diverse, so too are the topics that they will be presenting. The 185 scientific abstracts deal with subjects ranging from ecology to metagenomics, from Q-fever to climate change, from ocellated lizards to humans.

Vlieland, The Netherlands, is the ninth location of an EWDA conference. Previous conferences were held in Maisons Alfort, France (1994), Wrocław, Poland (1996), Edinburgh, U.K. (1998), Zaragoza, Spain (2000), Heidelberg, Germany (2002), Uppsala, Sweden (2004), Aosta, Italy (2006), and Rovinj, Croatia (2008). We chose Vlieland mainly because it is part of the Wadden Sea, which is the most pristine ecosystem in The Netherlands and recently designated as a UNESCO World Heritage Site. The Wadden Sea is known for its populations of harbour seals and grey seals, which may be seen regularly both from shore and at sea. At this time of the year, there also is a spectacular southward migration of millions of shorebirds. The Wadden Sea forms, as it were, a funnel between the extensive arctic breeding areas of North America and Eurasia and the two main wintering areas in West Africa, Banc d'Arguin in Mauritania and the Bijagós Archipelago in Guinée-Bissau.

However, Vlieland is not only special for its natural beauty, but also for its people. People already were living on Vlieland in the 13<sup>th</sup> century, when it was separated from the neighbouring Texel by the sea and became a separate island. Until the 15<sup>th</sup> century, Vlielanders lived mainly from hunting rabbits, cutting peat, and beachcombing. Gradually, fishing and merchant shipping gained in importance. The 17<sup>th</sup> and 18<sup>th</sup> centuries were a period of relative prosperity for the Vlieland, with a large part of the population active in the Baltic trade. In that period, Vlielanders also took part in whaling in Icelandic and Greenland waters, as still can be seen from the mandibles of bowhead whales used to mark graves in the village cemetery. This “Golden Century” was followed by a period of economic decline, that only changed with the growth of the tourist industry after 1945, which lasts to this day. We are pleased to have the support of the Vlieland community to make this conference a success.

Thijs Kuiken and Marion Koopmans

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This EWDA conference is sponsored by:



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# Programme overview

Detailed program of the 9<sup>th</sup> Conference of the European Wildlife Disease Association, Vlieland, The Netherlands, September 13-16, 2010

## Pre-conference workshops, Monday 13 September

**Location:** Strandhotel Seeduyn

09:00 – 12:00	Workshops	For details, see workshops
12:00 – 13:30	Lunch for workshop participants	
13:30 – 18:00	Workshops	For details, see workshops
20:00 – 23:00	Ice breaker , for all participants	

## Day 1, Tuesday September 14, Focus on public health

**Location:** Conference hall “De Bolder”

8:30 – 8:45	Mayor Yorick the Haan	Opening of the meeting and word of welcome by The Honourable Yorick the Haan, mayor of Vlieland
8:45 – 12:00	Chair persons: Marion Koopmans & Jim v Steenbergen	
8:45 – 10:00	<b>SESSION 1</b>	<b>Are wildlife diseases relevant for public health?</b>
	keynote	Prof. Dr. Ab Osterhaus Wildlife and emerging zoonoses: a real problem
	keynote	Dr. Jim van Steenbergen Wildlife and emerging zoonoses: not a real problem
	keynote	Prof. Dr. Herbert Prins Wildlife and emerging zoonoses: a reality from a conservation point of view
10:00 – 10:30	BREAK	
10:30 – 12:00	<b>SESSION 2</b>	<b>Zoonotic diseases</b>
10:30 – 10:40	1 J. Alison Peel	Potentially zoonotic viruses in straw-coloured fruit bats ( <i>Eidolon helvum</i> ) in the Gulf of Guinea Islands
10:40 – 10:50	2 Judith van den Brand	Absence of associated histopathologic changes in tissues of bats with Coronavirus infection
10:50 – 11:00	3 Bob McLean	Genetic relatedness of raccoons in Northeastern Ohio, USA: Implications for rabies spread
11:00 – 11:10	4 Danny Morick	Bartonella Species in the harbor seal ( <i>Phoca vitulina</i> ) and in seal lice ( <i>Echinophtirus horridus</i> )
11:10 – 11:20	5 Karoly Erdelyi	The Central European expansion of lineage 2 West Nile virus during 2008 and 2009
11:20 – 11:30	6 Francisco Ruiz-Fons	Modification of a commercial ELISA to detect antibodies against <i>Coxiella burnetii</i> in wild ungulates: application to population surveillance
11:30 – 11:40	7 Jolianne Rijks	Roe deer and the Dutch Q-fever epidemic
11:40 – 12:00	8 Scott H. Newman	FAO Embraces the One Health Challenge at the wildlife-livestock-human-ecosystem interfaces
12:00 – 13:30	LUNCH	
13:30 – 17:00	Chair persons: Thijs Kuiken & Menno de Jong	
13:30 – 15:00	<b>SESSION 3</b>	<b>Signaling (new) risks for public health: (how) does it work?</b>
13.30 – 14:00	keynote	Prof. Dr. Menno de Jong Emerging infectious diseases in an international context, a medical perspective
14:00 – 14:20	keynote	Dr. Alexandra Mailles Cowpox outbreak through animal trade
14:20 – 14:30	9	Katie Colvile Chlamydomydia psittaci infection in garden birds in England and Wales
14:30 – 14:40	10	Miklos Gyuranecz Investigation of the ecology of <i>Francisella tularensis</i> in an interepizootic period
14:40 – 14:50	11	Victor Simpson Borreliosis in a <i>Pipistrellus</i> sp. bat: Is this an emerging zoonosis?
14:50 – 15:00	12	R. Martinez Salmonella spp. and Shiga toxin-producing <i>Escherichia coli</i> prevalence in an ocellated lizard ( <i>Timon lepidus</i> ) research centre in Spain
15:00 – 15:30	BREAK	
15:30 – 17:00	<b>SESSION 4</b>	<b>Debate and Teasers</b>
15:30 – 16:30	Panel discussion “Are wildlife diseases relevant for public health?”	
16:30 – 17:00	Why you need to come see my poster: two minute teasers	
17:00 – 17:30	BREAK AND CHANGE OF LOCATION	
17:30 – 19:00	Poster session	<b>Location:</b> Strandhotel Seeduyn
19:30 – 21:30	Barbecue	
21:30 – 23:30	Auction	

**Day 2, Wednesday September 15****Location:** Conference hall "De Bolder"

8:30 – 12:00 Chair persons: Christian Gortázar &amp; Oliver Krone

8:30 – 12:00	<b>SESSION 5</b>		<b>Environmental aspects and ecology</b>
8:30 – 9:00	keynote	Prof. Dr. Sarah Randolph	Climate change and infectious diseases
9:00 – 9:10	13	Francisco Ruiz-Fons	A broad assessment of factors determining <i>Culicoides imicola</i> abundance: modelling the present and forecasting its future in scenarios of climate change
9:10 – 9:20	14	Adam Michel	Infections with <i>Babesia</i> spp. in free-ranging wild ungulates in Switzerland
9:20 – 9:30	15	Ezio Ferroglio	The role of foxes in <i>Leishmania infantum</i> epidemiology
9:30 – 9:40	16	Fabien Mavrot	Occurrence of healthy carriers of <i>Mycoplasma conjunctivae</i> and comparison of strains and mycoplasmal loads in asymptomatic and diseased wild Caprinae
9:40 – 9:50	17	Paul Jepson	Trends in contaminant exposure and potential health effects in UK-stranded cetaceans (1990-2008)
9:50 – 10:00	18	Andrew Brownlow	The porpoise of surveillance: 20 years of monitoring disease in Scottish cetaceans
10:00 – 10:30	BREAK		
10:30 – 10:40	19	Rebecca Vaughan	Disease risk analysis for conservation translocations: The reintroduction of the Eurasian crane ( <i>Grus Grus</i> )
10:40 – 10:50	20	Maud Marsot	The introduced <i>Tamias sibiricus</i> increases the prevalence of Lyme borreliosis agent in native reservoir hosts
10:50 – 11:00	21	Lucy Gilbert	Can wildlife management be used to control ticks and tick-borne pathogens?
11:00 – 11:10	22	Bob McLean	Farm yard and rural home visitation by white-tailed deer ( <i>Odocoileus virginianus</i> ): Implications for mitigation of disease transmission
11:10 – 11:20	23	Ruth Cromie	Developing practical guidance on the prevention and control of disease of wetlands
11:20 – 11:30	24	Martin Lange	Assessing CSF disease control measures using an individual-based model
11:30 – 12:00			
12:00 – 13:30	LUNCH		
13.30 – 19.30	SOCIAL ACTIVITIES		

**Day 3, Thursday September 16****Location:** Conference hall "De Bolder"

8:30 – 10:00 Chair persons: Joke vd Giessen &amp; Ezio Ferroglio

8:30 – 17:00	<b>SESSION 6</b>		<b>Wildlife disease reporting and surveillance</b>
8:30 – 9:00	keynote	Prof. Dr. Bruno Gottstein	Public health aspects of <i>Echinococcus multilocularis</i> : wildlife crossing domestic life in Europe
9:00 – 9:10	25	Christof Janko	<i>Echinococcus multilocularis</i> and habitat use of red foxes ( <i>Vulpes vulpes</i> ) in villages and small towns
9:10 – 9:20	26	Astrid Sutor	Prevalence Estimation of <i>Echinococcus multilocularis</i> among raccoon dogs ( <i>Nyctereutes procyonoides</i> ) in northern Brandenburg, Germany
9:20 – 9:30	27	Katsuhisa Takumi	Increasing risk of human alveolar echinococcosis in the Netherlands and possible control options
9:30 – 9:40	28	Andreas König	Fox tapeworm: an underestimated threat and a strategy to solve the problem
9:40 – 9:50	29	Sylvain Larrat	Preventing human trichinellosis acquired from walrus meat in the Canadian North: the successful example of the Nunavik Trichinellosis Prevention Program
9:50 – 10:00	30	Marieke Opsteegh	Seroprevalence of <i>Toxoplasma gondii</i> in wild boar in the Netherlands
10:00 – 10:30	BREAK		
10:30 – 12:00	Chair person: Wim vd Poel		
10:30 – 10:40	31	Pikka Jokelainen	<i>Toxoplasma gondii</i> killing European brown hares and mountain hares in Finland: Proportional mortality rate, seroprevalence, and genetic characterization
10:40 – 10:50	32	Anke Wiethölter	The National Research Platform for Zoonoses – a novel approach to intensify zoonotic disease research in Germany
10:50 – 11:00	33	Susanne Schex	Longitudinal study reveals stable occurrence of Hantaviruses in free-ranging rodents in South-Eastern Germany
11:00 – 11:10	34	Vicente Joaquin	Field epidemiology of wild boar livestock interactions in South Central Spain
11:10 – 11:20	35	Natacha Wu	Risk assessment for pathogen transmission from wild boar to outdoor pigs in Switzerland
11:20 – 11:30	36	Laura Fernández-Sirera	Surveillance of Pestivirus infection in wild ungulates from Andorra
11:30 – 11:40	37	Josanne Verhagen	Linking surveillance of avian influenza viruses in wild birds with outbreaks in poultry
11:40 – 11:50	38	Catherine Lutton	Estimating contact rate – density relationships for badgers ( <i>Meles meles</i> )
11:50 – 12:00			

12:00 - 13:30	LUNCH		
13:30 - 17:00	Chair persons: Dolores Gavier-Widen & Andrea Gröne		
13:30 - 13:40	39	Marie-Pierre Ryser-Degiorgis	First evidence of <i>Cytauxzoon</i> sp. infection in Eurasian lynx
13:40 - 13:50	40	Alvaro Oleaga	New techniques for an old disease: sarcoptic mange in the Iberian wolf
13:50 - 14:00	41	Andreas König	Scabies in Bavarian chamois populations and management recommendations
14:00 - 14:10	42	Jolianne Rijks	Set-up of vector-borne disease prevalence studies in roe deer
14:10 - 14:20	43	Charles van Riper III	Proximate and ultimate effects of blood parasites on the California Western Scrub Jay ( <i>Aphelocoma californica</i> )
14:20 - 14:30	44	Becki Lawson	The spread of finch trichomonosis, an emerging infectious disease, by migrating birds from Great Britain to Southern Fennoscandia
14:30 - 14:40	45	Nelson Marreros	Caprine lymphotropic herpesvirus infection associated with broncho-intestinal pneumonia in alpine Ibex
14:40 - 14:50	46	Orusa Riccardo	Isolation of avian pox virus in hooded crows in Italy
14:50 - 15:00	47	Trine Jensen	Novel Aleutian mink disease virus strain found in wild mink at Bornholm
15:00 - 15:30	BREAK		
15:30 - 15:40	48	Steven van Beurden	Possible role of pathogenic viruses in the decline of the wild European eel stocks
15:40 - 15:50	49	Christos Iacovakis	Update on genetic analysis and the epidemiology of EBHSV across Europe from 1999 to 2010
15:50 - 16:00	50	Bjoernar Ytrehus	<i>Bartonella</i> in deer ked ( <i>Lipoptena cervi</i> ) in Scandinavia
16:00 - 16:10	51	Benoît Lévesque	Seroprevalence of ten zoonotic infections in two Canadian Cree communities
16:10 - 16:20	52	Kristin Mueldorfer	Bacterial diseases in free-ranging European bats
16:20 - 16:30	53	Victor Simpson	Exudative dermatitis in red squirrels ( <i>Sciurus vulgaris</i> ) associated with <i>Staphylococcus aureus</i> ST 49 infection
16:30 - 16:40	54	Samoa Giovannini	Epidemic of salmonellosis in passerine birds in Switzerland and suspected spillover in domestic cats
16:50 - 17:00			
17:00 - 17:15		Prof. Dr. Thijs Kuiken	Closing of meeting
20:00 - 24:00	BANQUET DINNER		<b>Location:</b> Strandhotel Seeduyn

Please note that changes in the program might be made.

# Abstracts of oral presentations, by day and session

## KEYNOTE

### **Wildlife and emerging zoonoses: a real problem**

Albert D.M.E. Osterhaus<sup>1</sup>

Until the start of the last century, infectious diseases were a major cause of human mortality. In the following decades the burden of infectious diseases in the Western world decreased dramatically by the implementation of public health measures and later also by the introduction of antimicrobials and vaccination strategies. A major success was the eradication of smallpox in the 1970s through a worldwide WHO orchestrated vaccination campaign. Policymakers and scientists even speculated that all serious infectious diseases of humankind would soon be brought under control. Paradoxically in the past decades, the world was confronted with an ever-increasing number of emerging or re-emerging infectious diseases, most of which originated from wild animal reservoirs. Striking examples were the pandemics caused by influenza A viruses, HIV and SARS-coronavirus, that had spilled over from their animal reservoirs: birds, chimpanzees and bats respectively. In some of these introductions domestic animals played an intermediate role. Furthermore a long list of exotic names of viruses like Ebola-, Lassa-, Rift-Valley-, Hanta-, Crimea-Congo-, Hendra-, Nipah-, and West-Nile virus, highlights the origins of several viruses that crossed the species boundary from animals to humans with dramatic consequences in the past decades. Similarly, recent mass mortalities among wild aquatic and terrestrial mammals caused by known and newly discovered morbilliviruses, as well as outbreaks of hog cholera, foot-and-mouth disease and fowl plague among domestic animals, highlight this trend of newly emerging viruses in humans and animals.

Although improved detection and surveillance techniques, as well as increased media attention may have contributed to our perception of an increase in the incidence of outbreaks of virus infections in humans and animals, it has become also clear that major changes in our modern and globalizing society increasingly create new opportunities for virus infections to emerge: a complex mix of changes in social environments, medical and agricultural technologies and ecosystems continues to create new niches for viruses to cross species barriers and to rapidly adapt to new species.

In combating this global threat, we should make optimal use of new tools provided by the unprecedented advances made in molecular biology, epidemiology, genomics and bioinformatics. Especially the role of early warning systems based on state of the art serological, virus detection and virus discovery techniques, as well as targeted intervention strategies based on genomics data related to virus-host

interaction, have already shown to be instrumental in dealing with recent viral threats from the animal world like SARS and avian influenza.

## KEYNOTE

### **Wildlife and emerging zoonoses: not a real problem**

Dr. Jim van Steenberg

The public is afraid of any emerging infectious disease: it's contagious, it's unpredictable and it's deadly. The truth is, however, that for disease burden, as measured by morbidity and mortality, infectious diseases are not a true public health problem in the rich industrialized western world. If infectious diseases are not a true public health problem, emerging infectious diseases are less of a public health problem, and an even further selection, emerging zoonoses are not a real problem at all.

As we are participating in the European Wildlife Disease Association's discussion, it is appropriate to focus on Europe. There, the real killers are lifestyle diseases and other unpleasant aspects of modern life: road traffic accidents and suicides. In comparison with cancers, cardiovascular diseases and other serious killers, infectious diseases as a whole are not a problem. If all infectious diseases are grouped together they make a good third place in mortality. A major contribution to overall infectious disease mortality is the (pneumococcus) pneumonia of the elderly.

As you might know, in The Netherlands we have faced a large zoonotic epidemic: Q fever from 2007-2009. In 2009, the "hottest" year, 2317 cases were reported. In the Netherlands annually 120.000 persons are diagnosed with pneumonia.

This biggest Q fever epidemic ever recorded in history caused less than 2% of our annual pneumonia disease burden.

Once you are working in infectious disease control, you argue with policy makers that infectious diseases might not be a real public health problem at present (under control by sanitation, ample safe water and food, vaccination programs) but they might become a real public health problem in the immediate future. Indeed, there is a constant threat of emerging infections. This threat is a possible imminent public health problem, not a real problem at present. Real problems should be solved first, before spending time and money on possible problems that might arise in the future.

Of course, governments in the rich industrialized world have the responsibility to prepare the health care system of their countries for early detection of threats (either from inside or outside their country), and preferably -if possible- also to prevent the (re)emergence of diseases, regardless of their origin.

If we agree that a well developed society should not only look into real problems, but also into imminent problems, emerging infections are something to deal with. The question arises where these threats might come from, and to what size the future problem might grow.

As humans are just one of the many animal species in the world, it is far from surprising that a majority of new

infectious diseases originate from other animal species.

In The Netherlands it is more likely that we will face an emerging zoonotic disease from captive animals than from wild animals. We have 16.5 million inhabitants living on 33.883 km<sup>2</sup>. On this same densely populated area we have eight times as many animals in captivity: 3.890.000 cattle, 12.026.000 pigs, 1.213.000 sheep, 96.700.000 poultry. As all these animals have to be cared for, there will be much more close contact with these captive animals than with wildlife. Contact with wildlife is less, and although the numbers of rodents exceeds by far those of captive animals, for roe deer we only have 80.000 (compare the smallest husbandry sector in this country are dairy goats with 300.000 animals) Emerging zoonoses need attention from governments, in the first place those that originate from captive animals. To spend much money and time on early detection of zoonoses originating from wildlife is a luxury product, which only should be installed if real threats of real problems are tackled properly.

## KEYNOTE

### Wildlife and emerging zoonoses: a reality from a conservation point of view

Prof. Dr. Herbert Prins

Landscape can be thought as a geomorphologic skeleton enveloped by vegetation. This vegetation can occur in different formations, such as forest or grassland. Wherever in the temperate zone or in the tropics water is not limiting plant growth, trees will outcompete grasses and herbs by shading them out. Yet in savannas (or savanna-like vegetations) there is a stable equilibrium between these life forms. If trees are edaphically or climatically potentially dominant, they can be suppressed by fire, herbivory or man. Anything that thus changes the numerical abundance of herbivores or people has the potential of changing the biotic component of the landscape.

I first will show the effect of two diseases (anthrax and rinderpest) on the African savanna landscape. I then demonstrate the consequence of a classical zoonose (pest) on the temperate forest landscape of Europe. I will end by giving you an idea about cascading effects of tuberculosis on savanna landscapes in southern Africa: TB infection, perhaps originating in a human populations, moved from cattle to buffalo, and thence to lion. Changes in lion population structure then spills over in modifications in the behaviour of other herbivores (such as impala) which changes the spatial nutrient redistribution. This redistribution then upsets the competitive balance between shrubs, trees and grasses, leading to ever further cascading modifications of the landscape.

## ORAL PRESENTATION 1

### Potentially Zoonotic Viruses in Straw Coloured Fruit Bats (*Eidolon helvum*) in the Gulf of Guinea islands

Peel, Alison J<sup>1,2</sup>; Fernández Loras, Andrés<sup>2</sup>; Baker, Kate S.<sup>1,2</sup>; Hayman, David T.S.<sup>1,2</sup>; Gill, David<sup>2,3</sup>; Kamins, Alexandra<sup>1,2</sup>; Rossiter, Stephen J.<sup>4</sup>; Sargan, David R.<sup>1</sup>; Cunningham, Andrew A.<sup>2</sup>; Wood, James L.N.<sup>1</sup>

<sup>1</sup>University of Cambridge; <sup>2</sup>Zoological Society of London; <sup>3</sup>Imperial College; <sup>4</sup>Queen Mary;

**Key words:** Chiroptera, henipavirus, lyssavirus, population genetics, bushmeat

**Background:** Ongoing studies in continental Africa have identified the straw-coloured fruit bat, *Eidolon helvum*, as a reservoir for potentially zoonotic viruses (henipa- and lyssa-viruses) and a common source of bush meat. We have also determined that *E. helvum* exists as one large panmictic population with extensive gene flow throughout sub-Saharan Africa, with no evidence of segregation according to presumed migration routes. This population structure appears to have resulted in widespread seroprevalence to henipavirus and Lagos Bat Virus (LBV) across Africa. However, it is currently unknown whether isolated non-migratory populations of *E. helvum* in the Gulf of Guinea islands are also reservoirs of these viruses.

**Methods:** *E. helvum* bats were sampled on the islands of São Tomé, Príncipe, Bioko and Annobón to investigate further the genetic population structure and to assess the viral infection status of these populations. Interviews were conducted regarding bat consumption, including hunting, butchering and cooking methods, the structure of the commodity chain, offtake levels and the perceived risk of exposure to zoonotic pathogens.

**Results:** Island populations show evidence of genetic and morphological differentiation from the continental population, with two to three colonisation events to the islands. Potential interactions with human populations varied considerably between each island, from Bioko, where *E. helvum* roost in large numbers within the city, directly over human populations, but are 'ignored' and not targeted as a bushmeat species, to São Tomé, where bats are a favoured bushmeat species and roost far from urban populations. In the former, city residents may be at greater risk from aerosol-borne zoonoses, whereas in the latter, hunters may be at greater risk from bat bites or blood-borne zoonoses. Serological analyses are currently underway to determine the seroprevalences to henipavirus and LBV in these island bat populations.

**Conclusions:** Investigating the ecology and potential reservoir status of *E. helvum* in the Gulf of Guinea could provide great insight into zoonotic viral infection dynamics and subsequent risks to public health.

## ORAL PRESENTATION 2

### Absence of associated histopathologic changes in tissues of bats with coronavirus infection

van den Brand, Judith<sup>1</sup>; Leijten, Lonneke<sup>1</sup>; van der Kooij, Jeroen<sup>2</sup>; Dekker, Jasja<sup>3</sup>; Reusken, Chantal<sup>4</sup>; Kuiken, Thijs<sup>1</sup>

<sup>1</sup>Erasmus Medical Centre; <sup>2</sup>Norwegian Zoological Society; <sup>3</sup>Zoogdierverseniging; <sup>4</sup>National Institute for Public Health

**Key words:** Coronavirus, bats, pathology

**Background:** Bats are the reservoir of a number of viruses that cause emerging diseases in humans. One of these diseases, severe acute respiratory syndrome (SARS), was found to be caused by a coronavirus very similar to those endemic in bats in Asia. Subsequently, group 1 and 2 coronaviruses, including SARS virus-like coronaviruses, have been found in various bat species in other parts of the world. These viruses were found predominantly by PCR on fecal samples as well as by antibody detection in serum. However, it is not known how these coronaviruses affect the health of infected bats. Therefore, the goal of our study was to determine whether corona-virus-infected bat tissues had histopathological changes.

**Methods:** We screened lung and intestinal tissues from 36 bats from Germany, 15 bats from Norway and 33 bats from the Netherlands for coronavirus by RT-PCR and examined paired samples by light microscopy for histopathological changes. Tissues were obtained from bats found moribund or dead.

**Results:** We detected coronavirus in the lung, intestine or both of 13/36 (36%) bats from Germany, 2/15 (13%) from Norway and of 3/33 (6%) bats from the Netherlands. The species infected were *Nyctalus noctula* (12) and *Plecotus auritus* (1) in Germany, *Eptesicus nilssonii* (1) and *Myotis daubentonii* (1) in Norway, and *Pipistrellus pipistrellus* (3) in the Netherlands. No significant histopathological changes were detected in paired samples of infected lung and intestine.

**Conclusions:** Our results show the presence of coronavirus in bats in absence of noticeable histopathological changes. These findings suggest that coronavirus infections in bats may be subclinical.

## ORAL PRESENTATION 3

### Genetic Relatedness of Raccoons in Northeastern Ohio, USA: Implications for Rabies Spread

Berentsen, Are<sup>1</sup>; Wisely, Samantha<sup>2</sup>; Dunbar, Mike<sup>1</sup>; Fitzpatrick, Chadd<sup>1</sup>

<sup>1</sup>National Wildlife Research Center; <sup>2</sup>Kansas State University

**Key words:** Genetics, Ohio, Rabies, Raccoons, USA

**Background:** Raccoon (*Procyon lotor*) variant rabies is distributed throughout the eastern United States. Westward spread of the disease has historically been prevented by geographic barriers and distribution of oral rabies vaccine (ORV) baits. In 2004 a rabid raccoon was documented in northeastern Ohio, representing a breach in the ORV barrier. As a result, the movements of raccoons in northeastern Ohio needed to be examined to identify if there were natural barriers to impede further disease spread and whether corridors exist that may facilitate this spread. The objective of this study is to use molecular tools to investigate landscape-scale movement and dispersal patterns of raccoons in northern Ohio.

**Methods:** Epithelial tissue samples (ear clips) were obtained from 321 live-caught and salvaged raccoons from urban and suburban areas within 85 km of Cleveland, Ohio. DNA was extracted and 11 microsatellite loci were amplified. For spatial analysis animal locations were binned into urban, north-central-south, east-central-west and far-west sectors. Tests for Hardy-Weinberg equilibrium, sex-biased dispersal and spatial autocorrelation analysis were performed using the program Genalex, V 2.2.

**Results:** All loci were in Hardy-Weinberg equilibrium. Overall genetic diversity was high with an average 18 alleles per locus and an average expected heterozygosity of 0.86. Nine of 11 sectors contained private alleles, suggesting that the breeding population was larger than the sampled area. Spatial autocorrelation suggests average dispersal distance of 0.5 – 3.5 km, although this did not include eight instances of long distance dispersal.

**Conclusion:** Results suggest a large admixed population with dispersal among all sectors as well as into and out of outlying rural areas. At the current stage of analysis Cleveland does not appear to constitute a barrier to raccoon movements and appears to have sufficient habitat to support raccoons, and thus raccoon variant rabies. Additional habitat-based analysis is ongoing.

## ORAL PRESENTATION 4

### **Bartonella Species in the Harbor Seal (*Phoca vitulina*) and in Seal Lice (*Echinophthirius horridus*)**

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**Key words:** Bartonella, Echinophthirius horridus, Harbor seal, Phoca vitulina, Seal louse

**Background:** Bartonella species are hemotropic, gram-negative, emerging bacteria that are highly adapted to their mammalian reservoir hosts. In 2005, the first detection of Bartonella spp. in a marine mammal was reported, where Bartonella henselae DNA was detected in blood samples from two harbor porpoises (*Phocoena phocoena*). A recent report described the detection of two B. henselae strains in captive and free-ranging beluga whales. The aim of this study was to explore whether harbor seals (*Phoca vitulina*) and seal lice (*Echinophthirius horridus*) are exposed to Bartonella spp.

**Methods:** Thirty-five seal lice (*Echinophthirius horridus*) were collected from seven seals, during their rehabilitation period in the Seal Rehabilitation and Research Center at Pieterburen, The Netherlands (SRRC). Forty-eight spleen samples were collected during necropsies of other harbor seals that died during rehabilitation at the SRRC. DNA was extracted by DNA extraction kit. Molecular diagnosis of Bartonella spp. was performed by High resolution melt, real-time PCR amplifying partial loci of the RNA polymerase (*rpoB*) gene, and the intergenic spacer (ITS) region.

**Results:** One lice pool and one spleen sample were found positive for Bartonella spp. One hundred percent sequence similarity with Bartonella henselae was found in the ITS region, and 97% sequence similarity with Bartonella grahamii was detected in the *rpoB* gene. The Bartonella spp. identified in the spleen and lice were found to be identical to each other.

**Conclusions:** This is the first report describing the detection of Bartonella spp. from a seal or any other member of the pinnipedia order, and from seal lice. The 100% sequence similarity in the ITS of the Bartonella sp. identified with the zoonotic B. henselae warrants further investigation and characterization of this organism, which may be found to be of public health importance.

## ORAL PRESENTATION 5

### **The Central European expansion of lineage 2 West Nile virus during 2008 and 2009**

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**Key words:** West-Nile virus, wildlife, Europe, zoonoses

A lineage 2 West Nile virus (WNV) strain emerged in Hungary in 2004 and had caused sporadic cases of encephalitis in wild and captive Goshawks (*Accipiter gentilis*), some other birds of prey and mammals during the following years. After WNV became endemic in the area of the initial outbreak, a sudden and unexpected expansion of the pathogen was detected during 2008 and 2009.

Passive and targeted active monitoring of WNV related disease cases and mortality was conducted throughout the above period. Carcasses of potentially infected birds and blood samples from birds, horses and humans were examined. The presence of WNV was demonstrated in organ and blood samples by RT-PCR and immunohistochemistry, and sero-conversion was detected by competitive ELISA and IFAT in suspect clinical cases.

During 2008 WNV encephalitis was diagnosed in 25 dead Goshawks and other birds of prey, in 12 clinically ill horses, and 22 humans. The Westward spread of the virus had also reached the Eastern federal states of Austria, where WNV was detected in 15 wild birds. During the following year (2009) WNV outbreaks on the territories of Hungary and Austria were documented by positive WNV diagnoses in 16 wild birds, 4 horses and 6 humans. The WNV strains isolated in both years were closely related to the lineage 2 WNV strain isolated from the initial outbreak in 2004 (goshawk-Hungary/2004). The endemic presence and the unexpected expansion of WNV in central Europe raised serious public health and veterinary concerns. Ongoing studies of WNV epidemiology and ecology as well as WNV monitoring should provide a basis for the assessment of the potential for future geographic spread and overall European impact of this agent.



## ORAL PRESENTATION 6

### Modification of a commercial ELISA to detect antibodies against *Coxiella burnetii* in wild ungulates: application to population surveillance

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<sup>1</sup>NEIKER

Key words: *Coxiella burnetii*; Serology; Wildlife;

*Coxiella burnetii* is the causal agent of Q fever, a currently emergent disease in Europe. Domestic ruminants are the main reservoir but wildlife may also maintain *C. burnetii*. Little is known on the role of wildlife in the life cycle of this pathogen, mainly because of the lack of accurate surveillance tools. It was hence our aim to search on the feasibility of modifying a commercial ELISA to investigate the status of *C. burnetii* in wild ungulates and to apply this protocol in a Q fever endemic area in Spain.

A commercial ELISA test developed for domestic ruminants (ELISA Cox kit, LSI, Lyon, France) was modified. The main modification consisted on the substitution of the secondary antibody by a protein binding to most mammals' immunoglobulin G, i.e. protein G from *Streptococcus* spp. Cut-off, sensitivity and specificity were established by testing known *C. burnetii* IFAT antibody positive red and roe deer, and also by sheep and cattle sera positive/negative by ELISA and PCR. Roe deer (n=65), red deer (n=23) and European wild boar (n=158) sera collected in the Basque Country were tested by the modified ELISA.

The modified ELISA showed a high sensitivity and specificity at the established cut-off with sheep and cattle sera. Protein G has proved as a good secondary antibody to test wild ungulate sera by ELISA, for which we assumed sensitivity and specificity values to be similar in wild ungulates. Only 2 wild boar sera out of 246 samples tested (0.8±1.1%) reacted positive in ELISA.

We herein provide an approach for the use of a commercial ELISA test to search for the status of *C. burnetii* in wild ungulates. The modified ELISA could be a good, easy-to-perform and cheap tool for the surveillance of *C. burnetii* in wild ungulates. Wild ungulates in our study area seem to have a low contact rate with *C. burnetii*, which agrees with previous findings using PCR.

## ORAL PRESENTATION 7

### Roe deer and the Dutch Q-fever epidemic

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Key words: *Coxiella burnetii*, *Capreolus capreolus*, The Netherlands

The Netherlands are facing an unprecedented human Q-fever epidemic that started in 2007. Infected dairy goats are considered the main source of infection, with huge numbers of *Coxiella burnetii* being excreted after abortion, as has occurred on several farms since 2005. Many domestic and wild animal species are susceptible for *C. burnetii* infection, but information on *C. burnetii* infection in wildlife in the Netherlands is virtually inexistent. Roe deer (*Capreolus capreolus*) are the most numerous (> 60,000) and widespread cervid species in the Netherlands, and serological data from elsewhere suggests the species is susceptible to *C. burnetii* infection. The aim of this study is to examine sera and tissues of roe deer retrospectively for evidence of *C. burnetii* infection.

An indirect ELISA, with protein G as conjugate, is used to examine sera collected from several hundred roe deer shot in the Netherlands between January and June 2010. Cases with positive or dubious results are tested further by complement fixation test and for pathogens reported to serologically cross-react with *C. burnetii*. In addition, PCR is performed retrospectively on tissues of approximately 70 roe deer submitted to the Dutch Wildlife Health Centre for post-mortem examination between January 2008 and June 2010. The tissues tested are lung, liver, spleen, kidney and bone marrow when available.

The tests are on-going. Initial ELISA results are one positive and two dubious cases among 300 sera samples tested. Initial PCR results are one positive (liver) out of two deer tested. The preliminary serological results suggest that *C. burnetii* infection is not widespread among roe deer in the Netherlands. However the positive PCR case suggests that the infection can occur. To the best of our knowledge, this is the first time *C. burnetii* antigen has been shown in roe deer.

## ORAL PRESENTATION 8

### FAO Embraces the One Health Challenge at the wildlife-livestock-human-ecosystem interfaces

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In a globalized world where pathogens can travel the world in a day, emerging diseases, especially those affecting humans, livestock or wildlife, can have large negative socio-economic implications. Impacts can be severe for public health, livelihoods and food security, as well as for international trade and tourism. Climate change and loss of ecosystem resilience, furthermore, are paving the road for the emergence of a series of new, multidimensional conservation and health challenges.

The Food and Agriculture Organization of the United Nations (FAO) has been an integral partner in 3 Inter-Ministerial Conferences (New Delhi-2007, Sharm El Sheikh-2008, and Hanoi-2010) where the New Delhi Recommendation addresses the larger issue of emerging infectious diseases at animal-human-ecosystem interface and the Hanoi Declaration, based on global experience with H5N1 highly pathogenic avian influenza and pandemic (H1N1) reaffirmed the importance of international and regional cooperation, national political commitment, inter-sectoral collaboration, timely and transparent communication, and capacity building as essential to build a health system capable to address high impact disease threats that arise at the animal-human-environment interface. Within the Food Chain Crisis Management Framework – Animal Health, FAO has recently developed a One Health Programme entitled, “A Comprehensive Approach to Health – People, Animals and the Environment” which provides a strategic framework to guide the implementation of FAO’s vision for animal health which requires inputs and expertise from many Departments and Divisions with FAO including Forestry, Fisheries, Natural Resources, and Legal.

While more science is necessary to understand the complex relationships among disease emergence, transmission and ecological systems, science alone is not the solution. It is also essential to address the social and cultural dimensions of societies where issues concerning livestock, wildlife, humans and entire ecosystems intersect. Changes in thinking and behaviour must be encouraged, and future decision-making must be cognizant of the repercussions of poor natural resource management and their implications for civilization.

## KEYNOTE

### Emerging infectious diseases in an international context, a medical perspective

Prof. Dr. Menno de Jong

## KEYNOTE

### Cowpox outbreak through animal trade

Dr. Alexandra Mailles

A nationwide outbreak of cowpox virus infection, due to a unique viral strain, involving 20 cases, occurred in France in early 2009 among persons exposed to pet rats that were traced back to a same supplier in the Czech Republic. On January 16, 2009, the French national Public Health Institute (Institut de Veille Sanitaire, InVS) received reports of 3 patients in the Oise district with ulceronecrotic skin lesions and painful regional lymphangitis and lymphadenopathy of unknown origin. All three patients had had close contact with rats purchased at the same pet shop. On January 26, the Virology laboratory of La Timone hospital in Marseille identified a virus morphologically consistent with an orthopoxvirus by electron microscopy of skin lesion tissue from 3 patients. Additional PCR assay, and subsequent sequence data, indicated that the causative agent was a cowpox virus (CPXV).

## ORAL PRESENTATION 9

### Chlamydomphila psittaci infection in garden birds in England and Wales

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**Key words:** Chlamydomphila psittaci garden birds chlamydiosis

**Background:** The avian pathogen Chlamydomphila psittaci has the potential to cause severe zoonotic disease. In the UK, sporadic chlamydiosis outbreaks have been diagnosed in garden birds such as robins (*Erithacus rubecula*), dunnocks (*Prunella modularis*), Paridae (tit species) and Fringillidae (finches), but the prevalence of *C. psittaci* infection in these species is unknown. It has been estimated that over 12 million householders in the UK provide food for garden birds, and contaminated garden bird feeders or infected carcasses represent a possible source of zoonotic *C. psittaci* infection.

**Methods:** An outbreak of chlamydiosis in small passerines was diagnosed at a site in southern England in February 2009. Following this, the incidence of chlamydiosis in passerines in England and Wales was investigated retrospectively using archived frozen tissues from passerine carcasses that had been submitted to the Garden Bird Health initiative from 2005 to 2009. Cases selected for the study had pathological findings consistent with chlamydiosis at gross post mortem examination, but for which no cause was identified on routine microbiological testing. Tissues were analysed for *C. psittaci* infection using a combination of DNA ArrayTube microarray and PCR techniques.

**Results:** Twelve (80%) of 15 cases, from ten separate sites of mortality, were positive for *C. psittaci* infection: five dunnocks, four great tits (*Parus major*), two blue tits (*Cyanistes caeruleus*) and one robin. In all of nine cases in which genotyping was possible, the *C. psittaci* isolates were Genotype A. Concurrent infectious disease was present in four (33%) of the positive cases.

**Conclusions:** Chlamydiosis appears to be an under-diagnosed cause of passerine mortality in England and Wales. The prevalence of *C. psittaci* infection and of chlamydiosis in wild birds in Europe merits further investigation. Also, it is important to determine any risk to human health.

## ORAL PRESENTATION 10

### Investigation of the ecology of *Francisella tularensis* in an interepizootic period

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Majoros, Gabor<sup>1</sup>; Tirjak, Laszlo<sup>3</sup>; Erdelyi, Karoly<sup>2</sup>

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**Key words:** disease ecology, European brown hare, *Francisella tularensis* ssp. *holarctica*, *Haemaphysalis concinna*, zoonosis

**Background:** A one year study of the ecological cycle of *Francisella tularensis* was performed in an enzootic area during an inter-epizootic period, since the ecology of tularemia is still only partially understood with many open questions about reservoirs and vectors.

**Methods:** The study was based on multiple sampling of all major constituents of the disease cycle.

**Results:** Seroprevalence of tularemia in the European brown hare (*Lepus europaeus*) population was 5.1% (10/197) with low titers (1/10 and 1/20) and *F. tularensis* ssp. *holarctica* was isolated from four hares. The modification of the diagnostic 1/40 tube agglutination titer is suggested. *F. tularensis* was not detected in the trapped 38 common voles (*Microtus arvalis*), 110 yellow-necked mice (*Apodemus flavicollis*), 15 striped field mice (*Apodemus agrarius*) and a by-catch of 8 Eurasian pygmy shrews (*Sorex minutus*) and 6 common shrews (*Sorex araneus*). A total of 1106 *Ixodes ricinus* and 476 *Haemaphysalis concinna* ticks were collected from vegetation and 404 *I. ricinus*, 28 *H. concinna* ticks and 15 *Ctenophthalmus assimilis* and 10 *Nosopsyllus fasciatus* fleas were combed off small mammals. One *H. concinna* female and one nymph collected from the vegetation was infected with *F. tularensis* ssp. *holarctica* thus resulting a 0.42% (2/476) prevalence. *F. tularensis*-specific DNA was not detected in environmental water samples and the examined 100 sheep, 50 cows and 50 buffalos grazed at the study area were seronegative.

**Conclusions:** We hypothesize that during interepizootic periods *F. tularensis* ssp. *holarctica* persists only in the European brown hare – *H. concinna* cycle. *H. concinna* may not serve exclusively as an arthropod vector but it might also harbor bacteria for three–four years through multiple life stages and act as an important reservoir of *F. tularensis* ssp. *holarctica*. Since chronically infected hares shed live bacteria by urine, an additional airborne hare – hare cycle, may complement the main vector borne cycle. Rodent species probably do not serve as true reservoir hosts of tularemia.

## ORAL PRESENTATION 11

### Borreliosis in a *Pipistrellus* sp bat. Is this an emerging zoonosis?

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**Key words:** *Borrelia*, spirochaete, *Pipistrellus*, zoonosis, *Argas*

**Background:** Historically little attention has been paid to diseases of bats in the UK.

**Methods:** During 2004-2008 80 bats from SW England were examined post-mortem, organs showing gross lesions examined histologically and hearts retained at -20°C. Ten bats from other regions were examined in 2009 and 24 ticks collected from bats in Avon and Oxfordshire. DNA extracted from bat tissues and ticks was used in PCR assays targeting fragments of 16S rRNA, *glpQ* and *flaB* genes followed by sequence analysis.

**Results:** In 2008 a juvenile *Pipistrellus* species was found grounded in Cornwall. Despite rehabilitation efforts it died 12 days later. Necropsy revealed healed tears in wing membranes and an *Argas vespertilionis* tick attached to the dorsum. Body condition was good but it was anaemic with pale skeletal muscles. The lungs looked normal but there was excess blood tinged pleural fluid. Both liver and spleen were enlarged and dark, the adrenal glands enlarged, pale with focal haemorrhages; kidneys were pale with cortical speckling. A liver impression showed numerous Gram-negative spirochaete-like structures. Histological examination revealed hepatic congestion with scattered multifocal areas of hepatocyte necrosis and inflammation. Warthin-Starry staining showed numerous long, undulating, argyrophilic bacilli in liver lesions and in other organs. Sequence analysis of a PCR product from the liver identified the organism as a likely novel *Borrelia* species within the relapsing fever group. None of the 90 heart samples yielded *Borrelia* DNA but an *A. vespertilionis* tick from Avon gave a positive result and sequencing showed an exact match with that derived from the pipistrelle bat.

**Conclusions:** The pipistrelle bat died due to infection with a likely novel *Borrelia* species. As it is genetically close to *B. recurrentis*, *B. duttonii*, and *B. crocidurae* there is a strong likelihood that it can cause disease in humans. *Argas vespertilionis* can be infected, is known to bite humans, and is a likely vector.

## ORAL PRESENTATION 12

### Salmonella spp. and Shiga toxin-producing Escherichia coli prevalence in an ocellated lizard (Timon lepidus) research centre in Spain

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**Key words:** Shiga toxin-producing Escherichia coli (STEC), Salmonella spp., ocellated lizard, Serotyping, Pulsed-field gel electrophoresis

The genus Salmonella is the largest and most heterogeneous group of the medically important Gram-negative bacteria and a wide range of animal species have been identified as faecal reservoirs. High prevalences of intestinal carriage in reptiles and some studies of human reptile-associated salmonellosis have been reported but there is little knowledge about the epidemiological status of Salmonella in wild, free-living reptiles. Shiga toxin-producing Escherichia coli (STEC) strains have recently emerged as important food-borne pathogens and their pathogenic capacity resides in a number of virulence factors, including Shiga toxins (Stx1 and Stx2). The aim of this work was to study the epidemiological status of Salmonella spp. and STEC in an ocellated lizard research centre in southwest Spain to determine the lizards' potential risk to public health as a reservoir of these food-borne pathogens. Faecal and environmental samples were collected and examined for Salmonella spp. and STEC. Detection of isolates was performed using real-time PCR (RT-PCR) and characterisation using serotyping and pulsed-field gel electrophoresis (PFGE). 52% of samples were positive for Salmonella spp. using RT-PCR and seven isolates were obtained from samples from ocellated lizards and their environment, whereas no samples were positive for STEC. Salmonella isolates belonged to *S. enterica* subsp. *enterica* serovar 28:r:e,n,x and *S. enterica* subsp. *salamae* serovars 41:z10:z6 and 18:z10:z6. Indistinguishable and closely related PFGE types were found, which supported the existence of horizontal transmission between animals due to crowding of animals and the persistence of Salmonella in the environment. This work describes the absence of STEC and the high prevalence of Salmonella in ocellated lizards, which represent a potential reservoir of this food-borne pathogen. Also emphasise the need for improved prevention efforts and good hygiene practices in research centres, recuperation centres and zoos with reptiles to minimise the exposure of personnel and visitors to this pathogen.

## KEYNOTE

### Climate change or human activities as drivers of the emergence of zoonotic infections

Prof. Dr. Sarah Randolph

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Risk of zoonotic infections depends on the environmental hazard, such as infected vectors, and contact of humans with that hazard. Trade and travel now disperse exotic infectious agents around the globe with unprecedented force. Competent vectors, either recently introduced or commonly already present, provide opportunities for exotic pathogens introduced by travel and trade. At the same time, the correct combination of environmental conditions (both abiotic and biotic) makes many far-flung parts of the world latently and predictably, but differentially, permissive for persistent transmission cycles. For tick-borne pathogens endemic in Europe, the heterogeneous upsurge in incidence over recent decades was caused by a network of interacting factors, acting synergistically but with differential force in space and time. Human behaviour determined by socio-economic conditions evidently played a more significant role than abiotic and biotic environmental factors acting on enzootic cycles, although there is an explicit causal linkage from one to the other.

## ORAL PRESENTATION 13

### A broad assessment of factors determining *Culicoides imicola* abundance: modelling the present and forecasting its future in scenarios of climate change

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**Key words:** Biogeography; *Culicoides*; Climate; Wildlife; Vector

Bluetongue (BT) is still ongoing in Europe and threats of introduction of new serotypes from endemic areas in the African continent are to be considered. *Culicoides imicola* remains as one of the most relevant BT vectors in Spain and research on environmental determinants driving its life cycle is a key issue for prevention and control of BT. We aimed to improve the understanding of the biotic and abiotic determinants of *C. imicola* by modelling its present abundance, studying the spatial pattern of predicted abundance in relation with BT outbreaks, and investigating how the predicted current distribution and abundance patterns might change under future (2011-2040) scenarios of climate change according to the Intergovernmental Panel on Climate Change.

*C. imicola* abundance data from the bluetongue national surveillance programme were modelled with spatial, topoclimatic, host and soil factors. The influence of those factors was further assessed by variation partitioning. Furthermore, predicted abundance of *C. imicola* was projected to the future by replacing the current temperature and precipitation variables in the models with those expected for the future period.

The final models retained variables of the four factors. Variation partitioning evidenced that the pure effect of host and topoclimate factors explained a high percent (>80%) of the variation. The pure effect of soils was the next in importance explaining the abundance of *C. imicola*. A close link was confirmed between *C. imicola* abundance and BT outbreaks. The projection of the final *C. imicola* abundance models to future climatic scenarios showed an expected increasing total predicted abundance for each locality although not in a high extend.

This study is, to our knowledge, the first considering hosts in predictive modelling for an arthropod vector. Main findings for the near future show that there are no evidences to expect an important increase in the distribution range of *C. imicola* in contrast to an expected increasing abundance in the areas where it is already present in mainland Spain. What we may only be able to predict on the future scenario for orbiviruses in mainland Spain is that higher predicted *C. imicola* abundance may significantly change the replication rate of orbiviruses.

## ORAL PRESENTATION 14

### Infections with *Babesia* spp. in free-ranging wild ungulates in Switzerland

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**Key words:** *Babesia*, babesiosis, survey, Switzerland, wild ruminants

Babesiosis are tick-borne diseases caused by protozoans of the genus *Babesia*. In the past five years in Switzerland, an emergence of fatal babesiosis caused by *B. capreoli* has been observed in chamois (*Rupicapra r. rupicapra*). Subsequently, a pilot study identified the roe deer (*Capreolus c. capreolus*) as a probable reservoir host for this parasite. However, only few animals originating from two selected study sites had been sampled. The aims of the present study are to evaluate the overall prevalence of infection with *Babesia* species in free-ranging wild ungulates sampled from all of Switzerland, and to eventually assess risk factors for infection.

We collected EDTA blood and demographic data from 960 animals during the 2009-2010 hunting season. This included 261 chamois, 211 roe deer, 228 red deer (*Cervus elaphus*) and 260 Alpine ibex (*Capra i. ibex*). Polymerase chain reaction (PCR) with a broad specificity for *Babesia* species, targeting part of the 18S rRNA gene, was performed on individual DNA isolations.

Preliminary results show that 89 chamois (31%), 110 roe deer (52%), 73 red deer (32%) and 45 ibex (17%), were positive for *Babesia* infection. Sequencing of eight amplicons revealed the presence *B. capreoli*/*B. divergens* (n=5), *Babesia* sp. EU1 (n=2), and *Babesia* sp. CH1 (n=1).

Interestingly, obtained prevalences are higher as compared to the results of the pilot study. Furthermore, we report for the first time an infection in a mammalian host with *Babesia* sp. CH1 which had been identified in ticks in Switzerland. As a further step, PCRs specific for *B. capreoli*, *B. divergens*, or *Babesia* sp. EU1 will be performed, and the identification of other *Babesia* will be achieved by sequencing. Finally, we will compare demographic and geographical data in an attempt to identify risk factors for infection.

## ORAL PRESENTATION 15

### The role of foxes in *Leishmania infantum* epidemiology

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**Key words:** fox, *leishmania infantum*, epidemiology, zoonosis

**Background:** *Leishmania infantum* is a protozoan parasite responsible of human and canine visceral leishmaniasis in the Mediterranean area. The dog is the main reservoir and the infection is transmitted to vertebrate hosts by phlebotomine sandflies while the role of wild carnivores in the epidemiology of leishmaniasis is still controversial. So we decided to evaluate by PCR the infection status in foxes harvested in the province of Imperia (NW Italy), and to compare their PCR-RFLP strains with those found in infected dogs and in human beings.

**Methods:** During hunting seasons 2008-2009 spleens and lymph nodes were collected from 90 foxes culled from several municipalities of the province of Imperia as well as blood samples from 57 dogs with clinical signs living in the same municipalities where the foxes were harvested and from 40 PCR positive human beings. DNA was extracted from tissues and blood using the commercial kit GenomeElute, and PCR protocol used RV1 and RV2 primers and PCR positive products were digested with restriction enzymes BsiY I and MlnI. Resulting restriction fragments were separated on a 2% agarose gel and fragment size was estimated by comparison with pBR 322 HaeIII molecular weight standards Digest.

**Results:** Twenty-eight out of the 90 foxes (31.1%) were positive at PCR. The RFLP analysis of the amplicons evidenced the presence of 10 RFLP patterns in foxes, 26 from dogs and 12 from human beings. We did not evidence overlap of patterns belonging to foxes and dogs. On the contrary a strain common to foxes and a human being has been found.

**Conclusions:** Our results suggest that a high percentage of foxes is infected by *L.infantum* and that foxes can maintain *L.infantum* infection without the need of spill-over from infected dogs, even if strain common in foxes can be found in humans.

## ORAL PRESENTATION 16

### Occurrence of healthy carriers of *Mycoplasma conjunctivae* and comparison of strains and mycoplasmal loads in asymptomatic and diseased wild caprinae

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**Key words:** Alpine chamois, Alpine ibex, healthy carriers, infectious keratoconjunctivitis, *Mycoplasma conjunctivae*

Sheep are regarded as a reservoir for *Mycoplasma conjunctivae*. However, *M. conjunctivae* was recently detected in asymptomatic Alpine ibex, (*Capra i. ibex*) suggesting that the causative agent of infectious keratoconjunctivitis (IKC) may also be maintained in wild populations. The aims of our study were (1) to assess the occurrence of healthy carriers of *M. conjunctivae* in Alpine chamois (*Rupicapra r. rupicapra*); (2) to follow up the situation in ibex; (3) to investigate the relationship between severity of eye lesions and mycoplasmal load; (4) to compare *M. conjunctivae* strains from asymptomatic and diseased animals.

Eyes swabs of 220 chamois and 447 ibex, all clinically healthy and collected in different Swiss regions between 2008-2009, were tested with a rt-PCR. Mycoplasmal load was additionally determined in 102 positive animals showing different stages of IKC lesions. Finally, comparison of strains from 21 animals was performed.

*Mycoplasma conjunctivae* was detected in 5.5% of the healthy chamois and 5.6% of the healthy ibex. Prevalence of healthy carriers showed significant variations between years and regions in both species. Mycoplasmal load was significantly lower in samples from healthy carriers than from symptomatic animals, and eyes showing moderate or severe symptoms had significantly higher mycoplasmal loads compared to eyes with mild symptoms. There was no difference in *M. conjunctivae* strains between species or between asymptomatic and diseased animals.

Our results indicate that healthy carriers of *M. conjunctivae* do not occur only in ibex but also in chamois, and prevalences vary over time. It also appears that presence and severity of lesions are closely related to the quantity of *M. conjunctivae* in the eyes, and not to the strain. We assume that individual or environmental factors may influence the clinical expression of the disease. The role of healthy carriers in the maintenance of *M. conjunctivae* in wild populations remains uncertain.

## ORAL PRESENTATION 17

### Trends in contaminant exposure and potential health effects in UK-stranded cetaceans (1990-2008)

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**Key words:** PCBs Phocoena immunosuppression disease pollutants

Concerns exist about high exposure to persistent organic pollutants in some marine mammal species. During 1990-2008, a large and integrated dataset of pathological and toxicological data was developed mainly on UK-stranded harbour porpoises (*Phocoena phocoena*) using internationally standardised necropsy and contaminant analysis methodologies. Polychlorinated biphenyls (PCBs) (n=440), organochlorine pesticides (OCs) (n=426-463), trace metals (n=265-510), butyltins (n=312), brominated diphenyl ethers (BDEs) (n=415), perfluorooctane sulfonate (PFOS) (n=58) and perfluorooctanoic acid (PFOA) (n=58) were tested. Summed blubber concentrations of 25 chlorobiphenyl congeners (sum25CBs) between 1990 and 2005 were significantly higher and much more temporally stable than all other contaminants tested. OCs levels declined more rapidly over time than PCBs. Median concentrations of nine summed BDEs (primarily from the penta-mix PBDE product) peaked around 1998 but declined significantly thereafter (EU banned penta-mix in 2004). PFOS concentrations (<16 to 2,420 ng/g wet weight) were detected in porpoise livers but PFOA was undetected. In case-control studies, sum25CBs in healthy harbour porpoises that died of acute physical trauma (mean = 11,400 ng/g lipid) (n=276) were significantly greater than sum25CBs in animals that died due to infectious diseases (mean = 22,300 ng/g lipid) (n=182) (p<0.001). The association between elevated sum25CBs and infectious disease mortality only occurred at concentrations exceeding proposed thresholds for mammalian toxicity and independently of other potentially confounding variables including age, sex, season, region, year and two quantitative indices of nutritional status. Results are consistent with PCB-induced immunosuppression at naturally occurring concentrations. Adult female porpoises (n=96) had the lowest sum25CBs but many had levels associated with reproductive impairment in other mammalian species. Mean PCB levels in UK-stranded bottlenose dolphins (*Tursiops truncatus*) (n=15) and killer whales (*Orcinus orca*) (n=5) greatly exceeded levels associated with infectious disease mortality in porpoises. PCBs undoubtedly pose a serious conservation threat to bottlenose dolphins and killer whales in many European waters.

## ORAL PRESENTATION 18

### The porpoise of surveillance: 20 years of monitoring disease in Scottish cetaceans.

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**Key words:** Cetacean, surveillance, harbour porpoise, marine, pathology

Since 1989 a dedicated scheme for the monitoring and sampling of stranded cetaceans has been in operation in Scotland. In close collaboration with similar programmes running in England and Wales, the scheme has collated, analysed and reported data for all marine mammal strandings around the UK coast. This purpose of the scheme is to monitor the level of disease and contaminant burden in stranded marine animals, collect life history parameters and identify any substantial new threats to conservation status. The scheme relies on opportunistic sampling of stranded cetaceans. Species, morphometric data, size, location and body condition of the stranded animal is collected and, if feasible, the animal is collected for a post-mortem examination. Gross pathology, bacteriology, histopathology, and neurohistopathology are undertaken on most specimens collected for necropsy.

Harbour porpoise (*Phocoena phocoena*) account for the greatest number of strandings each year and comprise 52% of the total number of strandings. Between 1992 and 2008 the Scottish strandings scheme received reports of 1166 harbour porpoise. Of these, 632 animals were in suitable condition for necropsy. Excluding by-caught and live-stranded animals, the most common causes of mortality were starvation, infectious diseases (mainly pneumonias due to combinations of parasitic, bacterial and/or mycotic infections) and physical trauma. The objective of this work is to present these data and show the spatial and temporal variations in cause of death. Robust assessment of disease burden in this species potentially allows for indication of disease in sympatric cetacean populations, where data are more scarce and analysis complicated by factors such as reporting bias, over-dispersion or small sample size.



## ORAL PRESENTATION 19

### Disease risk analysis for conservation translocations: the reintroduction of the eurasian crane (*Grus grus*)

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**Key words:** Disease risk analysis, translocations

**Background:** In the past, methodology for qualitative disease risk analysis for translocations has focused on the importation of animals and animal products to protect domestic animal and human health (Murray et al. 2004). While we can extrapolate from these methods, for translocations undertaken for conservation purposes, additionally we need to consider i) parasite encounters throughout the translocation pathway, ii) changes in the pathogenicity of parasites carried by the translocated animals induced by stressors and iii) non-infectious hazards at the destination site. We outline our methods using the example of the reintroduction of the Eurasian crane from Germany to England.

**Methods:** We divided our risk assessment into four components as set out by Murray et al. (2004) and Leighton (2002) namely, (i) release assessment, (ii) exposure assessment, (iii) consequence assessment and (iv) risk estimation. We extrapolated from Murray's methods to consider, infectious transport and carrier hazards, host-immunodeficiency hazards and infectious and non-infectious destination hazards. We also investigated ways in which the parasites of Eurasian cranes can be conserved through modification of therapeutic regimes to ensure that native parasites are maintained in the reintroduced population. Risk management options for all identified hazards were recommended.

**Results:** Of the 23 hazards identified, one destination and host-immunodeficiency hazard (*Eimeria* sp) was classified as high risk. Five medium risk hazards were identified, and all other hazards were considered low or very low risk. We were unable to classify the risk of one carrier hazard, Inclusion Body Disease of Cranes Virus (IBDCV) owing to its unknown distribution in Eurasian cranes in Europe. We therefore advocated serological screening of the donor population, free-living cranes and captive Eurasian cranes in the UK.

**Conclusions:** The disease risks of translocations for conservation purposes can be effectively assessed using this feasible and practical method.

**References:**

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## ORAL PRESENTATION 20

### The introduced *Tamias sibiricus* increases the prevalence of Lyme borreliosis agent in native reservoir hosts

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**Key words:** reservoir host, introduced species, *Borrelia*

Recent studies have shown that the composition of host community can modify the dynamics of transmission of infectious diseases, but these studies have mainly been done in host communities settled in different habitats. How introduced vertebrates may act as a new host for native infectious agents has received little attention. Our objective was to analyze how the introduction of a potential reservoir species, the Siberian Chipmunk (*Tamias sibiricus*), modifies the prevalence of tick vector of a multi-host pathogen, the Lyme disease agent, in native reservoir hosts.

We compared the infection prevalence by *Borrelia burgdorferi* sl and tick infestation of native reservoir rodents (bank vole - *Myodes glareolus*, wood mouse - *Apodemus sylvaticus*) and ground insectivores birds in a suburban forest near Paris (Sénart, France) between 2 sites with and 2 sites without the introduced Siberian Chipmunk during the period of high tick density (May-June 2008).

The prevalence of infection was higher for native rodents on sites with (31%, n=45 for voles and 11%, n=37 for mice) than on sites without chipmunks (6%, n=35 for voles and 0%, n=37 for mice). There was no difference between the infestation rate by larval or nymphal ticks of voles and mice on sites with or without chipmunks. Moreover chipmunks were more infected (35%, n=20) and infested than native rodents. Too few engorged larvae were collected on birds to determine their infection prevalence. There was no difference of infestation rate by ticks between the two types of sites in birds.

In conclusion, rodents and birds hosted similar tick burden on sites with and without chipmunks, but rodents were more infected by *Borrelia* on sites with chipmunks. Since chipmunks are more infected than native reservoirs, and harbour more ticks, they seem to amplify the circulation of *Borrelia* between reservoir hosts. Adult ticks are the critical stage that determines tick abundance locally. Because chipmunks do not host adult ticks, the presence of chipmunks do not influence the burden of tick infesting native reservoirs. The effects of the introduction of chipmunks on the risk for Lyme disease in human are currently under investigation.

## ORAL PRESENTATION 21

### Can wildlife management be used to control ticks and tick-borne pathogens?

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**Key words:** ticks, control, deer, sheep, grouse

**Background:** Ticks are among the most important vectors of zoonotic pathogens in Europe. In the UK ticks and tick-borne diseases such as Lyme borreliosis and louping ill virus (part of the tick-borne encephalitis complex of viruses) are increasing. Here we aim to assess the potential for various different strategies to control ticks and tick-borne pathogens; specifically relating to *Ixodes ricinus* in Scotland. Current and potential methods to control ticks and tick-borne diseases in Scotland generally involve managing the key host species. For example: excluding deer using fencing, culling deer, culling mountain hares, and the use of acaricide-treated sheep as “tick mops”.

**Methods:** We tested the potential effectiveness of the above strategies by using a combination of replicated controlled field experiments, natural experiments, cross-sectional field sampling, and mathematical modelling.

**Results:** We found that while most of the tick and tick-borne pathogen control methods tested could be very effective under certain circumstances, deer abundance is often critical to the effectiveness of each method. For example, culling mountain hares could be an effective tool in controlling ticks and louping ill virus only in the absence of alternative hosts such as deer.

**Conclusions:** Controlling ticks and tick-borne pathogens can be very difficult due to the complexities of the multi-host system, and each method has associated ethical and practical issues. The presence or absence of the primary *I. ricinus* hosts (in Scotland these are deer) are critical to the effectiveness of other control methods.

## ORAL PRESENTATION 22

### Farm Yard and Rural Home Visitation by White-tailed Deer (*Odocoileus virginianus*): Implications for Mitigation of Disease Transmission

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**Key words:** bovine tb, contact, disease mitigation, farm visitation, white-tailed deer

**Background:** In 1994 and 2005 *Mycobacterium bovis* (bTB) was found to be endemic in free-ranging white-tailed deer (*Odocoileus virginianus*) populations in Michigan and Minnesota, respectively. Currently, the contact rate between cattle and deer, rates of farm visitation by deer, and co-use foraging resources by cattle and deer is not well understood. To evaluate the extent to which deer and livestock may come in contact and potentially share forage resources farm yard visitation by white-tailed deer was investigated. In addition, data were collected on farm yard and rural home locations, farming practices, pastures used by cattle, timing of use, timing of feeding and locations of stored cattle feed.

**Methods:** Female white-tailed deer (N=27) were fitted with global positioning system collars programmed to record geographic locations every two hours for one year. Deer were collared within or adjacent to the bTB infected zone in Michigan's Lower Peninsula.

**Results:** Deer visited 42.3+/-10.2% cattle yards and 34.5+/-8.4% rural homes within their annual home range. Yard visitation varied among deer with 15% of deer accounting for 76% of all yard visits. Multiple visits of yards in the same day were common. Most visits occurred at night (74.0+/-8.6%) with the majority of these (60.3+/-8.5%) occurring after midnight. Visitation of farm yards increased through spring and peaked during the fawning season.

**Conclusions:** These findings suggest that frequency and timing of deer visitation should be incorporated into mitigation and control efforts to guard against potential transmission of bTB between cattle and deer. Deer visitation of multiple farms may contribute to local area spread of bTB and other pathogens. Focusing mitigation efforts, lethal or non-lethal, on individual deer that are most likely to visit farms may reduce potential bTB transmission to cattle and between farms.

## ORAL PRESENTATION 23

### Developing practical guidance on the prevention and control of diseases of wetlands

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**Key words:** Wetland, disease, guidance, Ramsar

Healthy wetlands are vital for human, domestic animal and wildlife health. However, these varied habitats are at specific risk of emerging and re-emerging diseases due to: their association with high population densities of people, agriculture and industry; having been subject to substantial habitat modification, sites not being isolated (requiring a catchment approach); maintaining a high diversity of host taxa; and high proportions of invasive alien species and their associated parasites. Moreover, climate change is negatively affecting wetlands in a variety of ways.

The 10<sup>th</sup> Meeting of the Conference of the Parties (CoP) to the Convention on Wetlands (Ramsar) in 2008 focussed on 'Healthy Wetlands, Healthy People' and produced a significant body of work addressing human health issues in particular. A call from the CoP for better disease guidance resulted in Ramsar's Scientific and Technical Review Panel tasking the authors of this paper with producing practical guidance for wetland managers and decision makers on the prevention and control of animal disease in wetlands, especially those diseases having implications for human health.

A needs questionnaire for end-users was disseminated globally which identified a clear requirement for guidance and will help develop the outputs.

The aim of the guidance is to encourage well-informed decisions with regard to the prevention and control of animal diseases in wetlands so as to ensure 'wise use'.

The guidance hopes to achieve this by:

- explaining principles of disease prevention and control in wetlands;
- providing generic guidance on procedures and methodologies;
- providing generic information on a selection of priority diseases; and
- providing advice on incorporating disease prevention and control guidance into site management plans.

This guidance will hopefully be endorsed by the upcoming CoP in 2012. This is an opportunity to get key wildlife health messages to the global network of 159 Ramsar Contracting Parties and the authors would value input from the EWDA in development of this product.

## ORAL PRESENTATION 24

### Assessing CSF disease control measures using an individual-based model

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**Key words:** Classical Swine Fever, Hog Cholera, Disease control, Oral immunisation, Individual-based model

**Background:** Classical Swine Fever (CSF) is a viral disease in wild boars (*Sus scrofa*) and domestic pigs causing huge economic impact on individual farmers and national economies. The management of the disease became even more complicated in the last decades due to endemicity in wild boar populations in several European countries. Huge effort is paid on CSF control in wild boar populations by oral mass vaccination, but few is known about the efficacy of the applied measures to control or eradicate the disease. Furthermore, virulence as a crucial parameter for disease dynamics varies widely between CSF virus strains and is highly uncertain.

**Methods:** We implemented a spatially-explicit, individual-based wild boar population model, coupled with a CSF virus model on the level of individual traits. The model accounts for social behaviour of boar groups as well as individual variations in disease outcomes. Over a range of case mortality and duration of the infectious period (the virulence), we tested alternative spatial baiting strategies. We compared these scenarios regarding the performance of the management measured by final size of the infected area and long-term persistence.

**Results:** Our analysis showed that artificial immunisation can facilitate disease persistence under certain conditions. High success in virus eradication as well as prevention of disease spread was only possible with preventive vaccination in terms of baiting in front of the epidemic wave. Buffered vaccination effort was completely sufficient to exploit the effect of vaccination of the entire area which translates strategic needs into a practical management plan. A buffer radius corresponding disease spread distance of one year revealed suitable to fully exploit the potential of oral mass vaccination. **Conclusions:** Although preventive baiting strategies are not yet implemented in the field due to EU legislation but with marker vaccines in sight, we recommend buffered baiting of the infected area.

## KEYNOTE

### Public health aspects of *Echinococcus multilocularis*: wildlife crossing domestic life in Europe

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Alveolar echinococcosis (AE) is a severe helminth disease affecting humans as a result of infection with the larval stage of the fox tapeworm *Echinococcus multilocularis*. In Europe, a significant increase of prevalence since the 1990's is not only affecting the historically documented endemic area North of the Alps, but more recently also neighboring regions previously not known to be endemic. Several reasons account for this phenomenon, they include a general high increase of the fox population density in some European countries, the urbanisation of fox populations in some areas of Europe, and extension of the parasite life cycle to other definitive hosts such as racoon dogs (*Nyctereutes procyonoides*), but especially also domestic dogs that live in close physical contact with humans. Naturally infected foxes display highly variable and over-dispersed worm burdens, and worm burdens appear significantly higher in juvenile as compared to adult foxes. But even in a fox population with a high prevalence, only a relative low number of highly infected individuals are responsible for most of the egg contamination of the environment.

In the definitive host, egg production starts as early as 28 days after infection. After accidental egg ingestion by an intermediate host, larval maturation will occur practically exclusively within the liver tissue; subsequent metastases may affect adjacent or distant organs, such as lungs or brain. Proliferation occurs by exogenous budding of metacystode tissue with a progressive tumor-like growth, central necrotic cavities may develop and cause differential diagnostic problems. Many rodent species are known to be suitable intermediate hosts for *E. multilocularis* with varying significance in the maintenance of the life cycle respective to different regions. Furthermore, many non-rodent mammalian species (beside humans) have been described as accidental hosts of *E. multilocularis*. These include dogs, monkeys, pigs and rarely some other mammalian species. In all of these animals, the liver is affected primarily as well. In humans, untreated AE presents a high mortality (>90%) due to a severe hepatic destruction as a result of parasitic metacystode proliferation which behaves like a malignant tumor. Treatment is mainly based on surgical resection of parasitic masses and on longterm medication with albendazole, both combined provide a good prognosis to affected AE patients, especially at early stage of infection. Prevention of AE focuses primarily on veterinary interventions to control the extent and intensity of infection in definitive host populations, which may indirectly

be approached by controlling the prevalence in animal intermediate hosts also. The first includes regular medication with e.g. praziquantel and taking sanitary precautions for handling domestic dogs and to prevent infection and egg excretion, respectively. Regular praziquantel treatment of wild-life definitive host (e.g. foxes and racoon dogs) may contribute to lower the prevalence in affected areas. The societal and economic benefits of control of *E. multilocularis* would be a reduction in the incidence of AE in humans. Treatment of AE cases is expensive, for example in Switzerland it costs on average in excess of €100,000 to treat a single case of AE, as determined by Torgerson et al. (2008).

## ORAL PRESENTATION 25

### **Echinococcus multilocularis and habitat use of red foxes (*Vulpes vulpes*) in villages and small towns**

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**Key words:** zoonosis, red fox, fox tapeworm, habitat use

In recent years red foxes (*Vulpes vulpes*) have been recorded in villages and small towns more frequently. Detailed information about territorial use by foxes and the prevalence of foxes infected with the small fox tapeworm (*Echinococcus multilocularis*) is lacking for this habitat. Rising *E.*

*multilocularis* infection rates in foxes and the close proximity of the foxes to people have resulted in an increased risk of human infection, especially in urban areas.

Radio-tracking of 17 foxes showed that the animals spend 38% of their time within the settlement and 62% in areas outside the town (within a range of 500m). Foxes focused on the border between urban and rural areas and preferred the settlements and grasslands outside the villages. Within the settlements, the average distance of the located foxes had been 66.1m (max. 728.2m) to the village border.

About 43.1% of the foxes using villages and small towns had been infected with *E. multilocularis*. The infection rate is not significantly different from the 39.4% infection rate in foxes in open countryside, determined by the intestinal scraping technique ( $\chi^2=0.12$ ,  $df=1$ ,  $p=0.727$ ,  $n=98$ ). PCR analyses of faeces could also not proof significant differences between these two habitats ( $\chi^2=0.68$ ,  $df=1$ ,  $p=0.411$ ,  $n=95$ ).

We assume these similar infection rates are because of foxes get infected outside of the villages, where sufficient intermediate-host species are abundant, especially in the preferred grassland areas. As a result of the high *E. multilocularis* infection rates, foxes carry the parasite into villages and small towns. Comparing the number of people living here and considering the high fox abundances as well the potential risk of an infection in villages and small towns is higher than in rural areas. Furthermore, the frequent contacts between foxes and people are enforcing the infection risk as well.

## ORAL PRESENTATION 26

### **Prevalence Estimation of *Echinococcus multilocularis* among raccoon dogs (*Nyctereutes procyonoides*) in northern Brandenburg, Germany**

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**Key words:** *echinococcus multilocularis*, raccoon dog, germany

The human alveolar echinococcosis, caused by the larval stage of the small fox tapeworm *Echinococcus multilocularis*, is a lethal zoonotic infection with an incubation period in mean of 10 years.

This 2-4 mm sized parasite, living in the small intestines of carnivores, is widely distributed in the Northern Hemisphere. Its main definitive host in Germany and other European countries is the red fox *Vulpes vulpes*, a most widespread and abundant canid species. In the federal states of Germany, studies on the occurrence of *Echinococcus multilocularis* differ according to sample sizes, tested animal species and geographic distribution of the samples. In Brandenburg a yearly statewide monitoring of red foxes has taken place since 1991 and starting in 2000 the raccoon dog *Nyctereutes procyonoides* has been included in this investigation. The first evidence of *Echinococcus multilocularis* in two male raccoon dogs (*Nyctereutes procyonoides*) in northern Brandenburg, was provided by Thiess et al. in the year 2001. The raccoon dog is a medium-sized canid, originally distributed in East-Asia and its presence in Germany is proofed since the early 1960-ies.

Data on echinococcosis consist of the number of diagnosed positive and negative results directly linked in a Geographic Information System (GIS) to the corresponding communities. Due to the lack of samples and that positive cases did not appear in southern counties in raccoon dogs, evaluation was based on the summarized data of the years 2000 to 2008 only from five northern counties of Brandenburg. For estimation of prevalences we applied the Beta-binomial- model.

## ORAL PRESENTATION 27

### Increasing risk of human alveolar echinococcosis in the Netherlands and possible control options

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**Key words:** Echinococcus multilocularis, fox, surveillance, control, Mathematical model

**Background:** Alveolar echinococcosis (AE) is one of the most pathogenic parasitic zoonoses in central Europe. Humans are infected when accidentally ingesting the parasite eggs that are shed into the environment by infected foxes. The parasite was first detected in the Netherlands in 1996 and subsequently spreaded in the local population of foxes. Using the spreading of the parasite as a predictor, we assess the risk of human alveolar echinococcosis in the new endemic regions, and evaluate parasite controls.

**Methods:** Red foxes were collected from two provinces of Limburg and Groningen and analyzed by mucosal scrapings. Spatial-temporal dynamics of the parasite infection was modelled by a diffusion equation with a local exponential growth of the parasite population. The basic reproduction number (R<sub>0</sub>) for the parasite was derived and estimated from the worm burdens of the foxes collected in NL. The speed at which contour line of a constant mean parasite burden is advancing was estimated. The human risk in Limburg was simulated by a Monte Carlo approach based partly on the historical records of AE epidemiology in Switzerland. Effect of reducing the parasite lifespan by the application of anthelmintic treatment on foxes was evaluated using a mathematical model of the parasite transmission.

**Results:** Estimated reproduction numbers of the parasite were 1.6 in Limburg and 2.0 in Groningen. The infection front is advancing into the Netherlands at the speed of 2.7 km per year from the Belgium border and at the speed of 3.4 km from the German border. In Limburg, up to 30 human cases are predicted by 2030. The duration of the control is a critical factor for a successful parasite control.

**Conclusions:** The epidemiology of AE in the Netherlands might have changed from the period of zero risk in the past to the period of increasing risk in the coming years.

## ORAL PRESENTATION 28

### Fox tapeworm: An underestimated threat and a strategy to solve the problem

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**Key words:** Human wildlife conflict, urban wildlife, Vulpes vulpes, wildlife diseases, zoonosis.

In recent years, red fox (*Vulpes vulpes*) populations increased all over Central Europe. An increase in prevalence of the fox tapeworm (*Echinococcus multilocularis*) is specifically detectable in parts of southern Germany. As a result, the risk for humans getting infected with this parasite has also risen. Incurable and fatal if not treated. To ensure efficient use of resources it is crucial to know where counter-measures are most beneficial. To assist prevention efforts, a model was developed based on prevalence rates in foxes (*Vulpes vulpes*), fox population densities, fox defecation rates and human population densities. For example the model shows, that in 2005 the likelihood of contact was 45 times higher in the city of Munich than the Bavarian average.

That's why, anthelmintic treatments against *E. multilocularis* have been tested successfully as part of a public health program and led to a remarkable reduction in infection rates. Against this backdrop, three baiting programs were started in southern Bavaria. Prevalence rates of 29%, 40% and 54% were determined within the study sites, with regional maximum levels up to 80%. Within the first year, praziquantel containing baits were brought out every month (50 baits / km<sup>2</sup>) and within the second year in a six-week interval (50 baits / km<sup>2</sup>). Prevalence rates declined to 0% resp. 1% after first year and could be manifested on a low level in second year (0% resp. 2%). An applied baiting strategy, incorporating baiting by aeroplane in open landscapes and baiting by hand in settled areas are responsible for low prevalence levels. The key for success lies in a closed baiting network across the whole study area.

## ORAL PRESENTATION 29

### Preventing human trichinellosis acquired from walrus meat in the Canadian North: the successful example of the Nunavik Trichinellosis Prevention Program

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**Key words:** *Trichinella nativa*, Walrus, Inuit, Zoonosis, Prevention

**Background:** During the 1980's, several trichinellosis outbreaks caused by *Trichinella nativa* were documented in Inuit communities from Nunavik (Northern Quebec, Canada). Epidemiological investigations revealed that walrus meat, eaten either raw or fermented, was the source of these outbreaks. Since the walrus hunt is nutritionally and culturally important for Nunavik inhabitants, stakeholders decided to develop and implement a prevention program. The objective of the present communication is to review the Nunavik Trichinellosis Prevention Program (NTPP) from 1992 to 2009, and to discuss its impact on the occurrence of trichinellosis outbreaks in Nunavik.

**Methods:** As part of the NTPP, tongues from harvested walrus were submitted for *Trichinella* spp. detection using an enzymatic digestion assay. The results of these analyses and the appropriate food-management recommendations were communicated to local authorities. Quarantine status was lifted from meat originating from uninfected animals, which received approval to be eaten raw or undercooked; whereas it was recommended to destroy infected meat. The occurrence of human cases of trichinellosis in the communities was monitored by local health services with the assistance of the Nunavik Public Health Department during the entire study period.

**Results:** All walrus hunting communities participated in the NTPP. From 1992 to 2009, 20 positive animals were identified out of the 694 walrus tested (apparent prevalence: 2.9%). With the exception of one outbreak that occurred in 1997 following the consumption of infected meat during its quarantine period, none of the tested walrus have been linked to any human cases.

**Conclusion:** The absence of recent outbreaks of trichinellosis in Nunavik can be reasonably attributed to the NTPP. The success of the program depends on many facilitating factors such as the nature of the disease and its source, the existence of an efficient analytical method and the strong involvement of the different partners, including the direct resource users.

## ORAL PRESENTATION 30

### Seroprevalence of *Toxoplasma gondii* in wild boar in the Netherlands

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**Key words:** *Toxoplasma gondii*, wild boar, seroprevalence, the Netherlands

**Background:** *Toxoplasma gondii* is an important zoonotic pathogen that is best known as a cause of abortion or abnormalities in the newborn after primary infection during pregnancy. In addition, it can cause severe disease in the immunocompromised, and is an important cause of chorioretinitis in the immunocompetent. It was our aim to determine the prevalence of *T. gondii* in wild boar to investigate the possible role of their meat in human infections and to get an indication of the environmental contamination with *T. gondii*.

**Methods:** The presence of anti-*T.gondii* antibodies was determined by in-house ELISA in 509 wild boar shot in 2002/2003 and 464 wild boar shot in 2007. Most of the boar originated from the southern part of Limburg (n = 673) and "de Veluwe" (n = 241). A binormal mixture model was fitted to the log-transformed optical density values for boar less than 20 months old to estimate the optimal cut-off value (-0.67) and accompanying sensitivity (91.6%) and specificity (93.7%).

**Results:** The overall prevalence was estimated at 23.1% (95% CI: 19.9-26.3%). Logistic regression analysis with estimated age, region and year showed a significant increase in prevalence with age in years (OR 1.24, 95% CI: 1.11-1.38), but no significant differences between regions or sampling years.

**Conclusions:** The lack of variation between years and regions indicates a stable and equal infection pressure from the environment. The observed prevalence is much higher than the prevalence in pigs (0-5.6%) (Kijlstra et al., 2004; van der Giessen et al., 2007), which is likely due to more intensive contact with the environment compared to pigs. The high prevalence suggests that eating undercooked wild boar meat poses a risk of infection with *T. gondii*.

## ORAL PRESENTATION 31

### **Toxoplasma gondii killing European brown hares and mountain hares in Finland: Proportional mortality rate, seroprevalence, and genetic characterization**

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**Key words:** *Toxoplasma gondii*, toxoplasmosis, *Lepus europaeus*, *Lepus timidus*

*Toxoplasma gondii* is a protozoan parasite capable of causing disease in a wide variety of host species, including humans. Fatal toxoplasmosis is among the common infectious causes of death in hares (*Leporidae*, genus *Lepus*) in Finland, but recent detailed investigations of natural *T. gondii* infections in these animals have been lacking.

In the material examined post-mortem at Evira from May 2006 to April 2009, acute generalized toxoplasmosis was the immunohistochemically confirmed cause of death in 14 (8.1%) of 173 European brown hares (*Lepus europaeus*) and 4 (2.7%) of 148 mountain hares (*Lepus timidus*). The proportional mortality rates differed significantly between the two host species ( $P < 0.05$ ).

Sera from 116 of the brown hares and 99 of the mountain hares were screened with a commercial direct agglutination test for *T. gondii*-specific IgG antibodies. All the sera from cases of fatal toxoplasmosis had antibodies. In contrast, none (0%) of 107 European brown hares but 4 (4.2%) of 96 mountain hares that had died of other causes were defined as seropositive; the seroprevalences among non-cases differed significantly between the two host species ( $P < 0.05$ ). Our results thus support the hypothesis that brown hares are naturally susceptible to primary *T. gondii* infection: all the brown hares that had mounted an antibody response against the parasite had died from the infection.

Direct genetic characterization of the causative agent was performed on DNA extracted from formalin-fixed, paraffin-embedded tissue of the hares with fatal toxoplasmosis. Based on the results with six microsatellite markers, all the cases were caused by *T. gondii* genotype II; the size of the PCR product at the seventh marker varied. Genotype II is endemic in Europe and these results affirm its presence in Finnish wildlife: natural infections of *T. gondii* parasites belonging to this widespread genotype killed these 18 hares.

## ORAL PRESENTATION 32

### **The National Research Platform for Zoonoses – a novel approach to intensify zoonotic disease research in Germany**

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**Key words:** zoonoses, research, network, Germany

Zoonotic infectious disease emergence and spread do not respect geographical boundaries. Due to the rapid world population growth, the increasing mobility, the intensive animal husbandry and the climate change, the study of zoonoses becomes ever more important. In addition, zoonoses are caused by all types of agents: bacteria, viruses, parasites, fungi, and unconventional agents. They affect humans as well as domestic and free-ranging animals. Effective zoonotic disease research therefore requires an integrated approach that involves natural scientists of all fields to ensure a broad exchange of knowledge and experiences.

In this context the German National Research Platform for Zoonoses ([www.zoonosen.net](http://www.zoonosen.net)) is funded by the Federal Ministry of Education and Research since 2009. It represents a science oriented umbrella organisation for all scientists and research networks working on zoonotic infectious diseases in Germany. The objectives of the Platform are to build up a central information and service network, to foster the exchange between scientists all over Germany and to develop sustainable solutions to strengthen research, prevention, and therapy of zoonotic infectious diseases.

The activities designed to meet these objectives include the conception and organisation of workshops and symposia as well as the registration, documentation and standardisation of resources including the setup of databases containing an overview on zoonoses-related experts, research institutions, projects, funding programmes, existing samples, and cell lines. In addition, the Platform initiates innovative pilot-projects and interdisciplinary cross-sectional projects and establishes a central contact for scientists, politicians, and the general public providing independent information about zoonotic infectious diseases.

Meanwhile over 200 scientists of different research institutions are members of the National Research Platform for Zoonoses, working together to investigate and to combat zoonoses in humans, livestock, and wildlife.



### ORAL PRESENTATION 33

#### Longitudinal study reveals stable occurrence of hantaviruses in free ranging rodents in South-eastern Germany

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**Key words:** Hantavirus, rodents

**Background:** So far since 2001, many clinically apparent hantavirus infections in Germany are reported from Lower Bavaria, part of the federal state of Bavaria in South-Eastern Germany (n=123/3910, May 27th, 2010). We therefore initiated a longterm monitoring of hantaviruses in wild rodents in Lower Bavaria.

**Methods:** In 2004 and 2005 we started with screening the micromammalia population at several sites of this region where human hantavirus infections occurred. Since 2008 we investigate rodents from the National Park Bavarian Forest from where extensive climate, microclimate and vegetation data exist within the BIOKLIM project. Trapping of free-ranging small mammals was performed on an elevation gradient with the aim to correlate our findings with the data mentioned above. RT-PCR targeting hantaviral partial S-segment were used for molecular screening of RNA extracted from lung tissues and positive samples were sequenced. Serology was performed on serum and transudates using a commercial Puumala virus immunofluorescence test.

**Results:** Over a period of 5 years micromammalia were trapped in the area. Amongst them there were bank voles (*Myodes glareolus*), field voles (*M. agrestis*), yellow-necked (*Apodemus flavicollis*) and wood mice (*Apodemus sylvaticus*), and several insectivores like *Sorex* spp. Trapping indices were high in the years 2004/05 and 2010, whereas low in the years 2008/09. Hantavirus prevalences were higher in the peak years of human infection 2004, 2007 and are probably rising again this year 2010.

**Conclusions:** Longitudinal monitoring of small mammals is important because fluctuations of reservoir population (depending on various climate- and food source-related factors) and human infections are correlated and are the base for assessment of infection risk for the human population. This project is part of the VICCI network and funded by the Bavarian Ministry of Health.

### ORAL PRESENTATION 34

#### Field epidemiology of wild boar-livestock interactions in South Central Spain

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<sup>4</sup>VISAVET-Universidad Complutense de Madrid

**Key words:** wild boar, domestic livestock/wildlife interface, spatial epidemiology, tuberculosis

**Background:** The pattern of persistence and spread in contiguous populations of wild boar under different management and epidemiological risks may well determine the degree of interactions between individuals at the population and inter-population level, and between wild boar and domestic livestock. This is essential to predict the consequences of wildlife - livestock interactions and to implement disease control actions.

**Methods:** During 2009 and 2010 we captured and tagged 15 wild boar in the Montes de Toledo range, South-central Spain. Adult and juvenile wild boar were provided with a GPS-GSM collar and piglets were marked with transponders. We also sampled over 300 wild ungulates (mainly wild boar and red deer) during the 2009/10 hunting season for blood and tissues in order to establish the prevalence of selected diseases (tuberculosis and viral diseases). Data on livestock health were obtained from the official veterinary services. These data were integrated and analyzed regarding the local patterns of disease persistence and transmission in wild ungulates.

**Results:** A preliminary analysis on habitat use by wild boar shows a wide range in home range area, which varies with management (i. e. food provision) and the presence of barriers such as big game fencing. Nonetheless, our results evidenced the capacity of wild boar to undercross fences. Maximum distances travelled in a day were up to 14 km. Ranging by wild boar coming from hunting areas in cattle grazing areas was detected.

**Conclusions:** These aspects are crucial for disease control in wildlife and useful to design epidemiological management units depending on the pathogen, hosts, environment conditions and anthropogenic factors. The sustainable management of big game wild boar as potential disease reservoirs for diseases can seriously compromise livestock health, and is revealed as key a sustainable use of wild ungulates by hunting, and regarding public health.

## ORAL PRESENTATION 35

### Risk assessment for pathogen transmission from wild boar to outdoor pigs in Switzerland

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**Key words:** *Brucella suis*, domestic pig, risk assessment, Switzerland, wild boar

The risk of transmission of pathogens from free-ranging wild boar to domestic pigs kept outdoor is an increasing concern in Switzerland. Domestic pigs are officially free of major reportable diseases such as brucellosis, however, *Brucella suis* has been reported in wild boars. The aims of this study were to assess the risk for pathogen transmission from wild boar to outdoor pigs, and to identify risk factors to propose adapted protection measures for outdoor farms.

Expansion potential of the wild boar range towards areas with numerous outdoor piggeries was evaluated using data from camera surveillance of wildlife overpasses, road kills and hunting bags. Prevalence of *B. suis* infection in wild boar was estimated by culture, serology and PCR. Contacts between wild boar and pigs and characteristics of farms at risk were recorded by questionnaire surveys, visits and camera surveillance. Of these piggeries, 11 were tested for *B. suis* infection.

We found no indication of an expansion of the wild boar range within the past 10 years, but wild boar have recently been recorded on 11/19 wildlife overpasses. Of 252 tested wild boar, 99 (39.3%) had been exposed to *B. suis* infection (28.0% positive by PCR/culture). Contacts between wild boar and pigs were reported in 39/84 farms (46.4%). Hybrids were identified in 15 farms (17.9%). An infection with *B. suis* was detected in one piggery. Preliminary results of the risk factor analysis suggest a higher risk for sexual contacts with wild boar for curly-hair hogs than other breeds.

Our data indicate a non-negligible risk for contacts between wild boar and pigs, in particular curly-hair hogs, and thus for a spillover of *B. suis* and likely other pathogens on domestic pigs. This risk could increase in the next future as wild boar cross anthropological barriers and their population might expand its range.

## ORAL PRESENTATION 36

### Surveillance of Pestivirus infection in wild ungulates from Andorra

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**Key words:** pestivirus, Andorra, wild ungulates

Since 2001, mortality associated with a Border Disease Virus (Genus Pestivirus, Family Flaviviridae) in Pyrenean chamois (*Rupicapra pyrenaica*) has been reported in the Spanish and French Pyrenees. The aim of the present study was to investigate pestivirus infection in wild ungulates from Andorra.

Sera from 284 mouflons (*Ovis aemon*), 30 Pyrenean chamois, 13 roe deer (*Capreolus capreolus*) and 5 wild boars (*Sus scrofa*) were investigated. Serological analyses to detect pestivirus-specific antibodies were performed using an ELISA antibody test. Positive sera were subsequently tested with a comparative virus neutralization test (VNT) for neutralizing antibodies against BVDV-1 strain NADL, BDV strain 137/4 and a BDV strain chamois. Virological analyses were performed with an ELISA antigen test. In order to confirm the positive or inconclusive results RT-PCR was performed. Two of 30 (6.6 %) chamois were positive with the ELISA antibody test. No antibodies were found in the other species. VNT confirmed the ELISA results of the two animals. The antibody titres were significantly higher against the BDV strains when compared with the BVDV strain, but no significant difference between the titres against the two BDV strains was confirmed. Inconclusive results were obtained in 29 sera (21 mouflons, 4 chamois, 3 roe deers and 1 wild boar) with the ag-ELISA test and they were all negative to RT-PCR. The overall seroprevalence found in Pyrenean chamois in Andorra was low when compared with other areas of the Catalan and French Pyrenees. These results indicate that pestiviruses were not circulating in the Andorran ungulates, although they were surrounded by severely affected chamois populations. This low seroprevalence may predispose this population to a potential epizootic in case the BDV get contact with this population. Monitoring pestivirus infection is therefore of importance for the conservation and management of Pyrenean chamois in Andorra.

## ORAL PRESENTATION 37

### Linking surveillance of avian influenza viruses in wild birds with outbreaks in poultry

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**Key words:** avian influenza virus, wild bird, poultry, risk factors, surveillance

**Background:** When an outbreak of avian influenza in poultry occurs, the exact source and route of entry of the virus is often unknown. Wild birds are the reservoir of avian influenza viruses and therefore suspected to be the source of avian influenza virus outbreaks in poultry. In this study we test the hypothesis that wild birds introduce avian influenza viruses in poultry, and that mainly poultry kept outdoors and close to high wild bird densities will be affected. The increased awareness of wild birds as potential spreaders of highly pathogenic avian influenza (HPAI) H5N1 has resulted in intensive sampling of wild birds. The intensive sampling of both wild birds and commercial poultry in a poultry dense and relatively small area rich in wild birds like the Netherlands offers a unique opportunity to investigate the role of wild birds in the introduction of avian influenza viruses in poultry.

**Methods:** We performed a temporal, spatial and genetic analysis on avian influenza viruses isolated from poultry and wild birds to clarify the degree of intra- and inter avian species relatedness. As part of the spatial analysis we compare poultry farms tested (sero)positive for avian influenza virus and negative farms with regards to farm characteristics and distance to high wild bird densities.

**Results:** Preliminary evidence suggests that the detection of an increased prevalence of avian influenza viruses in wild birds does not correlate with outbreaks in poultry, and that avian influenza virus subtypes detected in poultry have not been detected more frequently in wild birds in the year prior to the outbreak in poultry.

**Conclusions:** The results of this study will contribute to our knowledge on the relevance of avian influenza monitoring in wild birds, and to improve the current avian influenza virus wild bird surveillance schemes and control measures of possible future outbreaks.

## ORAL PRESENTATION 38

### Estimating contact rate-density relationships for badgers (*Meles meles*)

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**Key words:** contact rates, *Meles meles*, *Mycobacterium bovis*, population density, disease

**Background:** Understanding the contact rate—density relationship is fundamental for understanding disease transmission in wildlife populations and for determining the likely effectiveness of any culling-based disease control strategies. Badgers act as a host species for bovine tuberculosis (bTB) in the UK, and much control has been based on the assumption that reducing numbers of badgers will reduce contact rates and hence reduce disease transmission. However, field data suggest there is no simple relationship between bTB prevalence and badger density. Here we combine data from two field sites with contrasting population densities to determine the nature of the contact rate—density relationship for badgers.

**Methods:** We fitted proximity data loggers to 26 badgers at a high-density site (Woodchester Park, Gloucestershire, UK) and 11 badgers at a medium-density site (Dalby Forest, North Yorkshire, UK). We quantified the contact patterns in terms of contact rate and contact duration, and used social network analysis to investigate network connectivity and the constancy of associations over time.

**Results:** Daily contact duration and frequency, adjusted for the number of badgers wearing collars, were highly correlated with one another. There were no significant differences in the levels and patterns of contact, or in contact networks, between the two study sites.

**Conclusions:** Contrary to previous assumptions, the results are consistent with there being no positive relationship between contact rates and population density. This could have important implications for managing bTB in badgers, since alterations to population density, as caused (e.g. by culling or fertility control) may therefore be unlikely to reduce disease spread.

## ORAL PRESENTATION 39

### First evidence of *Cytauxzoon* sp. infection in Eurasian lynx

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**Key words:** *Cytauxzoon*, Eurasian lynx, *Lynx lynx*, prevalence, Switzerland

*Cytauxzoon felis* usually causes fatal cytauxzoonosis in domestic cats in the USA, while persistent subclinical infections are common in bobcats (*Lynx rufus*). In the past decade, *Cytauxzoon* spp. have been increasingly detected in other felid species. In Europe, single infected cats were reported from Spain and France, and the parasite was shown to be widespread in asymptomatic Iberian lynx (*L. pardinus*) in Spain. The aim of this study was to investigate free-ranging Eurasian lynx (*L. lynx*) for infections with *Cytauxzoon* sp. Samples were collected from 40 free-ranging lynx (19 trapped alive, 21 found dead) from 1998-2007 in Switzerland, and tested by PCR analysis.

Overall prevalence was 30% (95% CI: 16.6-46.5). Lynx from the Jura population were significantly more often infected (57.1%, CI 28.9-82.3) than lynx from the Alpine population (16.7%, CI 4.7-31.6). There were no significant differences in prevalence between sexes, age classes, alive versus dead lynx, and healthy versus diseased lynx. Differences between seasons were believed to be due to bias.

To our knowledge, this is the first report of *Cytauxzoon* sp. infection in Eurasian lynx. Infection appears to be common in Swiss lynx, and no associated disease condition has been recorded in this species so far. This suggests that the Eurasian lynx is another natural host of *Cytauxzoon* sp., and that this hemoparasite might be much more widespread than originally assumed. However, as no fatal case has been reported from domestic cats in Europe, the existence of low pathogenic strains has to be considered. Difference in prevalence between the Jura Mountains and the Alps could be due to known differences in climate and other environmental variables influencing vector occurrence and lynx exposure. Nevertheless, the existence of a tick vector in Europe remains to be investigated. Vector competence is a known key factor for transmission of pathogenic parasitic forms.

## ORAL PRESENTATION 40

### New techniques for an old disease: sarcoptic mange in the Iberian wolf

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**Key words:** mange, wolf, diagnosis, camera traps

1. Confirmation of sarcoptic mange in 6 wolves (*Canis lupus*) from Asturias (Northern Spain) during 2008 suggested the need for monitoring and research to characterize this parasitic disease and evaluate its implications and possible risks for wolf populations.
2. Between January 2008 and May 2010, 43 wolves (hunted in population control programs or killed by road traffic) were submitted for necropsy as part of a Wildlife Disease Surveillance Program in Asturias. Different study methods were applied in order to improve knowledge on the epidemiology and temporal/spatial distribution of the disease:
  - a. Evaluation and comparison of different diagnostic techniques (serology, mite isolation, histopathology, immunohistochemistry and skin digestion) using samples from animals submitted for necropsy.
  - b. Serological study of the presence of antibodies against *Sarcoptes scabiei* in sera collected from wolves since 2004 (n=88).
  - c. Assessment of pictures (provided by researchers and Rangers) of wolves hunted or found dead since 1993, in order to detect previously unnoticed mange compatible lesions.
  - d. Use and evaluation of camera-trapping as a valuable tool for wolf sarcoptic mange epidemiological research.
3. Both photographic and serological data confirmed the presence of sarcoptic mange in Asturian wolves since at least the beginning of the study (2004). Serology and necropsy data suggested an increase in sarcoptic mange prevalence (with seroprevalence peaks of 21.01% in 2008 and 37.50% in 2010) and incidence (6 and 3 wolves with sarcoptic mange confirmed on necropsy in 2008 and 2010 respectively, whereas no wolf showed mange compatible lesions on necropsy from 2004 to 2007, n=47).
4. The apparent increase in prevalence and incidence observed during recent years suggests the need for further studies on this wolf parasitosis. Such studies should include the epidemiological relationship with sarcoptic mange outbreaks in sympatric red foxes, and the possible effects on wolves' reproductive rate, pup survival and populations.

## ORAL PRESENTATION 41

### Scabies in Bavarian chamois populations and management recommendations

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**Key words:** *Rupicapra rupicapra*; *Sarcoptes scabiei* var. *rupicaprae*; population density; hunting bag; game management.

In Bavaria (Germany), scabies was registered for the first time in the year 1824 among the chamois population of the Berchtesgaden region, and disappeared 6 years later. For the next 119 years, the chamois populations in the Bavarian Alps seemed not to be infected by scabies. But in 1949 scabies occurred again in the Bavarian chamois populations east of the River Inn, with origin in the Berchtesgaden region. Scabies has been recorded repeatedly since then. Whereas between 1949 and the early 1990s scabies had a great impact on the development of the chamois populations in the Bavarian Alps east of the river Inn, the disease has had little significance since 1995. As it is assumed that there is a link between high population density and the occurrence of scabies among chamois, hunting bags (as indicators of population density) were compared with the recorded scabies cases (shot or deceased game) among chamois populations in the Bavarian state forests east of the river Inn. Data collected from the Berchtesgaden Forest Office were further analysed according to age and sex parameters. There is a highly significant, inverse, non-parametric correlation between recorded incidences of scabies and hunting bags in the whole study area and in the area of Berchtesgaden Forest Office. The results of the analysis show that bucks were slightly more likely to be registered with scabies than does ( $p=0.018$ ). Young animals (yearlings and juveniles) played only a secondary role in terms of scabies prevalence. Both the historical background of scabies in the Alps and the present study support the above-mentioned hypothesis that the incidence of scabies in chamois is related to high chamois population densities. The spread of scabies can therefore be slowed down or prevented by increasing game bags over a large area.

## ORAL PRESENTATION 42

### Set-up of vector-borne disease prevalence studies in roe deer

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**Key words:** Roe deer, prevalence, Epizootic Hemorrhagic Disease Virus, Bluetongue virus, Game management unit

The population of roe deer (*Capreolus capreolus*) in the Netherlands increased from +3,000 in 1930 to >60,000 today. The roe deer are scattered all over the country. The population is managed by 308 game management units (WBEs) collectively covering the Netherlands. WBEs obtain hunting permits through 14 fauna management units (FBEs). In 2009, a pilot study started to improve the representativeness of roe deer in disease prevalence studies. WBEs and FBEs were asked for their participation in this study on Bluetongue virus (BTV) and Epizootic hemorrhagic disease virus (EHDV).

Five-hundred sampling packages were assigned to WBEs, using a random sampling system based on the number of deer counted per WBE. Volunteers took samples from roe deer shot in the *Culicoides* activity-free period between late 2009 and early 2010. Samples were submitted with information on date, location, sex, age class, health, and type of sample. Blood samples were tested by PCR for EHDV, and ELISA for antibodies (Abs) against BTV.

The return rate was 73% (366 out of 500). Most of the samples were taken from the thoracic cavity, and were hemolytic. Samples were from does and calves, since bucks were not shot in this period. All 366 samples were tested negative for both EHDV and BTV.

Though the representativeness of roe deer in the collected samples could be improved, this pilot study has shown an acceptable participation of WBEs and FBEs in roe deer disease prevalence studies. No evidence was found for on-going EHDV infection in roe deer, or seroprevalence for BTV in these mainly solitary living deer species. The implications for the role of roe deer in the epidemiology of vector-borne diseases, like the 'ruminant' orbiviruses transmitted by species of *Culicoides*, will be discussed.

## ORAL PRESENTATION 43

### Proximate and Ultimate effects of blood parasites on the California Western Scrub Jay (*Aphelocoma californica*)

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**Key words:** Blood-parasites, population-impacts, ENSO, mark-recapture, Corvid

We found that the impact of blood parasites had differing short-term versus long-term consequences to the Western Scrub Jay in Central California USA. The Western Scrub-Jay is a medium-sized Corvid, approximately 27–31 cm (11.5 in) in length (including its tail), with a 39 cm (15 in) wingspan, and about 80g in weight. This species has a blue head, wings, and tail, a gray-brown back, whitish streaked throat, and grayish under-parts. A 10-year study was conducted on prevalence and incidence of blood parasites in a California colour-banded population. Young survived laboratory hematozoan challenge infections at 1-month and 1-year of age. Wild adults were found with 61% prevalence in the population, with *Plasmodium* having the highest prevalence (57 of 65 infected birds), *Leucocytozoon* a moderate prevalence (21 of 65 infections), and *Haemoproteus* the lowest level (3 of 65). Multiple infections were found in 15 birds. The ENSO weather pattern had a significant effect of prevalence over the decade of this study. A mark recapture analysis demonstrated that the level of infection and number of blood parasites significantly negatively influenced survival of adult scrub jays. Although proximately able to survive hematozoan infection, ultimately these blood parasites were the most important factor determining long-term survival of an individual Western Scrub Jay.

## ORAL PRESENTATION 44

### The spread of finch trichomonosis, an emerging infectious disease, by migrating birds from Great Britain to southern Fennoscandia

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Hamnes, Inger<sup>3</sup>; Tyler, Kevin<sup>5</sup>; Chantrey, Julian<sup>6</sup>; Hughes,

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**Key words:** trichomonosis migration finch *Trichomonas gallinae*

**Background:** Trichomonosis was recognised as an emerging infectious disease (EID) of British Passeriformes in 2005. Epidemic mortality occurred in the autumn of 2006 and in subsequent years leading to a decline in the breeding population of greenfinches (*Carduelis chloris*) and chaffinches (*Fringilla coelebs*). In summer 2008, finch trichomonosis incidents were first diagnosed in southern Fennoscandia. The importance of bird migration as a mechanism of spread for this parasitic EID from Great Britain to continental Europe was assessed.

**Methods:** A combination of opportunistic and systematic surveillance schemes for garden bird mortality incidents was used to investigate the distribution of the trichomonosis epidemic across Great Britain. Ring return data for the greenfinch were also analysed to assess the spatial distribution of the trichomonosis epidemic. Sequence data were compared for *Trichomonas gallinae* strains from Britain and Fennoscandia. Historical ring return data for multiple species known to be susceptible to the infection were examined to assess patterns of bird migration.

**Results:** Seasonal (autumn) epidemic mortality due to finch trichomonosis occurred annually from 2006 to 2009, with spread from the western to the eastern counties of England. Both garden bird mortality reports and contemporaneous ring return data supported this epidemic distribution.

Comparison of British and Fennoscandian parasite strains found no sequence variation between regions examined: the ITS1/ 5.8S/ ITS2 region and part of the 16S rRNA gene. Historical ring return data identified migrating chaffinches as the most likely primary parasite vector.

**Conclusions:** Data from surveillance, ring returns and sequencing support the hypothesis that *Trichomonas gallinae* spread to the Fennoscandian finch population through

migration of infected chaffinches from eastern England. Continued disease surveillance, in combination with census monitoring, is required to determine the impact of this EID on European finch populations.

## ORAL PRESENTATION 45

### Caprine lymphotropic herpesvirus infection associated with broncho-intestinal pneumonia in alpine ibex

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**Key words:** Alpine ibex, Herpesvirus, pneumonia, Switzerland

Pneumonia has been repeatedly diagnosed in Alpine ibex (*Capra ibex ibex*) from two Swiss colonies for about two decades. Affected animals, mostly adult males, showed chronic wasting and separated from the herd. The most distinctive pathological finding was a chronic lymphoplasmacytic broncho-interstitial pneumonia (Ibex-LPBIP) characterized by a prominent plasmacytic infiltrate. Additionally, parasitic pneumonia and suppurative bronchopneumonia were often observed, as well as multi-organic amyloidosis. Both parasitological and bacteriological investigations, including specific testing for *Mycoplasma* spp., remained unsuccessful at identifying a primary agent. Our aim was to assess whether a viral infection could be associated with the Ibex-LPBIP.

Lung samples from 31 ibex (18 sent for necropsy and 13 hunted) collected between 2007-2010 were included in this study. Animals were classified as cases (N=12) and controls (N=19) according to the presence or absence, respectively, of the histopathological features of Ibex-LPBIP. Screening for Herpesviruses by nested PCR was carried out on all samples, followed by sequencing of the amplicons. Presence of Bovine Adenovirus (BAV), Parainfluenza 3 virus (PI-3), Bovine Respiratory Syncytial Virus (BRSV) and Coronavirus was tested by immunohistochemistry on 21 samples.

Eighteen samples were positive for caprine lymphotropic herpesvirus (CpLHV). CpLHV was significantly more frequent in cases than in controls. Furthermore, 15 CpLHV-positive animals originated from the two colonies with a frequent history of pneumonia, and the three remaining came from a geographically related colony. Positive results were also found for BAV (1 control) and PI-3 (1 case and 2 controls). There was no evidence of multiple viral infection. This is the first report of CpLHV in Alpine ibex. This novel Herpesvirus was recently described in domestic goats, and its pathogenic potential is unknown. However, our results show an association between CpLHV infection and Ibex-LPBIP, suggesting a possible involvement of CpLHV in the pathogenesis of pneumonia in Alpine ibex.

## ORAL PRESENTATION 46

### Isolation of avian pox virus in hooded crows in Italy

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**Key words:** Avipoxvirus, Hooded crow, Virus isolation, Pathological findings

**Background:** Avian Pox virus (APV), member of the family Poxviridae and genus Avipoxvirus, is now considered an emerging disease described worldwide due to the recently increased case report frequency and the involvement of newly affected bird species. Currently, 10 species and 3 tentative species are recognized in the Avipoxvirus genus, but the exact number of species, strains, or variants that actually exist within the genus is unknown. Little information is available about pox strains in wild and captive birds. The present report describes APV isolates in some young hooded crows (*Corvus corone cornix*), killed during the implementing crows containment plan approved by Piedmont Region in 2009.

**Methods:** In response to a suspected poxvirus infection the Pinerolese veterinary district dispatched 5 carcasses of hooded crows to National Reference Centre for Diseases of Wild Animals (Ce.RMAS). The laboratory diagnosis was carried out by histopathologic examination, virus isolation on chorioallantoic membranes (CAMs) of embryonated chicken eggs and Agar Gel Precipitation on CAMs homogenate. During necropsy, skin samples of two carcasses were fixed in 10% neutral buffered formalin for histopathological investigation, performed with routine process and Hematoxylin-eosine staining. Other skin samples were kept fresh for virus isolation.

**Results:** All 5 carcasses were affected by nodular proliferative cutaneous lesions and hard brown masses diffusely covering the legs, feet and toes and, in one case, the beak. Processed cutaneous nodules showed the following histological characteristics: superficial ulceration with serocellular crusts, epithelial hyperplasia with ballooning degeneration, presence of eosinophilic intracytoplasmic inclusion bodies.

Three carcasses produced evident pocks on chorioallantoic membranes and gave positive result to AGP.

**Conclusions:** On the basis of our knowledge, this is the first documented case of Avipoxvirus in hooded crow. Sequence detected by phylogenetic analysis of the isolates will give us genetic distances between APV of the present study and other APV reference sequences available in international web databases.

## ORAL PRESENTATION 47

### Novel Aleutian mink disease virus strain found in wild mink at Bornholm

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**Key words:** Aleutian mink disease virus, AMDV, wild mink, Neovison vison

A strain of Aleutian mink disease virus (AMDV) different from the AMDV found in farmed mink (*Neovison vison*) and wild mink in other countries was found on the remote Danish island, Bornholm. During a health monitoring of Danish wild mink (2008-2009), a high seroprevalence of AMDV was identified in the population of wild mink at Bornholm. Screening for ADV by counter-current immune electrophoresis, revealed 58 mink out of 126 mink from Bornholm had antibodies against AMDV. In comparison, only 4 mink out of 237 wild mink from the rest of Denmark had antibodies against AMDV. Spleen and mesenteric lymph nodes from the seropositive mink were tested by a PCR, amplifying a 328 base pair fragment of the NS1 gene of AMDV. None of 17 wild mink from outside Bornholm were tested positive in the PCR, whereas 28 of 38 wild mink from Bornholm were positive by PCR. Sixteen of the PCR positive samples were sequenced and the analysis revealed a new variant of ADV clustering in group A. At necropsy, all the mink except one appeared healthy and in good condition. Apparently, the AMDV infection detected in wild mink did not compromise the general health status and only one mink had histopathological changes characteristic for AMDV infection. In contrast, AMDV infection in farmed mink usually induces a number of clinical syndromes: decreased reproduction, immune complex-mediated glomerulonephritis and arteritis and acute pneumonia in kits. Indeed, no AMDV infection had been detected among farmed mink on the island for more than a decade, despite continuous serological monitoring. Our observations suggest that a genetically distinct and pathogenetically different strain of AMDV has evolved in the wild mink population on the island of Bornholm.



## ORAL PRESENTATION 48

### Possible role of pathogenic viruses in the decline of the wild European eel stocks

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**Key words:** European eel decline viruses

**Background:** The European eel (*Anguilla anguilla*) has an extraordinary catadromic lifecycle with the spawning grounds in the Sargasso Sea located more than 5,500 km away from the growth habitats in the freshwater lakes and rivers of Europe. Since the 1980s wild European eel stocks declined as much as 99%. The cause of this massive decline is unknown, but probably involves fisheries, pollution, migration barriers and diseases. This study describes the presence and possible consequences of pathogenic viruses in wild European eels in the Netherlands.

**Methods:** From 1999 to 2007, almost 200 wild European eels from several rivers and lakes in the Netherlands were necropsized and tested for the presence of viruses. Organ suspensions were inoculated on the eel kidney 1 cell line at 15, 20 and 26 °C for two passages. If cytopathic effect became evident, the causative virus was characterized by subsequent immunological assays and PCR.

**Results:** Anguillid herpesvirus 1 was found during the entire monitoring period in eels originating from all parts of the Netherlands. Rhabdovirus *anguilla* was only detected in eels originating from two locations from 1999 to 2001. In none of the eels Eel virus European was found. Clinical signs were not necessarily correlated with virus infection.

**Conclusions:** While significant mortalities may result from Anguillid herpesvirus 1 and Rhabdovirus *anguilla* infections in eel culture, the pathogenicity of these viruses in wild eels is still unclear. Experimental infections do not provide a decisive answer. In addition, ambient water temperatures in relation to optimal virus growth temperatures, and the stressful consequences of migration should be taken into consideration. The results of this study do not suggest pathogenic viruses to be a major factor in the decline of the wild European eel stocks, but viruses might play a role as part of a multifactorial cause.

## ORAL PRESENTATION 49

### Update on genetic analysis and the epidemiology of ebhsv across Europe from 1999 to 2010

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**Key words:** hares, molecular epidemiology, EBHS

European brown hare syndrome (EBHS) affects wild and farmed hares of the species *Lepus europaeus* and *Lepus timidus*. The disease was first reported during 1980s, and occurred simultaneously in many European countries. We report the first results on the prevalence and genetic diversity of EBHSV infection across Europe. A total of 1347 liver samples from hares found dead or shot from 16 countries (Greece, Denmark, Switzerland, Austria, Bulgaria, Germany, United Kingdom, Serbia, Croatia, Poland, France, Netherlands, Italy, Spain, Turkey and Israel) between 1999 and 2010 were collected and tested by reverse transcription-PCR for EBHSV. Furthermore, phylogenetic analysis was performed in order to study the molecular epidemiology of the syndrome in Europe for the past 11 years. Sequencing analysis was performed on 212 nt of a 265bp RT-PCR fragment of the region coding for VP60. So far, EBHSV has been detected in 251 (18,63%) of the hare samples tested. Currently, the highest prevalence has been recorded in Greek, Danish and Bulgarian hare samples. Alignments were performed on 205 EBHSV isolates and on 39 RHDV's. This analysis confirmed that the EBHSV and RHDV isolates displayed 34-41% nucleotide variation. In the genomic region analysed the maximum nucleotide variation was 15% for the 205 EBHS viruses. Phylogenetic analysis showed that all isolates were clustered in two major branches, further divided in sub-clusters. So far, isolates cluster in time and space and our results demonstrate that old isolates still exist, contributing to genetic diversity and to the evolution of new strains.

## ORAL PRESENTATION 50

### **Bartonella in Deer Ked (*Lipoptena cervi*) in Scandinavia**

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**Key words:** Bartonella; *Lipoptena cervi*; Deer Ked; zoonosis; vector;

**Background:** Infections with several *Bartonella* spp. have been recognized as emerging zoonotic diseases in humans. Knowledge about reservoirs, vectors and transmission of these bacteria is hence needed. Recently, *Bartonella schoenbuchensis* was found in the midgut of the Deer Ked (*Lipoptena cervi*) and it was suggested that it could be transmitted to humans. The deer ked is a blood-sucking ectoparasite prevalent in Europe and Asia. In Scandinavia, its distribution was previously restricted to Denmark and Southern Sweden, but during the last decades it has showed a remarkable increase in abundance and has expanded its range northwards in Sweden and into Norway. Cervids are the main hosts for deer ked, but the insect attacks several animal species and humans.

**Methods:** Deer Ked imagines and pupae and tissue samples from moose were collected during autopsies performed in order to investigate an outbreak of alopecia in Scandinavian moose. DNA was extracted from the samples by use of Qiagen DNA mini kit (Qiagen) according to the manufacturer's protocol, and all extracts were subjected to Real-Time PCR. The PCR products were sequenced and compared to known isolates of *Bartonella* spp.

**Results:** We describe the findings of *Bartonella* spp. in Deer Ked imagines and pupae collected from moose (*Alces alces*) in Norway, while neither bacteria nor bacterial DNA was found in the host animals.

**Conclusion:** *Bartonella* spp. are present in the abundant and invading deer ked, a species that attacks both several animal species and humans. The *Bartonella* spp. seem to be transmitted from imagines to pupae. More research is needed to evaluate the role of *Lipoptena cervi* in the transmission of *Bartonella* to animals and humans and the possible pathogenicity of these bacteria.

## ORAL PRESENTATION 51

### **Seroprevalence of ten zoonotic infections in two Canadian Cree communities**

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**Key words:** seroprevalence, serology, zoonose, Cree

**Background:** In Canada, aboriginal populations as well as trappers may be more at risk of contracting zoonoses. These diseases are commonly difficult to diagnose, as their symptoms are non-specific. We evaluated the seroprevalence of ten zoonotic agents among the population of two Cree communities of Northern Quebec.

**Methods:** Participants were randomly selected. They were asked to answer three questionnaires (individual questionnaire (demographics and lifestyle), wildlife and zoonose exposure, traditional food frequency) and blood samples were also collected. ELISA methods were used for the detection of antibodies against *Trichinella* sp., *T. canis*, *E. granulosus*, *T. gondii*, *Leptospira* sp., *C. burnetii*, Sin Nombre virus and California serogroup viruses (Jamestown Canyon (JC) and snowshoe hare (SSH)). The detection of antibodies against *F. tularensis* was performed by means of a tube agglutination test. Medical records were verified for those people who had a positive serology. Univariate and multivariate logistic regression analyses were conducted to verify the relation between positive serologies and many variables.

**Results:** Seroprevalence rates were comparable between the two communities. Nearly half the individuals tested (n=251; 146 women, 105 men) were seropositive (n=113) for at least one zoonosis. The highest seroprevalence rates were for *Leptospira* sp. (23%), *F. tularensis* (17%), and the California serogroup viruses (JC and SSH viruses) (10 %). The other zoonoses (*T. gondii*, *C. burnetii*, *E. granulosus*, *T. canis* and *Trichinella* sp.) had seroprevalence rates lower than 5%; no exposures were identified to Sin Nombre Virus. Overall, seropositivity was related to age, gender, hunting and owning a dog. There was no medical history suggestive of overt diseases.

**Conclusions:** Considering the high seroprevalence rates for some pathogens, physicians should consider these agents when confronted with difficult or confusing diagnoses. In particular, the bacterial zoonoses should be ruled out in individuals with high or prolonged fever.

## ORAL PRESENTATION 52

### Bacterial diseases in free-ranging European bats

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**Key words:** bacteria, chiroptera, pathogens, pathology, wildlife

**Background:** In the last decades, many studies have shown that bats act as reservoir hosts of several infectious agents that can affect humans and wildlife or domestic animals. Meanwhile, the impact of pathogens on individual bats is largely unknown and has been much neglected. Most studies are limited to the identification of isolated microbial agents, therefore information concerning bacterial diseases and histo-pathological changes in chiropteran species are rare to non-existent.

**Methods:** In the present study about 300 deceased free-living bats among 18 species were collected in different German geographical regions (southern Bavaria, Berlin greater metropolitan area, eastern Brandenburg, eastern Lower Saxony) in cooperation with bat researchers and protectionists. The bat carcasses were subjected to a post-mortem exploration and investigated by histo-pathological and bacteriological methods. Virological examinations are performed in external cooperation.

**Results:** The histo-pathological investigation revealed inflammatory changes in one or more internal organs in 50 % of the bats with the main focus on interstitial pneumonia. A total of 28 different bacterial genera were cultured from bats including several bacterial species which are known as primary cause of diseases in humans and other animals.

**Conclusion:** By comparing bacteriological results and histo-pathological findings, we found that microbial agents indeed have an impact on free-ranging bats succumbing to infectious diseases, as 9 bacterial species were clearly associated with inflammatory changes and at least 16 % of all bats had died because of bacterial infection.

## ORAL PRESENTATION 53

### Exudative dermatitis in red squirrels (*Sciurus vulgaris*) associated with *Staphylococcus aureus* ST 49 infection

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Agency; <sup>6</sup>Wight Squirrel Project; <sup>7</sup>JSPCA; <sup>8</sup>Imperial College

**Key words:** squirrel, dermatitis, squirrelpox, mange, *S. aureus*

**Background:** Red squirrel (*Sciurus vulgaris*) populations on mainland Britain have declined dramatically in recent decades. The principal cause is infection with squirrelpox virus which is carried asymptotically by introduced grey squirrels (*Sciurus carolinensis*). Viable red squirrel populations survive on the Isle of Wight and on Jersey where grey squirrels are absent. Squirrels dying with skin disease were seen on both islands in 2007-08

**Methods:** Eleven squirrels were examined post-mortem and tissues examined histologically. In three cases bacteriology, electron microscopy and PCR analysis for squirrelpox was performed. *Staphylococcus* isolates were typed by Multilocus Sequence Typing. Ectoparasites were identified morphologically.

**Results:** Squirrels had scabby lesions involving lips, nose and eyelids, sloughing of skin over the feet and patchy alopecia. Numerous mites were present in the fur, especially around the muzzle. Most were *Dermacarus sciurinus hypopi* with occasional *Metalistophorus pagenstecheri* and, in one case, a few harvest mite *Neotrombicula autumnalis* larvae. Facial lesions in two cases yielded *Staphylococcus aureus* ST49 in pure culture. Histologically there was exudative, ulcerative dermatitis with epidermal hyperplasia, hyperkeratosis and superficial staphylococcal pyoderma. Fungi were absent. In two cases there were intracytoplasmic inclusions in the epidermis suggestive of poxvirus infection but there was no ballooning degeneration, electron microscopy was negative for virions and a PCR for squirrelpox proved negative. Internal organs showed no significant lesions.

**Conclusions:** The squirrels were suffering from exudative, ulcerative dermatitis and superficial staphylococcal pyoderma. Examinations for viruses, including squirrelpox, were negative. Although the skin lesions resembled those of mange the mites were not considered to be pathogenic. The primary cause of the dermatitis was not established but the involvement of *S. aureus* ST49 merits consideration. This sequence type has not been previously recorded from wild animals but it is known to be carried by humans and infection could possibly have been acquired from garden feeders.

## ORAL PRESENTATION 54

### Epidemic of salmonellosis in Passerine birds in Switzerland and suspected spillover in domestic cats

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**Key words:** salmonellosis, oesophagitis, passerine birds, cats

A die off of passerine birds was reported in various areas of Switzerland between February and March 2010. In parallel, two pet clinics reported an increased number of domestic cats with clinical signs including fever, anorexia, and occasionally dolent abdomen after the presumed consumption of passerine birds. Pathological and microbiological investigations were performed in order to determine the birds' cause of death, and to establish a possible causal relationship with the cats' illness.

Sixteen passerine birds including 14 Eurasian siskins (*Carduelis spinus*), 1 European goldfinch (*C. carduelis*) and 1 Greenfinch (*C. chloris*) were submitted for full necropsy. Bacteriological examination was carried out on samples from crop, intestines, lung and kidney from dead birds and on rectal swabs from 8 cats with the above-mentioned clinical signs.

At gross examination, 14 out of 16 birds presented light tan nodules (1-2 mm in diameter) scattered through the oesophagus/crop. Histologically, a necrotizing and ulcerative oesophagitis/ingluvitis was observed. The nodules were composed of viable and degenerating histiocytes and heterophils, with presence of both intra- and extracellular bacterial rods. Bacterial cultures yielded *Salmonella enterica* subsp. *enterica* serovar Typhimurium in 12 out of the 14 birds with esophageal nodules, while *Salmonella* sp. isolation in the remaining two birds with esophageal lesions was unsuccessful. The two *Salmonella* sp.-negative birds with no esophageal nodules died of other diseases (Toxoplasmosis and fungal coelomitis, respectively). Analysis of the cats' rectal swabs revealed the presence of *S. Typhimurium* in all cases.

Necropsy and bacteriological findings were consistent with a systemic infection caused by *S. Typhimurium*. Isolation of the same serovar from dead birds and ill cats is suggestive of a spillover from birds to cats through predation. Further characterization and analysis of *S. Typhimurium* isolates are on-going in the attempt to validate this hypothesis.



# Abstracts of poster presentations, by session

## 1. BACTERIAL ZOONOTIC DISEASES

### POSTER PRESENTATION 1

#### Epidemiology study of the natural infection of wild boars (*Sus scrofa*) by *M. bovis* and *M. caprae*.

WL., García-Jiménez<sup>1</sup>; JM., Benítez-Medina<sup>1</sup>; P., Fernández-Llario<sup>1</sup>; F., Bermejo<sup>1</sup>; M., Cortés<sup>1</sup>; A., García-Sánchez<sup>2</sup>; R., Martínez<sup>1</sup>; D., Risco<sup>1</sup>; L., Gómez<sup>1</sup>; J., Hermoso de Mendoza<sup>1</sup>

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Key words: wild boar (*Sus scrofa*), *M. bovis*, *M. caprae*

The epidemiology of tuberculosis (TB) in extensive breeding of livestock in the Mediterranean area directly depends on the degree of interaction between domestic and wild species, especially wild boar.

The present study has been carried out on wild boar from different estates located in the southwest of Spain. Sampling was done during the 2007-2008 hunting season and accounted for 50 samples with TB-like lesions. 26 samples came from wild boar living in areas where cattle was the predominant livestock, whereas the remainder 24 came for areas where goats were the predominant domestic species. Two grams of each retro-pharyngeal lymph node sample were homogenized and decontaminated by hexadecyl pyridinium chloride method. Two Lowenstein-Jensen slants, with piruvate and without glycerol were inoculated in parallel. The identification of *Mycobacterium tuberculosis* Complex was accomplished by PCR and genotyping by Spoligotyping. Histopathology was carried out by fixing tissues in 4% formalin , being further embedded in paraffin, cut in 5µm thick sections and stained by Hematoxillin-Eosine as well as by Ziehl-Neelsen techniques.

Remarkable results were the identification of 11 *M. bovis* different Spoligotyping profiles, being SB0121 (10/50= 20%) the most frequent, and 2 profiles of *M. caprae*, being SC1081 (18/50= 36%) the most frequent. Regarding to histopathology, it was observed that between the *M. bovis* infection samples only four wild boar gave positive results by Ziehl Neelsen, whereas 18 out of 24 *M. caprae* infection samples had positive results by Ziehl Nielsen.

The mycobacterial species involved may have an important in the kind of lesions produced, taht can have important consequences in the epidemiology of tuberculosis.

### POSTER PRESENTATION 2

#### *Mycobacterium bovis*, *Mycobacterium tuberculosis* and *Mycobacterium avium* infections in wildlife animals in the Bieszczady region (Poland)

Bartoszek, K<sup>1</sup>; Orłowska, B<sup>1</sup>; Anusz, K<sup>1</sup>

<sup>1</sup>Warsaw University of Life Sciences

Key words: tuberculosis, wildlife, zoonosis

**Introduction:** Tuberculosis has been present in humans and animals all over the world since antiquity and it still remains one of the most important infectious disease. Tuberculosis is caused by bacilli members of the *Mycobacterium tuberculosis* complex. The first case of tuberculosis in wildlife in Poland was reported in 1956 in roe deer. Frequent infections in Zoo animals were also diagnosed. In 1996, the first case of bovine tuberculosis in European bison (*Bison bonasus caucasicus*) in the Bieszczady region was reported.

**Methods:** Bacteriological investigations on postmortem specimens collected from 215 animals (5 bisons, 154 deers , 2 roe deers , 45 boars, 2 badgers, 6 wolfs, 1 lynx) in Bieszczady region in years 2006-2009 ,were conducted. A culture method using Lowenstein-Jensen and Stonebrink medium, biochemical test (niacin test) and bacterioscopy (Ziehl-Neelsen staining) were performed for the isolation and identification of the tuberculosis mycobacteria. Samples were analyzed by bioassay in guinea pigs.

**Results:** Mycobacteria were isolated in 10 of 215 examined animals. 3 strains were identified as *M. tuberculosis* (3 wolfs), 3 *M. bovis* strains (1 badger, 2 bisons) and 4 *M. avium* strains ( 4 deer).

**Conclusions:** Tuberculosis is an old problem but still actual health hazard, not only for people but also domestic and wild animals. It is important to take into consideration endangered species like bisons or wolfs and the possibility of disease transmission from domestic animals and humans to wildlife and inversely. Knowledge of the epidemiological chain will be very helpful in protection of endangered species.

### POSTER PRESENTATION 3

#### Case report of *Edwardsiella tarda* in a grey seal (*H. grypus*) representing a zoonotic risk

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<sup>1</sup>Seal Rehabilitation and Research Centre

**Key words:** *Edwardsiella tarda*, Grey seal, Zoonotic risk, SRRC

On May 9th, 2010, a dead male adult grey seal (*Halichoerus grypus*), of 118 kg body weight and 219 cm length, was found on the beach of the Wadden island Vlieland (53°14'N, 4°54'E).

The next day postmortem examination was performed at the Seal Rehabilitation and Research Centre, Lenie't Hart, (SRRC), Pieterburen, the Netherlands. General examination revealed poor body condition and no external lesions were observed. Gross pathological findings showed diffuse necrotic rhabdomyolysis, with a gelatinous butter appearance of the muscle in the anterior area of the body. In the pericardium, right lung and pleura 0.5-1 cm granulomatous lesions were observed, together with diffuse congestion and oedema of the right lung. Generalized lymphadenopathy was noticed. Bacteriological isolation was attempted from all mentioned organs and isolates recovered were identified as *Edwardsiella tarda*.

The source of the *E. tarda* which had infected the seal is unknown. *E. tarda* is the only recognized pathogenic species of its genus in humans. It has been associated with a number of infections, including sporadic cases of gastroenteritis, wound infections, abscesses, meningitis and cases of septicemia or bacteriemia. Although, in humans extraintestinal manifestations of *E. tarda* infections are rare, mortality rate has been as high as 50% in some studies. This is the first report of *Edwardsiella tarda*, as a cause of death in a pinnipeds. Due to the high zoonotic risk it is important to remark the function of the SRRC collecting carcasses and analysing the cause of death in seals stranded on the Dutch coast to protect public health.

### POSTER PRESENTATION 4

#### Clonal relationships and antimicrobial resistance in *Salmonella enterica* strains isolated from wild animals in Spain

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**Key words:** *Salmonella*; antimicrobial resistance; wildlife

*Salmonella* colonizes the gut of a wide range of animals, from insects to mammals. Although non typhoid salmonellosis is the most common food borne disease in the North hemisphere, little is known about the importance of wildlife in its epidemiology. Seventeen salmonella strains (4,5,12:i:-, Anatum, Bredeney, Choleraesuis, Enteritidis, Mikawasima and Typhimurium serovars) isolated between 1998 and 2008 from wild animals at Veterinary Teaching Hospitals of the Universities of Extremadura and Cordoba (South West Spain) were analyzed. Fourteen different pulsetypes were evidenced by pulse field gel electrophoresis (PFGE) according to PulseNet protocols as performed at VISAVET Health Surveillance Centre (Complutense University of Madrid). Minimum inhibitory concentrations (MIC's) for 23 antimicrobial agents were calculated and eleven strains resulted multidrug-resistant (according to EUCAST cut off values) whilst only four were totally sensitive to all tested antimicrobials. For the eight quinolone resistant strains, genes *gyrA*, *gyrB*, *parC* and *parE* were analyzed to detect the presence of mutations in their quinolone resistance determining regions (QRDR). Five strains share S83Y alleles of *gyrA*, whereas a single case of the allele S83F was found in a salmonella isolated from a white stork (*Ciconia ciconia*). In addition, the occurrence of class 1 integrons (*int1*) was revealed in the 47% of strains and correlated to the ACSSuT resistance profile. Four genes associated to *int1* were sequenced (*aadA2* + *blaPSE1*; *aadA1* + *dhfrVII*; *dhfrVII*; and *aadA1*) thanks to amplification of gene-cassettes from conserved regions (CS). These results support the high prevalence of antimicrobial resistance in *Salmonella* strains isolated from wildlife, with some determinants (*gyrA*-S83F and ciprofloxacin resistance, among others) closely related to those found in antibiotic resistant human *Salmonella* strains. Thus, wild fauna might play an important role for the spreading of antibiotic resistance.

## POSTER PRESENTATION 5

### Compiled data on extended spectrum $\beta$ lactamase producing *Escherichia coli* from wild animals in Germany

Grobbel, Mirjam<sup>1</sup>; Ewers, Christa<sup>2</sup>; Bethe, Astrid<sup>2</sup>; Beutlich, Janine<sup>3</sup>; Guerra, Beatriz<sup>3</sup>; Goedecke, Andreas<sup>4</sup>; Heidemanns, Katrin<sup>2</sup>; Luebke-Becker, Antina<sup>5</sup>; Ulrich, Rainer G<sup>6</sup>; Wieler, Lothar H<sup>5</sup>; Guenther, Sebastian<sup>5</sup>

<sup>1</sup>Institute of Zoo and Wildlife Research; <sup>2</sup>Institute for Microbiology and Epizootics; <sup>3</sup>Institute for Risk assessment; <sup>4</sup>ProRing e.V.; <sup>5</sup>Institute for Microbiology and Epizootics; <sup>6</sup>Friedrich Loeffler Institute

**Key words:** antimicrobial resistance, ESBL, rats, rodents, wild birds

**Background:** Decades of extensive use of antimicrobials gave microorganisms the chance of adaptation. Extended spectrum  $\beta$  lactamase (ESBL) producing *Escherichia coli*, resistant to many frequently used antimicrobial agents, are now emerging in both, human and veterinary medicine. We describe various approaches that have been performed to determine these bacteria in wild and synanthropic animal species in Germany.

**Methods:** A total of 172 *E. coli* isolates from organ and fecal samples of birds from rural districts of Hesse and Thuringia, 188 faecal isolates from rodents from rural regions all over Germany, and 211 isolates from urban brown rats from Berlin have been collected. Phenotypical confirmation of ESBL production was performed as given in CLSI guideline M31 A3. Further

characterization of positive isolates included sequencing of  $\beta$  lactamase (*bla*) genes, serotyping, multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE).

**Results:** ESBL producing *E. coli* were detected in four (2.3%) of the bird samples (two Eurasian Blackbirds, one Rock Pigeon, one Greater White fronted Goose), in one sample from a rat (0.5%) and in none of the small wild rodents. Interestingly phylogenetic analysis revealed the spread of a CTX M 15 producing clone of sequence type (ST) 648 among bird species with different habitats. The CTX M 9 producing *E. coli* isolate from the urban brown rat belonged to serotype O25:H4 and ST131, a clone currently spreading in human medicine worldwide.

**Conclusions:** Wild animals are possible circulators of ESBL producing *E. coli*, sharing STs with known pathogens from humans and therefore representing a potential zoonotic threat. Both, ST648 from wild bird and ST131 from a rat represent a link between multiresistant strains in human medicine and those present in wild animals. To unravel the relevance of wild avian and rodent hosts as possible sources of ESBL producing Enterobacteriaceae and to determine their contribution to the spread of antimicrobial resistant *E. coli*, detailed epidemiologic studies are urgently needed.

## POSTER PRESENTATION 6

### Emerging tularemia in the European brown hare (*Lepus europaeus*) in Sweden: characterization by pathology and immunohistochemistry.

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<sup>1</sup>National Veterinary Institute (SVA)

**Key words:** Tularemia, European brown hare, necrosis, *F. tularensis*.

Tularemia is endemic in the north of Sweden and it has typically affected mountain hares (*Lepus timidus*). In recent years, tularemia with a different epidemiological presentation emerged in Sweden. The emerging forms occurred in the European brown hares (*Lepus europaeus*) in centre-south parts of the country. The aim of this study was to describe the pathology of tularemia in the European brown hare, in comparison with tularemia in the mountain hare to better understand the epidemiology of *F. tularensis* infection.

Tissue samples were obtained for histopathological and immunohistochemical examination from nine European brown hares and four mountain hares, all found dead. Gross changes with multiple necrosis and enlargement of the spleen, liver and/or bone marrow were seen, but several hares showed no changes. Histopathological examination showed necrotic foci in the liver, spleen and bone marrow in all the hares. In some of the hares, necrotic foci in the lungs, lymph nodes, intestine and adrenal glands were additional findings. The majority of the lesions were acute. In three European brown hares there were mild associated inflammation, and in one liver a small granuloma was detected. Immunohistochemistry for detecting and locating *F. tularensis* demonstrated extra- and intracellular bacteria, mostly in association with the necroses.

It was concluded that the pathological presentation of tularemia was similar in both hare species, consistent with a multi-organ septicemic form. No differences in the pathology were observed in the hares from various geographic areas. Several hares that had died of tularemia showed no gross lesions. Chronic forms of tularemia such as those observed in Germany (Sterba and Krul, 1985) were not detected. Differences in the pathological presentation of tularemia in European brown hares in Europe may be attributable to differences in natural or acquired immune resistance to the bacteria or to different virulence of the bacterial strains.



## POSTER PRESENTATION 7

### Garden bird salmonellosis – is prevalence reflected in suspected spill-over hosts, including man?

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**Key words:** salmonellosis, hosts zoonosis, trends

Garden bird salmonellosis – is prevalence reflected in suspected spill-over hosts, including man?

**Background:** The current theory concerning garden bird salmonellosis is that in the UK the disease for several decades has been caused by S Typhimurium phage types, 40, 56 and 56 variant. These bacteria are regularly isolated from birds utilising garden feeders in the UK and it is assumed that these organisms are host-adapted to these species.

The phage types also occur, sporadically, in a wide range of domesticated animals, in wildlife, in pets, and in humans in the UK. These are considered to be spill-over hosts. The majority of infections in these spill-over hosts are sub-clinical however clinical disease does occur periodically particularly in horses and cats. Clinical infection in man, we assume is generally mild but may still necessitate visits to the doctors.

**Methods:** Using data from the VLA Diseases of Wildlife Scheme (VLADoWS) on salmonellosis in wild birds since 2000 coupled to the VLA national database for salmonella in food producing animals and incorporating human data for these Stm phage types we qualitatively compare the trends in infection rates across the species over the past ten years.

**Results:** At the time of writing analysis of data is on-going.

**Conclusions:** In the past 3 years there have been indications that the prevalence of salmonellosis in garden birds has been declining (as reported by the UK organisations investigating the disease). It would therefore be expected that the pattern of Stm isolations in other species would decline in step with the garden birds. This presentation hopes to assess this prediction, and then to draw conclusions.

## POSTER PRESENTATION 8

### Investigations of *Coxiella burnetii* infections in animals and ticks in Slovenia

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**Key words:** *Coxiella burnetii*, animals, ticks, PCR, ELISA

**Background:** *Coxiella burnetii*, the etiologic agent of Q fever, is a zoonotic pathogen. The disease agent, an intracellular bacterium is transmitted to animals also by ticks. Transmission to humans occurs as a consequence of close contact with infected domestic animals. Occasional outbreaks of Q fever occur in Slovenia. To research the transmission potential of ticks and occurrence of the pathogen the following study was conducted.

**Methods:** Ticks were sampled by flagging vegetation and from domestic animals in Slovenia, where Q fever was noted occasionally. Additionally blood was collected from animals on these farms. Some ticks were also collected from wild game in the regions, where our study took place. DNA was isolated from pooled ticks and animal blood using Qiagen BioSprint 15 kit. Probe specific PCR detecting a 66 bp portion of the transposase gene (IS1111) was used for *C. burnetii* detection in tick and animal samples. Additionally serological survey of animal samples was performed using ELISA kit (Idexx, Switzerland).

**Results:** 701 ticks, from wild and domestic animals and vegetation were screened for presence of the pathogen. 8 ticks were positive, but we were unable to confirm the infection by sequencing. 151 sheep and cattle were checked molecularly and serologically. We didn't detect bacterial DNA in any of the analyzed samples, but antibodies were detected in 51 of the 151 animals.

**Conclusions:** Overall prevalence of the pathogen is low, but regional analysis shows that the regions, where epidemics occur in most cases is also the one where *C. burnetii* was detected in ticks, as well from vegetation, as from animals. Also it might be that the concentration of the pathogen is too low to detect. Serologically a high percent of animals was positive, indicating a very active transmission in the area or stables and showing a high risk for human population.

## POSTER PRESENTATION 9

### Isolation of *Salmonella* spp. from Red Fox (*Vulpes vulpes*) and Badger (*Meles meles*) in Lombardy (North Italy)

Chiari, Mario<sup>1</sup>; Zanoni, Mariagrazia<sup>1</sup>; D'Incau, Mario<sup>1</sup>; Salogni, Cristian<sup>1</sup>; Giovannini, Stefano<sup>1</sup>; Alborali, Loris<sup>1</sup>; Lavazza, Antonio<sup>1</sup>

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**Key words:** red fox, badger, salmonella

**Background:** *Salmonella* has been isolated from a wide variety of wild animals, both mammals and birds. It may cause death in some of them, mainly small passerines, whereas some wild carnivores as red fox (*Vulpes vulpes*) and badger (*Meles meles*) may act as indicator species to determine the presence of *Salmonella* in the local environment.

**Methods:** In Lombardy the agreement among official veterinary service, public administrators and hunter associations, made possible sampling the carcasses of foxes (*Vulpes vulpes*) and badgers (*Meles meles*) found dead or hunted, between June 2009-May 2010. The presence of rabies was firstly excluded on all samples (511 red foxes and 18 badgers) and then further analysis were done. *Salmonella* was isolated from faecal samples using both the mandatory methods for *Salmonella* monitoring and control plan for primary productions (Annex D ISO 6579:2002) and the in-house isolation procedure based on the enrichment with Rappaport-Vassiliadis Broth and plating on Hecktoen enteric agar. *Salmonella* identification was performed with biochemical tests (growing on TSI, ONPG test, identification by multitest kit) and serotyping.

**Results:** *Salmonella* was isolated from 29 foxes (6%) and 2 badgers. Sixteen different serotypes were identified: 12/31 (39%) isolates belonged to serotypes (Typhimurium, Enteritidis, Infantis) commonly found in men. Others serotypes could be either sporadically found in man (Derby, Muenchen, Napoli, Livingston) or often found in water or in wild animals, in particular reptiles (*Houtenae*, *Diarizonae*, *Anatum*, *Veneziana*).

**Conclusions:** The behaviour and feeding habits of animals influence the likelihood of being infected with *Salmonella*. Foxes and badgers, that are at the top of the food chain in our region, could be infected by eating carcasses contaminated by *Salmonella* or by different anthropogenic environmental contamination, as foodstuff residues. Therefore, wild carnivores are an important reservoir of pathogenic serotypes of *Salmonella*, and may be a risk for human and livestock.

## POSTER PRESENTATION 10

### Preliminary studies on *Brucella suis* infection in wild boars *Sus scrofa*, hares (*Lepus granatensis* and *L. europaeus*) and hunting dogs in Aragon, Spain<sup>\*</sup>.

Martinez, David<sup>1</sup>; Dieste, Lucia<sup>2</sup>; Revilla, Miguel<sup>3</sup>; Muñoz, Maria Pilar<sup>2</sup>; Arnal, Maricruz<sup>4</sup>; De Miguel, Maria Jesus<sup>2</sup>; Barberan, Montse<sup>4</sup>; Marín, Clara<sup>2</sup>; Fernandez de Luco, Daniel<sup>4</sup>; Blasco, Maria Jose<sup>2</sup>

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<sup>2</sup>CITA; <sup>3</sup>Universidad de Zaragoza facultad de veterinaria;

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**Key words:** *Brucella suis*, wild boar, hare, dog, Aragon

Previous studies shown that *Brucella suis* was present in wild boar in Aragon. During 2008-2010, 943 hunted wild boars, 7 hares (5 *L. europaeus* and 2 *L. granatensis*) and 294 hunting dogs coming from 28 out of the 33 counties of Aragon were sampled.

Wild boar blood, mandibular lymph node and spleen were obtained from hunted animals whenever possible. Testes were sampled sporadically. Only blood of hunting dogs and viscera of hares could be sampled. Wild boar sera were tested serologically using an indirect ELISA (iELISA). Serum from dogs and thoracic fluid from hares were tested using the Rose Bengal agglutination test (RBT).

A total of 199 wild boar samples out of 934 (21.30%) resulted iELISA positive. Tissue samples from all these seropositive animals were cultured. A total of 36 (18.09%) yielded *B. suis* biovar 2 cultures, belonging all strains isolated to the main two haplotypes present in Spain. Two infected animals had gross testicular lesions. Culture positive animals were from 17 counties. Thoracic fluid from hares was unsuitable for RBT testing and all tissue samples were negative in bacteriological culture. Twenty nine (9.8%) out of 294 dogs were RBT positive, suggesting that brucellosis could have been transmitted to some of these animals.

These results confirm a high prevalence and a widespread distribution of *B. suis* in wild boar in Aragon. Additional research should be conducted to assess the involvement of hares and dogs.

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## POSTER PRESENTATION 11

### Serological survey of *Leptospira* infection in the small mammals population of Northern Portugal

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**Key words:** Leptospirosis, *Leptospira*, small mammals, serology, Portugal

**Background:** Leptospirosis is a re-emerging infectious disease of animals and man with a worldwide dispersion. Small mammals, particularly rodents, are maintenance hosts for some pathogenic leptospires and their local abundance can be an indicator of the potential *Leptospira* transmission to humans and livestock. This study is the first serological survey developed in these mammals from Northern Portugal, mainly to assess the relative importance of different serovars in each species trapped.

**Material/Methods:** Sera from 282 live-trapped individuals, 231 *Mus musculus*, 23 *M. spretus*, 18 *Rattus norvegicus*, 3 *R. rattus*, 4 *Apodemus sylvaticus* and 3 *Crocidura russula*, were analysed by the *Leptospira* microscopic agglutination test. Risk factors for the presence of *Leptospira* antibodies were assessed by nominal logistic regression analysis.

**Results:** One hundred and fifty-five (55%) rodents showed to be infected by leptospires (at 1:30 or higher), with the following seroreactivity rates: *Mus musculus* 54.6%, *M. spretus* 56.5%, *Rattus norvegicus* 55.6%, *R. rattus* 100% and *Apodemus sylvaticus* 75%. The most prevalent presumptive serogroups were Sejroe (41.3%), Pomona (25.2%) and Ballum (19.4%). No significant statistical differences in prevalence were detected between species and regarding sex. However, mature animals were shown to have a significant ( $p < 0.05$ ) higher serology than immature (54.2 and 45.8%, respectively; OR=1.88; CI=1.86-1.91). The insectivore *Crocidura russula* showed no specific *Leptospira* antibodies.

**Conclusions:** The high seroprevalence of infected rodents confirms the wide dispersion of leptospires in Northern Portugal, and alerts for the potential relevance of these wild natural hosts to the health of humans and livestock. Serological results by presumptive serogroup were not coincident with the serogroup of the field strains previously obtained from the same animals, suggesting the importance of isolation to confirm the causative agents. Sexual maturity state seems to be a risk factor for the leptospiral antibody status.

## POSTER PRESENTATION 12

### Tularaemia in European brown hare (*Lepus europaeus*) in France from 2003 to 2008 : temporal and spatial distribution, pathological aspects

Anouk, Decors<sup>1</sup>; Lesage, Célia<sup>1</sup>; Mailles, Alexandra<sup>2</sup>; Madani, Nora<sup>3</sup>; Moinet, Marie<sup>3</sup>; Mastain, Olivier<sup>1</sup>

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**Key words:** tularaemia, *Lepus europaeus*, zoonosis, distribution, pathological aspects

The European brown hare (*Lepus europaeus*) is supposed to play an important role in the ecology of tularaemia in France and to be a significant source of human infection. The aim of this present study is first to better understand the epidemiological link between the human and hare cases in France by comparison of the data of the two epidemiological surveillance networks: SAGIR and InVS from 2003 to 2008. The second aim is to describe pathological findings on naturally infected European brown hares collected within the SAGIR network.

The analysis of the temporal evolution of the human and hare cases suggests a variability of the epidemiological situation in relation with the geographic situation. In some regions, there is a simultaneity between the evolution of human and hare cases of tularaemia. This result emphasizes the hypothesis of the existence of a common source of contamination and may place the European brown hare as a sentinel of tularaemia. Infection of hare by a systemic route is suggested by the analysis of the main associations of lesions described during necropsy. All this results have to be confirmed by further studies but show the interest to investigate the epidemiology of tularaemia of European brown hare (e.g. prevalence, infection rate of hares obviously in good health) to better understand the place of the hare in the human transmission in France and to implement efficient preventive actions.

## POSTER PRESENTATION 13

### Zoonotic gastrointestinal bacteria in chamois (*Rupicapra rupicapra*) and cattle in Austria

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**Key words:** prevalence, chamois, *Rupicapra rupicapra*, cattle, zoonotic bacteria

Numerous studies on the prevalence of zoonotic bacteria in domestic animals have been conducted and their role as reservoirs has been extensively studied. However, knowledge regarding wild animals as potential carriers of zoonotic infectious agents is scarce.

To gain more insight we conducted a survey on free-ranging chamois (*Rupicapra rupicapra*) and 53 cattle grazing on an alpine pasture in the Northern Limestone Alps during June-August 2009. We conducted five samplings collecting individually assignable, fresh fecal specimen from cattle (total n=217) and fresh to dry fecal samples from several chamois (total n=24) every two to three weeks. After enrichment, PCR was employed to screen for four bacteria (*Salmonella* spp., thermophile *Campylobacter*, *Yersinia enterocolitica*, enterohemorrhagic *E.coli*) and two protozoa (*Cryptosporidium parvum*, *Giardia duodenalis*). According to a recent literature study, these pathogens have been shown to be the most relevant microorganisms causing gastrointestinal illnesses in humans in Austria.

Interestingly, our survey showed high prevalence for *Yersinia enterocolitica* and enterohemorrhagic *E.coli* over the entire sampling period in both, cattle and chamois. Besides, *Salmonella* spp. was also found in all five samplings in both species. Thermophile *Campylobacter* was detected in low numbers on four sampling occasions in cattle, but was only discovered in one sampling in chamois. *Cryptosporidium parvum* was found on one occasion in cattle and was absent in chamois. No samples tested positive for *Giardia duodenalis*.

Since the study site serves as a recreational area, the results of this study exemplify a potential public health risk. The sampling regime demonstrated that some microorganisms were present over the entire study period in cattle as well as in chamois. Given that all pathogens exhibit a high survival rate in the environment, environmental transmission is an important risk source. Therefore, awareness of risk factors among recreational users must be created and further studies evaluating wild animals as potential reservoirs of zoonotic agents should be encouraged in future.

## 2. VIRAL ZOOONOTIC DISEASES

### POSTER PRESENTATION 14

#### Avian Influenza Surveillance in Wild Birds in the United States: Environmental Sampling for the Rapid Detection of Avian Influenza Viruses

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<sup>1</sup>National Wildlife Research Center, USDA

**Key words:** avian influenza, wild birds, surveillance, USA

**Abstract:** The National Wildlife Research Center (NWRC), WS, USDA, was part of the National Strategy for Pandemic Influenza in the United States from April 2006 to March 2009 to increase and expand surveillance for the early detection of highly pathogenic avian influenza (HPAI) H5N1 virus that was circulating throughout the Asian, European, and African continents. The surveillance system was designed to detect HPAI H5N1 virus in wild migratory birds that have the potential to bring in the virus from Asia or Europe and spread it throughout North America. As part of this early detection system, the NWRC developed testing methods, sampling protocols, and guidelines to analyze avian fecal samples collected by Wildlife Service biologists initially in 50 states and the U. S. territories. A total of 101,539 samples were collected and analyzed by rt-RT-PCR in 20,859 pools with 4.51%-6.66% of the pools AI-M+. Only low pathogenic avian influenza (LPAI) viruses were detected and 41 different AI subtypes were isolated from fecal samples with H4N6, H3N8, H3N6, and H11N9 as the dominant subtypes. Experimental infection studies of LPAI in waterfowl revealed that fecal samples were equivalent to oral and cloacal swabs in detecting virus shedding. This monitoring effort was successful in diagnosing AI viruses in environmental samples and has proven to be a rapid and cost effective surveillance method that can be used as a first phase surveillance tool to select locations for subsequent live bird sampling, for supplemental sampling, or in urban and similar areas where live bird sampling is not feasible. The AI national sampling data was integrated with historical continental waterfowl banding data to determine pathways and focal areas where LPAI viruses are disseminated by native waterfowl species as a model for more targeted surveillance in the future for the potential detection and movement of HPAI.

## POSTER PRESENTATION 15

### Characterization of the cellular infiltrate in brains from wild birds naturally infected by highly pathogenic avian influenza (HPAI) H5N1

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**Key words:** avian influenza, brain, H5N1, inflammation, natural

During the outbreak of highly pathogenic avian influenza (HPAI) H5N1 in Sweden in 2006, disease and mortality was observed in a number of wild bird species. Encephalitis was one of the most consistent findings in necropsied birds; however the distribution and severity of the inflammation varied. In agreement with other descriptions of natural and experimental infection with HPAI H5N1, the neuropathology included multifocal to diffuse areas of gliosis and inflammation in the grey matter, neuronal degeneration, neuronophagia, vacuolation of the neuropil, focal necrosis, perivascular cuffing, and meningitis. This study characterized the cellular infiltrate in brains of 40 birds of various species with encephalitis naturally infected with HPAI-H5N1.

Selected sections of brain were examined for the presence of T-lymphocytes, B-lymphocytes, macrophages, and astrocytes expressing glial fibrillary acidic protein (GFAP). These selected sections were evaluated in relation to corresponding sections stained with H&E and with immunohistochemistry for Influenza A viral antigen. Variations were mostly observed in the amount of lymphocytic inflammation in the grey matter and the types of perivascular cuffs. Some cuffs appeared more acute with a predominance of lymphocytes and others were more chronic with more macrophages and plasma cells. The severity and temporal pattern of the inflammation and thus the type of cell infiltrate seemed to vary among different species. The differences in cell infiltrates suggest that some species die in a more acute stage of the disease while others have time to develop a more severe inflammation, re-emphasizing the differences in susceptibility to HPAI-H5N1. However, since very few individuals of each species were evaluated, individual variation in susceptibility and inflammatory cell response could not be excluded. Since neurotropism is a key feature of HPAI-H5N1 infection, the characterization of the inflammation in the brain is an important feature in understanding the pathogenesis of the disease.

## POSTER PRESENTATION 16

### Environmental Factors associated to the Reproduction Ratio of Avian Influenza Epidemics in Europe in 2005-2008

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**Key words:** Wildbirds, risk factors, spatial analysis, infectious diseases surveillance, avian influenza

**Background:** The disease reproductive ratio (R0) is a key parameter for understanding disease dynamics and as an indicator of the magnitude of disease transmission. High values of R0 are likely associated with the presence or absence of factors that, respectively, promote and prevent disease spread. Because environmental factors are known to influence the dynamics of diseases in wildlife (Wobeser 2007), the identification of factors that influence the value of R0 are critical for the formulation of predictive models of disease transmission and spread.

**Methods:** Application of a scan-based permutation model resulted in the identification of nine spatiotemporal clusters of disease in the H5N1 HPAI epidemics reported in wildbirds in Europe from January 2006 through January 2009 (Iglesias et al, 2010a). Subsequently, the values of R0 were computed for each of the nine clusters in the absence of population data (Iglesias et al 2010b). The objective of this study was to identify those environmental and demographic factors influencing the within-cluster values of R0. The environmental and biological factors evaluated for each cluster included climatic data (temperature, humidity) land cover (artificial, forest or agriculture areas, wetlands) (Corine 2000), whereas the demographic factor was the affected species in the outbreak. Descriptive statistics of the association between environmental and demographic factors were computed for each cluster using a GIS software. Factors significantly associated with R0 were identified by fitting a multivariate general linear model to the data.

**Results:** Differences in land use, humidity ranges, and affected species (presence of swans and coots) significantly increased the between-clusters difference in the estimates of R0.

**Conclusions:** Results presented here will help to formulate and parameterize spread models for the disease in wildbirds and to identify areas at high risk for AI in wild birds in Europe.

## POSTER PRESENTATION 17

### Evidence for role of waterfowl hunting in spread of HP H5N1

van den Ende, Marinus<sup>1</sup>

<sup>1</sup>Conseil Santé

**Key words:** waterfowl hunting spread HPH5N1

Evidence for role of waterfowl hunting in spread of HP H5N1  
Evidence is provided from surveys in Turkey for hunting and dressing practices as the cause of the spread of HP H5N1 virus from wild ducks to domestic poultry.

**Background:** Surveys were conducted in provinces which had HP H5N1 virus outbreaks in 2005/06, and on sites of new outbreaks in 2007 and 2008

**Methods:** Role of HPAIV introduction by wild birds was assessed through retrospective surveys and investigations of new outbreaks, based on standard HP AI outbreak questionnaires. For all interviews “participatory epidemiology” methods were practiced.

**Results:** Extensive inquiry failed to reveal evidence for introduction of virus by movements of animals or people, trade in live poultry or poultry products, or by gifts of the same. In three out of five villages where a HP H5N1 virus outbreak had occurred in the previous winter(s), waterfowl hunting was practiced. In the two other villages, and in six villages where no outbreak had occurred, no hunting practices were reported (Fisher’s exact test,  $P = 0.06$ ).

Investigation results of 8 new outbreaks in 2007/2008 were strongly suggestive of HP H5N1 introduction through entrails of hunted waterfowl.

**Conclusions:** Findings point at scraps from hunted waterfowl as a source of infection HP H5N1 outbreaks in backyard poultry. Findings are consistent with the potential role of “long distance carrier” of HP H5N1 which can be played by some duck species, as recently demonstrated (ref). Not only waterfowl showing signs of disease or found dead, but also apparently healthy birds that have been hunted are a potential source of HP H5N1 virus. By identifying waterfowl hunting as a risk factor for the introduction of HP H5N1 virus, it now may be possible to eliminate this route of transmission by changing behaviour in risk areas.

## POSTER PRESENTATION 18

### Five years monitoring for AIV and APMV circulation in non reservoir birds and mammals in Northern Italy

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**Key words:** orthomyxoviruses, paramyxoviruses, wild birds, mammals

**Background:** Avian influenza viruses (AIV) find their natural reservoir in aquatic wild birds, but have demonstrated their ability to adapt to and become sustained in mammals as well. Similar to AIV, velogenic (highly virulent) strains of avian paramyxoviruses (APMV) are thought to arise from lentogenic strains, derived from wild birds. This study is aimed to assess AIV and APMV sensitivity of non-reservoir bird species and mammals, usually considered at low risk of infection. Birds samples (cloacal swabs and organ pools) collected during National Influenza A Monitoring Plan on wild birds in Northern Italy from september 2005 to april 2010, were tested for AIV and APMV-1 in our laboratory.

**Methods:** For virus isolation, performed only on bird species, samples omogenated in 1:10 w/v PBS with antibiotics were inoculated in SPF embryonated chicken eggs. The HA positive allantoic fluids were subsequent screened for AIV and A PMV, using specific antisera, in a differential haemagglutination inhibition test. For molecular testing, performed on all birds and mammals samples collected, viral RNA was extracted and analyzed by virus isolation in SPF embryonated chicken eggs and real time RT-PCR for the detection of AIV RNA (M gene).

**Results:** A total of 1294 wild birds samples, not including reservoir species as Anseriformes and Charadriiformes, were collected. For type A influenza virus just 1 cloacal swab was found positive in july 2007 from a *Fulica atra* (Gruiformes Order). For APMV-1 a total of 17 birds were found positive in the period considered. None of the 267 mammal samples divided in 208 *Sus scrofa*, 48 *Vulpes vulpes*, 11 *Stenella coeruleoalba* resulted AIV positive.

**Conclusions:** Evidence of the involvement of any mammal species analyzed in the ecology of AIV was not found during this study, but further investigations are needed to evaluate this potential risk.

## POSTER PRESENTATION 19

### Increasing trend of contact with hepatitis E virus in red deer from south-western Europe

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**Key words:** epidemiology, hepatitis E, red deer, reservoir, zoonosis

\*(oral presentation)

**Background:** Hepatitis E (HE) is an important public health problem caused by HE virus. An increasing number of HEV infections are being identified in industrialized countries where wild and domestic animals could act as reservoirs. The objectives of the study were to search for HEV in red deer (*Cervus elaphus*) from different areas of Spain by means of serology and PCR, and to describe HEV epidemiology and time trends. We hypothesised that red deer would show a widespread contact with HEV in the Iberian Peninsula.

**Methods:** Sera from 968 Iberian red deer were collected between 2000 and 2009 and tested for anti-HEV IgG antibodies by means of an ELISA. Eighty one individuals were tested for HEV detection by RT-PCR. Eight HEV RT-PCR positive samples were selected for sequencing.

**Results:** Overall, 101 sera (10.4%) were positive for IgG. No significant differences in HEV seroprevalence were observed between sex and age classes. Contact with HEV increased with time. Significant differences were also found for IgG seroprevalence considering management types. Eleven out of 81 samples were RT-PCR positive (13.6%). All deer sequences from this study belonged to genotype 3 and shared 99% nucleotide identity with Spanish domestic swine strains.

**Conclusions:** This is the first demonstration of HEV infection in Iberian red deer and confirms that HEV circulates actively among deer populations in Spain. Although it has been previously shown that red deer are susceptible to HEV, this is the first large serosurvey in this species in Europe. Data also shows an increasing prevalence trend in the last decade. Red deer in Spain are infected with HEV, although at lower rates than wild boar and domestic pigs, and a proportion of them have detectable blood levels of HEV RNA, and may act as a potential source of HEV infection in humans.

## POSTER PRESENTATION 20

### Long term surveillance in wild birds for AIV early detection

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**Key words:** influenza A virus, surveillance, wild birds

**Background:** Wild aquatic birds carry the entire variety of influenza A virus (AIV) subtypes, usually of low pathogenicity (LPAI), but some have gained virulence by mutation after transmission and adaptation to susceptible gallinaceous poultry. Since 2005, following CE directives, IZS PLV in coordination with Public Health Direction of Piedmont and Ce.R.M.A.S. has started the Avian Flu Control Plan on wild birds, based on active and passive monitoring. Here we are presenting up-to date sampling datas collected from september 2005 to april 2010.

**Methods:** For virological diagnosis we performed virus isolation in SPF embryonated chicken eggs and viral nucleic acid identification by one step Real time RT-PCR protocols for gene M AI, H5, H7 and H9 subtypes. The serological detection of H7 and H5 subtype antibodies was performed by inhibition of hemagglutination test.

**Results:** Between July and September 2007 we isolated 3 AIV from a mallard: H10N1 and H3N8 from organ pool, H5N2 LPAI from cloacal swab. Two mallard cloacal swabs resulted type A influenza RT-PCR positive. In august 2007 we found anti-H7 antibodies in the serum of 3 mallards. In february 2008 one mallard serum showed anti-H7 antibodies and in july, august we isolated subtype H4N6 from cloacal swabs of 2 mallards. One positive cloacal swab for type A influenza was found from a *Fulica atra* (Ord. Gruiformes) in 2009 and in february 2010 a H4N6 strain was isolated on eggs from a mallard intestine.

**Conclusions:** Influenza virus surveillance in wild birds could provide both "early warning" signals for AIV introduction in new regions and access to strain for characterization. Long term surveillance is crucial to gain understanding of LPAI viruses and their hosts ecology in order to assess the role of feral birds in the dynamics of infection in a densely populated poultry area.

## POSTER PRESENTATION 21

### Pathology of an Experimental West Nile Virus Infection of Red Legged Partridge (*Alectoris rufa*)

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**Key words:** West Nile virus; Red legged partridge; Experimental infection; Mediterranean species; Histopathology

**Background:** West Nile virus (WNV) is an arthropod-borne virus of the genus *Flavivirus*, family *Flaviviridae*, of the Japanese-Encephalitis virus complex. It cycles naturally and silently between birds and mosquitoes, with horses and humans as accidental host. Recently it has emerged as zoonotic pathogen in Europe, Africa and the North American continent. In Europe, it has demonstrated pathogenicity in wild birds, mainly raptors. The red legged partridge is an autochthonous Mediterranean species, with an important economic and ecological value, that is frequently raised in outdoor operations. An experimental study was set up in order to evaluate the susceptibility of the species to WNV infection and disease, and its possible role as a reservoir. This study evaluates differences in lesion distribution and severity using two different Mediterranean isolates, with demonstrated different pathology for mice

**Methods:** Twenty-eight birds were divided in two groups and inoculated, subcutaneously, with 104 UFP/0.1ml DMEM of two strains of West Nile Virus isolated in Spain (Spain 2007 (Sp07) and Morocco 2003 (Mo03)). Tissue samples were collected during necropsies of naturally dead and euthanized animals, fixed in 10% neutral-buffered formalin and processed for histopathologic examination.

**Results:** Macroscopic lesions were limited in both groups and included ascites, enlarged liver and kidneys, pallor of the myocardium, and liver and brain congestion. Histologically we noted multiorganic involvement. Both groups had congestion and perivascular edema in the brain and cerebellum, pulmonary congestion and inflammation, myocardial necrosis and myocarditis and lymphoid depletion in the spleen and bursa. Numerous necrotic foci related to mixed inflammatory infiltrates were detected in the liver, spleen, intestine, pancreas and kidney mostly in partridges infected with Mo03.

**Conclusions:** The red legged partridge proved susceptible to WNV infection and developed lesions similar to those described in other birds. Higher pathogenicity of the isolate Mo03 was reflected by more severe lesions and involvement of the kidneys and gastrointestinal tract.

## POSTER PRESENTATION 22

### Pattern of influenza virus attachment in the lower respiratory tract of marine mammals

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**Key words:** Marine mammals, Influenza, Pattern of viral attachment, lower respiratory tract

Influenza virus infections in marine mammals range from single cases to large epidemics, and from subclinical to fatal infections. In humans, the pattern of virus attachment (PVA) partly determines efficiency of transmission and pathogenicity of influenza virus infections. Specifically, efficient transmission is associated with attachment to upper respiratory tract and trachea, while high pathogenicity is associated with attachment to bronchioles and alveoli. To study the effect of PVA on these factors in marine mammals, we collected respiratory tract tissues from trachea to alveoli from harbour seals, grey seals, harbour porpoises, and bottlenose dolphins (n = 3 per species). By use of virus histochemistry, we determined the PVA to these tissues of avian influenza viruses (A/H4N5 and A/H7N7) and human influenza viruses (A/H1N1, A/H3N2, and B).

Our results showed that avian influenza viruses attached most abundantly to trachea and bronchi of harbour and grey seals, and to alveoli of harbour porpoise and bottlenose dolphin. Human influenza B virus attached to trachea of harbour and grey seals, but not to trachea of harbour porpoise and bottlenose dolphin. Human influenza virus attached poorly to trachea of all four species, and variably to lower parts of the respiratory tract.

These results correspond in part with observations of influenza in marine mammals. The PVA of avian influenza viruses and human influenza B virus corresponds with the recorded epidemics of these viruses in harbour seals, and with their absence in harbour porpoise and bottlenose dolphin. Also, the failure of human influenza A viruses to attach to trachea fits with the absence of records of in these marine mammal species. In conclusion, knowledge of the PVA to respiratory tract contributes to our understanding of the variation in transmission efficiency and pathogenicity of influenza in marine mammals.



## POSTER PRESENTATION 23

### Raccoon Rabies Research Using Remote Download GPS Collars in an Urban Environment

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**Key words:** GPS, Ohio, rabies, raccoon, urban environment

**Background:** In 2004 raccoon variant rabies moved west from the state of Pennsylvania into Ohio, USA. To prevent further spread, USDA/Wildlife Services expanded the Oral Rabies Vaccination (ORV) boundary west toward Cleveland, Ohio. To assist the ORV Program to better understand how rabies might move through an urban area such as Cleveland, and to help develop the best vaccination strategy to stop its spread we studied raccoon movements in urban areas of Cleveland. Raccoons may inhabit culverts or sewer pipes that are inaccessible to humans. As a result, transmitters programmed to drop off or requiring recapture may be lost and the data never recovered. To combat this problem we used remote-download GPS collars, which allowed us to retrieve data without collecting the collar.

**Methods:** Raccoons were live-captured in cage traps and chemically restrained. Lotek 7000 SLU GPS collars were attached. The GPS was programmed to take a nightly location at 2300 hours. Locations were downloaded every six weeks using a three-element UHF antenna and entered into ArcView v3.3. Ninety-five percent fixed kernel home ranges were calculated.

**Results:** Ten raccoons were radio collared. One raccoon went missing shortly after release. We obtained 1506 locations from nine individual animals. Average home range was 19.2 ha (range 0.8-63.1 ha). GPS fix success rate was 84%.

**Conclusion:** Raccoons appear to be maintaining discrete home ranges within patches of forested habitat. This includes small "green belts" and individual trees surrounding urban homes. We suggest ORV baiting take place in patches of available raccoon habitat in Cleveland versus broadcast baiting over wide areas in urban environments. Use of remote-downloading GPS collars in urbanized landscapes appears well-justified.

## POSTER PRESENTATION 24

### Results of an ongoing study on the role of wild boars as an infection source for domestic animals and humans

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Artois, Mark<sup>4</sup>; Sofia, Marina<sup>5</sup>; Yon, Lisa<sup>6</sup>; Birtsas,

Periklis<sup>7</sup>; Hutchings, Mike<sup>8</sup>; Sokos, Christos<sup>1</sup>; Iacovakis,

Christos<sup>1</sup>; Valiakos, George<sup>1</sup>; Gavier-Widen, Dolores<sup>9</sup>;

Giannakopoulos, Alexios<sup>5</sup>; Leontides, Leonidas<sup>5</sup>; Billinis,

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<sup>9</sup>National Veterinary Institute

**Key words:** wild boars, epidemiology, infectious diseases

We report the first results from an ongoing survey in Greek wild boars for multiple viral and bacteriological pathogens known to affect wild and domestic animals and/or humans. Blood and organ samples were collected from 150 wild boars (*Sus scrofa*) shot during the hunting seasons of 2006 - 2008. So far, sera have been tested by enzyme-linked immunosorbent assay for the presence of antibodies against porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type - 2 (PCV-2), H1N1 influenza virus, H3N2 virus, Aujeszky virus, *Actinobacillus pleuropneumoniae* (AP), *Mycoplasma hyopneumoniae* (MHY), *Salmonella suis*, *Mycobacterium paratuberculosis*, *Chlamidophila abortus*, *Neospora caninum*, *Toxoplasma gondii* and *Trichinella spiralis* and for BVDV antigen. Further, tissue samples were examined by PCR for *Mycobacterium bovis* and PCV-2. Additional PCR tests will be done for BVDV, *Mycobacterium paratuberculosis* and *Toxoplasma gondii*.

Antibodies against PCV-2, Aujeszky, Salmonellosis, *Toxoplasma gondii*, AP and *Trichinella spiralis* were detected in 24%, 21%, 4%, 3% 76% and 3% of the tested sera, respectively. BVDV antigen has been found in 89% of wild boars. All sera were negative for the presence of antibodies against PRRS, H1N1 virus, H3N2 virus, MHY, *Mycobacterium paratuberculosis*, *Chlamydomphila abortus* and *Neoplasma caninum*. Tissue samples tested by PCR for *Mycobacterium bovis* were negative, while twenty percent of those tested for PCV-2 were positive.

These results indicate that wild boars may be carriers of several pathogens such as Aujeszky, Salmonellosis, *Toxoplasma gondii*, AP and *Trichinella spiralis* for domestic pigs in regions where domestic pig farms neighbor with hunting areas. Further, the results of this ongoing survey suggest that wild boars may be involved in the epidemiology of BVDV. Seropositivity in wild boars may be ascribed

to either the existence of another pestivirus or the likely reservoir role of wild boars for BVDV. Additionally, wild boars may act as a reservoir for PCV-2.

The research leading to these results has received funding from the European Union Seventh Framework Programme (2007-2013) under grant agreement n° 222633 (WildTech).

## POSTER PRESENTATION 25

### **Seasonal ecology and epidemiology of low pathogenic avian influenza virus of migratory waterfowl in Alaska.**

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**Key words:** influenza, seasonal, Alaska, epidemiology

Viral segments of avian origin have been present in all major human pandemic viruses identified since the early 20th century. Most studies of avian viruses in wild birds take advantage of behavioral patterns that permit sampling of large numbers at specific times and locations during annual migratory patterns. However, the development of useful models for understanding and predicting the contribution of avian viruses or birds as vectors of pandemic human viruses will require a more detailed understanding of viral dynamics within communities of migratory waterfowl. Toward this end we have undertaken a long-term study of avian influenza at Minto Flats State Game Refuge in Interior Alaska. This study aims to sample waterfowl in each life-history stage of the breeding season, from their arrival in Alaska during spring to their migratory departure in fall. A variety of capture methods are being employed to examine within- and among-season variation in viral prevalence, and assess relationships between infection status and body condition, survival, and reproductive success in multiple duck species. Cloacal and oropharyngeal samples are screened by RTPCR for the presence of influenza. Positive samples are inoculated into embryonating chicken eggs and cultured samples sequenced to determine viral subtype. Our results thus far are consistent with the expectation that viral prevalence is highest in hatch-year dabbling ducks during fall staging in August. However, the subtype composition of viral infection changes over time and, contrary to current dogma, we have also detected infection in very young birds and in after-hatch-year adults during the pre-nesting and nesting stages of May and June. Our results indicate that a proportion of hatch-year birds are becoming infected as ducklings, prior to fall staging, and that viral transmission and local epidemiology may depend in part on the composition of viruses present in the early stages of the breeding season.

## POSTER PRESENTATION 26

### Survey of several zoonotic pathogens in domestic ducks of a public park from Madrid (Spain)

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**Key words:** zoonotic, duck, pathogens

Duck populations, very common in urban parks and gardens, may carry zoonotic pathogens representing a health risk for humans. A field study to detect pathogenic agents in feral ducks was conducted in a Park of the city of Madrid. The targeted agents were: Enterobacteriaceae, Campylobacter jejuni and C. coli, Chlamydomphila psittaci, Newcastle disease virus (NDV) and Avian Influenza virus (AIV).

During November 2009 eight domestic ducks were captured and sampled. Tracheal swabs for AIV and cloacal enema were collected for all determinations.

Detection of Campylobacter jejuni and C. coli was determined by a multiplex PCR previously enriched in Bolton broth. Chlamydomphila psittaci was detected by real time PCR based on TaqMan<sup>®</sup> probe, and AIV and NDV were analysed with two different real time RT-PCR tests based on TaqMan<sup>®</sup> probe. Enterobacteriaceae, including Salmonella spp., were isolated in a specific media and identified by biochemical strips (RapId One, Oxoid<sup>®</sup>).

The most frequent agents found were: Escherichia coli (6/8), AIV (4/8), Salmonella spp. (1/8) and C. jejuni (1/8). Other Enterobacteriaceae found were: Enterobacter sakazakii (1/8), Klebsiella oxytoca (1/8) and Citrobacter freundii (1/8). All of the AIV were serotyped by sequencing and were classified as low pathogenic AIV. No individuals were positive to Chlamydomphila psittaci, Campylobacter coli and Newcastle Disease Virus.

The results obtained confirmed the presence of potentially zoonotic pathogens in feral ducks from parks and gardens. Periodical surveys and epidemiological studies should be performed to establish the risk of transmission to the human population that visit public parks and gardens.

This work was partially funded by the Council of Madrid and the Agreement INIA-MARM CC08-020.

## POSTER PRESENTATION 27

### West Nile Virus in a key wild bird species of the Mediterranean ecosystem: Pathogenicity of two recent Western Mediterranean West Nile virus isolates in experimentally infected red-legged partridges

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**Key words:** West Nile virus, experimental infection, red-legged partridges

**Background:** West Nile Virus (WNV) is a Flavivirus that is maintained in a bird-mosquito transmission cycle and accidentally infects humans, horses and other mammals. It has recently reemerged as zoonotic pathogen in Africa, the Middle East, Asia, and southern Europe, and was introduced into North America in 1999. There, native bird species are highly susceptible to WNV infection with high mortalities. Especially birds of prey have sporadically also been affected by WNV in Europe. The red-legged partridge (*Alectoris rufa*) is a gallinaceous bird indigenous to the Iberian Peninsula. It plays a key role in the Mediterranean ecosystem and constitutes an economically important game species being frequently raised in outdoor operations.

**Methods:** Two groups of ten red-legged partridges each were experimentally infected with two different WNV isolates from the Western Mediterranean: Morocco/2003 and Spain/2007. Three non-infected partridges were contacts to each group and eleven partridges were kept as controls. We evaluated clinical outcome, duration and intensity of viremia, virus shedding, persistence in organs, contact transmission, and serologic response to WNV.

**Results:** Clinical signs were observed in all inoculated birds but were more severe and started one day earlier in partridges inoculated with Mo03. Mortality occurred between 5dpi and 9dpi and was 70% in partridges inoculated with Mo03 and 30% in birds infected with Sp07. Partridges from both groups showed mosquito-infective viremia between 2-5dpi. Virus could be detected in all organs evaluated between 3-8dpi. Oral and cloacal shedding of WNV was observed 3-7dpi, while in feather pulp virus was detected until 14dpi. Antibodies against WNV were detected by cELISA from 6dpi. Neutralizing antibodies were detected from 10dpi. No contact bird showed clinical signs, seroconverted or tested positive for WNV genoma

**Conclusions:** These results demonstrate the susceptibility of the red-legged partridge to WNV infection and clinical disease, and their potential role as reservoir.

## POSTER PRESENTATION 28

### West Nile virus infection and survival of semi-captive lesser scaup (*Aythya affinis*) ducklings

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**Key words:** West Nile virus, survival, ducklings

**Background:** Intraspecific variation in disease-associated mortality is well-documented in wildlife populations and provides a foundation for understanding epidemiological processes. For example, temporally variable exposure and susceptibility to disease-causing agents may subject offspring of seasonally-reproducing organisms to changing disease risk as the breeding season progresses. However, temporal changes in disease risk have received little attention in studies of seasonally declining offspring survival.

**Methods:** In 2007, we recorded West Nile virus (WNV)-associated mortality in semi-captive lesser scaup (*Aythya affinis*, hereafter, scaup) ducklings in Saskatchewan, Canada. We used data obtained from this outbreak to estimate daily survival rates (DSR) for 34 ducklings using known-fate survival models and to assess the importance of number of days post-release (age; maximum 28 days), hatch date, and immunogenic challenge. WNV and vector monitoring data were obtained from the local health agency to measure temporal patterns in risk of infection.

**Results:** In the best-approximating model, the number of days post-release was organized by 7 4-day periods; survival was highest in the three periods (DSR > 0.93) following release and lowest in the last two periods (DSR < 0.74). An advantage of early hatching was detected (Beta = -0.882, 95% confidence interval = -1.41 to -0.35). However, survival was unrelated to immune challenge response. Furthermore, prevalence of WNV-infected mosquito vectors tracked closely the timing of duckling mortality.

**Conclusions:** This study demonstrated that survival of scaup ducklings was inversely related to hatch date during a WNV outbreak and identified a proximate variable (prevalence of infected vectors) that could partially explain this pattern. Our findings are consistent with the hypothesis that seasonally variable exposure to disease could affect patterns of offspring survival in wildlife populations.

## 3. PARASITIC ZOOONOTIC AND WILDLIFE DISEASES

### POSTER PRESENTATION 29

#### Are rats reservoirs for *Trichinella spiralis*?

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Grasset, Aurélie<sup>1</sup>; Vallée, Isabelle<sup>2</sup>; Boireau, Pascal<sup>2</sup>;

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**Key words:** *Trichinella spiralis*, rat, experimental infection, Mathematical model

**Background:** Trichinellosis is a parasitic zoonotic disease caused by *Trichinella* spp. and transmitted by eating raw or undercooked meat with muscle larvae (ML) of infected omni-or carnivore animals. Eleven different *Trichinella* species are known, and of these *T. spiralis* has a worldwide distribution. *T. spiralis* has been isolated from 11 mammalian host species in Europe. The diversity of susceptible wildlife species does not mean that all species are equally important for the maintenance of the parasite's life cycle. We studied the hypothesis that rats might act as a reservoir species and we studied rats as a proxy for wildlife sustainability of *Trichinella*.

**Methods:** We developed a model for dose-dependent infection of *T. spiralis*. Infection is defined as the establishment of ML in the muscle cells as a result of successful mating of adults in the intestine and is detected by the digestion of muscle samples. To augment the model, infection experiments were performed using doses between 10 ML and 16,000 ML and the larvae per gram (LPG) of 66 rats in total were quantified. A series of events in which a new host ingests meat containing ML was simulated by means of a Monte Carlo simulation. Results are compared with a novel mathematical approach that characterizes the state of parasite persistence from the experimental infection study.

**Results:** All rats that were inoculated with a low dose of 10 ML were infected. LPG increased non-linearly with doses reaching initially a constant mean between doses of 400 and 2,000 ML. LPG increased further to reach a higher mean between doses of 8,000 ML and 14,000 ML. In the simulated between-host dynamics, most rats were infected with ca. 200 LPG. When intense cannibalism is assumed, intermittent bursts of heavily infected rats (ca. 6,000 LPG) were observed. When 1.4g or less meat is consumed, the parasite disappeared eventually from the rat population.

**Conclusion:** *T. spiralis* transmission persists in a population of rats when they cannibalize their own species.

## POSTER PRESENTATION 30

### A case report of *Eimeria gilruthi* infection in Laristan mouflons (*Ovis orientalis laristanica*)

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**Key words:** Laristan, *Eimeria gilruthi*, abomasal coccidiosis

**Background:** During routine necropsies in four Laristan mouflons (*Ovis orientalis laristanica*) from a captive population in Qatar, macroscopic changes of the abomasal mucosa have been detected. Further investigation revealed *Eimeria gilruthi*, also described as *Globidium (Gastrocystis) gilruthi* as cause of the changes. *E. gilruthi* has been described in sheep and goats, but not in wild species until now. This study describes the macroscopic, cytological and histopathological findings of abomasal coccidiosis in four necropsied Laristan mouflons.

**Methods:** After the necropsy of all dead animals, faecal samples were examined for parasites and a sample was sent for sporulation and McMaster to identify coccidia species. In addition impression smears of abomasal changes were taken for cytological examination and tissue samples were collected for histopathological investigation.

**Results:** All animals showed macroscopic changes in the mucosa of the abomasum ranging from congestion to ulcerations and cyst-like structures. In the abomasal impression smears spindle-shaped merozoite like structures were found. Histopathology results revealed several large protozoal developmental stages (schizonts) within the mucosa, lymphoplasmatic infiltration of propria and severe edema and infiltration with lymphocytes and plasma cells of submucosa. Further, sedimentation and flotation of faeces demonstrated the presence of coccidian-eggs. Sporulation revealed the following *Eimeria* species: *E. ovinoidalis* (51.5%), *E. bakunensis* (18.5%), *E. faurei* (17%), *E. parva* (10.5%), *E. crandallis* (1.5%), *E. intricata* (0.5%) and *E. pallida* (0.5%).

**Conclusions:** This is the first description of *E. gilruthi* in Laristan mouflons. Even though abomasal coccidiosis is described as mostly non-pathogenic in domestic species, obvious macroscopic lesions were found in the abomasum accompanied by developing stages of the parasite. Although not the primary cause of death, it may have supported an infection with other agents and could lead to death in stressed or immunosuppressed animals.

There is only little known about *E. gilruthi*; the only stage found until now are giant schizonts in abomasums and sometimes duodenum in necropsies; faecal examination can not reveal an infection with *E. gilruthi*. Therefore further biomolecular investigations of *E. gilruthi* would be necessary to get a better understanding about the life-cycle, systemic classification, potential pathogenicity and diagnostic in living animals.

## POSTER PRESENTATION 31

### Clinical evaluation and specific immune response in domestic rabbits (*Oryctolagus cuniculus*) after experimental infestation with the mite *Sarcoptes scabiei*

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**Key words:** rabbit, mange, experimental infestation, IgG, ivermectin

**Background:** A Sarcoptic mange epizooty has recently been reported for the first time in the European wild rabbit in Majorca (Balearic Islands, Spain) and other areas of NE Spain, which may be a threat to the conservation of the wild rabbit and their predators.

**Methods:** To improve knowledge of the host-sarcoptes immunological interaction six domestic rabbits were experimentally infested by direct contact for 48 h with rabbits infested with *S. scabiei* var. *cuniculi* (obtained from a naturally infested wild rabbit from Majorca). At week 8 post-infestation (PI) two of the rabbits were treated with ivermectin to assess the effect of treatment on serum antibody profiles. Clinical signs were examined and antibody levels (IgG) assessed using an ELISA based on a recombinant antigen.

**Results:** After initial infestation an increase in IgG levels (above the cut off value 0,030) was detected between weeks 4 and 6 PI, which continued to progressively increase until about one week before death when the levels had remained constant or had begun to drop slightly. Mange lesions first appeared at week 6 PI as crusts in the hind and forelimbs, starting at the root of the claw and advancing up the paw. Crusts in the nostrils and ears were observed in two rabbits. A couple of weeks before death body condition deteriorated with apparent weight lost.

Following ivermectin treatment antibody levels decreased drastically (about 86.2% of the IgG present in serum disappeared), although levels never went down to pre-infestation levels. Mange lesions disappeared progressively and there were no visible lesions of mange by three weeks post-treatment.

**Conclusion:** We have shown that sarcoptic mange is a potential threat to rabbit populations, capable of causing rapid death. Antibody levels increased throughout the experimental infestation but were unable to control infestation. Treatment of infested rabbits with ivermectin controlled lesions and allowed full recovery.

## POSTER PRESENTATION 32

### Cluster of cysticercosis (*Taenia pisiformis*) in European Brown hares in Northern Italy

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<sup>1</sup>IZSLER; <sup>2</sup>ATC BO 1

**Key words:** Cysticercosis, European Brown hares, Northern Italy

**Background:** The cestode *Taenia pisiformis* (Bloch, 1780) occurs in the small intestine of domestic and wild carnivores (dog, fox and rarely in the cat). The intermediate hosts are lagomorphs, primarily rabbits and hares but also rodents. In this work the Authors report a cluster of cysticercosis due to *Cysticercus pisiformis* in hares from an area where this pathology has been previously found sporadically.

**Methods:** Between October 2008 and February 2010 47 brown hares, hunted or found dead in north area of Bologna province, were collected and submitted to necropsy at the laboratories of IZSLER. Furthermore, 8 foxes from the same location were necropsied and checked for the presence of intestinal helminths. Cysticerci and adult taenids were morphologically identified following standard taxonomic keys.

**Results:** At necropsy, 20 (42.5%) hares from eight municipalities were found infested by *C. pisiformis*. These animals showed considerable weight loss and bacteriological and virological investigations showed negative results. Cysticerci were found in liver, abdominal cavity and mesentery of the lower digestive tract. All the animals were infested by more than 20 cysticerci. Most of the cases (10/20) were located in two neighbouring municipalities. Only 1 fox out of 8 examined carried two adults of *T. pisiformis* in the small intestine. The other 7 foxes were infested by *Mesocestoides lineatum* and *Toxocara canis*.

**Conclusions:** The increased prevalence of *C. pisiformis* in the study area may have different causes such as: level of environment contamination, dispersion of eggs, egg survival, age and immune response of the host, as well as densities of both definitive and intermediate hosts. In the study area, the hare population decreased of 39,4% in 2008-2009, whilst the fox population increased. Furthermore the practice allowing dog to eat the viscera of game animals may affect the prevalence of infection in the hare population.

## POSTER PRESENTATION 33

### Demonstrating freedom from *Echinococcus multilocularis* in Sweden, Norway and Finland

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**Key words:** *Echinococcus multilocularis*; Surveillance; Documenting disease freedom

**Background:** *Echinococcus multilocularis* (EM) is an emerging zoonotic parasite in Central Europe. Introduction of the parasite to previously disease free areas, like Svalbard in Norway and Hokkaido in Japan, has also occurred. At present, five EU member states, including Sweden Norway mainland and Finland, consider themselves free from EM and national requirements for dogs and cats to be treated against EM before entering the country are in place. However, the EU Commission has indicated that due to the cost and inconvenience these requirements are considered disproportionate.

To be able to keep the present legislation there is a need to document the probability of freedom from EM.

**Methods:** Probability of disease freedom was estimated using a methodology for objective quantitative analysis of multiple complex data sources. The model was adapted to include surveillance of several different species, thereby requiring definition of separate design prevalences for each species. Survey data from different surveillance systems as regards EM in foxes, rodents, out-door pigs, wild boars, dogs and humans from each country (Sweden, Norway and Finland) was collected from 1st January 2000 to 31st December 2009. Because data on the combined surveillance system sensitivity for humans was not possible to obtain, the contribution of this surveillance system component was included in the model as an increased prior probability of freedom in year 2000.

**Results and Conclusion:** Preliminary results of the model will be presented. Relevance of documenting disease freedom on country basis for EM as well as strengths and weaknesses of the present model, especially the inclusion of different species, will be discussed. The definition of design prevalences will also be discussed as the output of the model is highly dependent on this parameter.

## POSTER PRESENTATION 34

### Detection of *Toxoplasma* antibodies in naturally exposed red foxes (*Vulpes vulpes*) in south-east France

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**Key words:** Toxoplasmosis, Fox, Epidemiology, France,

**Background:** Toxoplasmosis caused by the coccidian parasite *Toxoplasma gondii* is one of the most widespread parasitic infections of warm-blooded animals, including humans.

Nearly one third of humanity has been exposed to this parasite. The infection is usually common in wild carnivores.

In order to assess the circulation of *Toxoplasma gondii* in the ecosystem of the vast Canjuers military camp, south-east France, we carried out a serological survey in red foxes.

**Methods:** In 2008 and 2009, muscle samples were collected from 47 foxes killed during operations aiming at regulating their number, in the military camp of Canjuers. During the necropsies, gender (male or female) and age (young or adult) were recorded. The samples were kept frozen at -20°C until being processed. In the laboratory, a multi-species serological test, ID Screen Toxoplasmosis Indirect® (IDVET, Montpellier, France), was applied on fluids (after ½ dilution) obtained from the muscle samples.

**Results:** *Toxoplasma gondii* antibodies were evidenced in 29.8% (14/47) (CI 95%: 17.3-44.9) of the animals tested. No significant difference was observed according to gender but the prevalence in adults (55.6%) was significantly higher than in young animals (13.8%) (Khi2=9.3 ; p<0.002).

**Conclusions:** Having a carnivorous diet, red foxes are highly exposed to this common parasite. In this particular ecosystem, infection of red foxes can occur by ingestion of meat from domestic (sheep...) and more probably wild animals (rodents, wild boars, roe deers, birds...) containing muscular cysts. Other routes of transmission include cannibalism, transplacental infection and ingestion of surface water containing oocysts shed in felid feces. The higher prevalence in adults could result from a continuous exposure during lifetime. *Toxoplasma* infection of red foxes illustrates the circulation of the parasite in this ecosystem and further investigations are required to better understand the complex epidemiological features of the parasitic cycle.

## POSTER PRESENTATION 35

### Development of a pcr assay to identify *Baylisascaris transfuga* in bear faecal samples

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**Key words:** *Baylisascaris transfuga*, Bear, *Ursus arctos*, Ascarids.

The intestinal nematode genus *Baylisascaris* infects a variety of mammals and can pose a zoonotic risk. The species that infects raccoons, *B. Procyonis*, is a serious health concern as it is responsible for ocular, visceral and neural larva migrans in more than 100 species, including humans. The species from bears, *B. transfuga*, has a wide geographical and host range. This parasite is traditionally identified via morphological evaluation of adult nematodes, eggs and larvae. The development of a highly specific molecular assay to rapidly identify *B. transfuga* would be helpful in monitoring the prevalence of the parasite in the field. In this study, a PCR-based assay for detection and identification of *B. transfuga* eggs in bear faeces was developed. Ninety six faecal samples were collected from wild brown bears (*Ursus arctos*) in Croatia and first analyzed by sedimentation/flotation. Using these methods, *Giardia*, *Eimeria*, *Cryptosporidium*, ciliates, *Syngamus*, strongyle eggs and larvae and *B. transfuga* eggs were identified. An efficient method for DNA extraction from *B. transfuga* eggs was developed and the PCR optimized using DNA extracted from *B. transfuga* adults. The PCR detected a 301-bp *Baylisascaris* specific product from both adult *B. transfuga* and faecal samples spiked with *B. transfuga* eggs. It is anticipated that this molecular tool could be used to monitor the prevalence of the parasite in wild bear populations and may be used to indicate the actual zoonotic risk for human operators who get in contact with brown bears and bear faeces.

## POSTER PRESENTATION 36

### Echinococcosis in an European beaver (*Castor fiber*) in Austria

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**Key words:** beaver, echinococcosis

Echinococcosis is a mostly fatal helminthic zoonosis in humans due to the infection with the larval stages of *Echinococcus* species. The disease is endemic in Europe and the red fox (*Vulpes vulpes*) is considered the main reservoir. In urban Vienna the prevalence of *Echinococcus multilocularis* in foxes is approximately 1%, for the rural areas it is suspected to be as high as 35%. As most important alternate hosts vole species and muskrat (*Ondatra zibethicus*) are discussed, but many more species are suspected. There are various reports of other accidental host species.

An approximately 2 years old, male, European beaver (*Castor fiber*) was presented at the pathology department of the Research Institute of Wildlife Ecology, Vienna (FIWI). Although the beaver was rather light (13,4 kg), the abdomen of the animal appeared big and pear shaped. Necropsy revealed a highly enlarged liver (3,33 kg) with multi focal to coalescing cysts. Only some 5 – 10% of the original liver tissue was still present. All abdominal organs were massively covered with fibrinogenous material. The heart and lungs were atrophic most probably due to the pressure from the enlarged liver. Tissue samples taken at necropsy were fixed in 7% buffered formalin and embedded in paraffin wax. For pathohistological examination, tissue sections were cut at 3 µm thickness, mounted on glass slides and stained with hematoxylin and eosin (H&E) according to standard procedures. Several cysts, calcareous corpuscles and parts of the parasite were seen. PCR for species differentiation is currently ongoing. This is the first report of echinococcus in a beaver in Austria.

## POSTER PRESENTATION 37

### Findings and investigations on the trematoda, *Alaria alata* (Goeze, 1792) in France

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**Key words:** *Alaria alata*, Trematoda, Trichinella, Boars

*Alaria alata* (Diplostomidae, Trematoda) is a cosmopolite parasite found in red foxes (*Vulpes vulpes*), the main definitive host in Europe. In contrast only few data are reported in wild boar (*Sus scrofa*) a paratenic host. Recently, several countries have discovered this parasite in boars (Germany, Romania, Croatia). Two countries have decided to consider these boars as unfit for human consumption in front of a potential zoonotic risk (Germany, Switzerland). American *Alaria* species have been reported as causal agent of illness and death in human. However little is known about *Alaria alata* in Europe. In France, since a recent report in 2003, more than a hundred detections in boars are made every year. The aim of this oral communication is to describe the importance and distribution of *Alaria alata* mesocercariae in this paratenic host .



## POSTER PRESENTATION 38

### First evidence of hemoplasma infection in free-ranging namibian cheetah (*acinonyx jubatus*)

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**Key words:** cheetah, free-ranging, hemoplasma, hemotropic mycoplasma, Namibia

**Background:** Hemotropic mycoplasmas (hemoplasmas) are the causative agents of feline infectious anemia; they can induce acute hemolysis associated with anorexia, lethargy, dehydration, weight loss and sudden death in domestic cats. Hemoplasma infection has been documented worldwide in domestic and free-ranging cat species with high prevalence in Iberian lynxes (*Lynx pardinus*), Eurasian lynxes (*Lynx lynx*), European wildcats (*Felis silvestris silvestris*), African lions (*Panthera leo*) in Tanzania and domestic cats in South Africa. Feline hemoplasma infection may be associated with infections of feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV). Canine distemper virus (CDV) was frequently detected in hemoplasma-positive lions. The prevalence of hemoplasma has not yet been investigated in free-ranging felids in southern Africa.

**Methods:** In this study we screened 73 blood samples from 61 cheetahs in central Namibia for presence of hemoplasmas using quantitative real-time PCR.

**Results:** Only one of the cheetahs, a CDV antibody positive female, tested PCR-positive. Phylogenetic analysis based on partial sequencing of the 16S rRNA and RNaseP gene revealed that the isolate belongs to the *Mycoplasma haemofelis/haemocanis* group.

**Conclusions:** This is the first molecular evidence of a hemoplasma infection in a free-ranging cheetah. We currently have no evidence to suggest that hemoplasmas are a threat to the cheetah population in central Namibia because prevalence was low, no clinical sign of hemoplasma infection was apparent in any of the cheetahs, the hemoplasma and CDV positive female lived for 4 years post sampling, and the potential deteriorating effect of co-infection, e.g. with FIV, FeLV and CDV, is low due to the low prevalence of the latter infectious agents among cheetahs in central Namibia.

## POSTER PRESENTATION 39

### First finding of trichinella infection in baltic grey seal *halichoerus grypus*

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**Key words:** *Trichinella*, grey seal, Baltic Sea

**Background:** Zoonotic nematode parasites of the genus *Trichinella* are known to infect marine mammals in arctic seas. Several seal species reportedly occasionally harbour *Trichinella*, e.g. bearded seal *Erignathus barbatus*, hooded seal *Cystophora cristata* and ringed seal *Phoca hispida*. The Baltic Sea is a subarctic sea inhabited by grey seals *Halichoerus grypus*, Baltic ringed seals *P. h. botnica* and harbour seals *Phoca vitulina*. The grey seal population has increased in the northern part of the sea in the last decade and limited hunting is now legal in Finnish and Swedish waters. Interest in the use of seal meat is also increasing which requires active surveillance of zoonotic diseases in grey seals. *Trichinella* infection is of special interest because of the parasite's high prevalence in terrestrial wildlife.

**Methods:** Muscle samples (mainly tongue) were collected from hunted grey and ringed seals or in few cases seals found dead during 2008-2009. From each animal, 20 g of muscle was examined by digestion method. Samples were pooled in batches of 5 and when positive result was obtained, confirmatory samples were digested again individually. The species of *Trichinella* larvae was identified by multiplex-PCR. **Results:** A total of 169 grey seals were examined and 1 (0.6%) was positive with a density of 0.2 lpg. *T. nativa* species was identified from a 13 year-old female grey seal. All 59 samples of ringed seals were negative.

**Conclusions:** This is the first finding of *Trichinella* infection in the Baltic grey seal, and to our knowledge, first finding of natural infection in grey seals worldwide. *Trichinella* spp. are very prevalent in Finnish wildlife, especially in red foxes *Vulpes vulpes* and raccoon dogs *Nyctereutes procyonoides*. Spill-over from the usual terrestrial hosts to rarer host species, such as seals and birds, is not unexpected. Meat inspection of seals should always include *Trichinella* examination.

## POSTER PRESENTATION 40

### Infection of red foxes (*Vulpes vulpes*) by *Trichinella britovi* in south-east France

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**Key words:** *Trichinella britovi*, Fox, Epidemiology, France,

**Background:** Nematodes of the genus *Trichinella* are one of the most widespread zoonotic pathogen around the world but local epidemiological cycles are not always well known.

In 2003, a focus of trichinellosis (*Trichinella britovi*), due to the consumption of underdone wild boar meat, was reported near the military camp of Canjuers, Haut-Var area, south-east France. As wild carnivores concentrate *Trichinella* spp, we conducted a longitudinal survey on the fox population of this area, in order to assess the circulation of the parasite.

**Methods:** From 2006 to 2009, diaphragm pillars muscle samples were collected from 108 red foxes killed in the camp of Canjuers, by a military hunting society authorized to regulate their population. In the laboratory, *Trichinella* larvae were evidenced using an artificial digestion technique (European Commission, Directive 2075/2005). Then larvae species found in positive samples were identified using a multiplex PCR test.

**Results:** Prevalence of *Trichinella* infection in red foxes was 2.7 % (3/108). The identified parasite was *Trichinella britovi*. The three infested foxes had a low parasitic load: 2.4 and 0.8 larvae/g (LPG) respectively in two foxes screened in 2007 and 0.5 LPG in a fox screened in 2008.

**Conclusions:** *T. britovi* is the etiological agent of sylvatic *Trichinella* infection in temperate regions of Eurasia. This species has also been detected in wolves, brown bears, jackals, raccoon dogs, mustelids, sylvatic and domestic swine, brown rats and horses. Having a predominantly carnivorous diet, the red fox concentrates the parasite in a given ecosystem. As micro-mammals (fieldmice...) represent 95 % of its intake, they could play a role in the transmission among the red fox population, as well as cannibalism. Additional epidemiological surveys are required to fully understand the local natural cycle of the parasite and the risk for humans.

## POSTER PRESENTATION 41

### Outbreak of coccidiosis in a red squirrel (*sciurus vulgaris*) spanish breeding center

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Neves, Elena<sup>1</sup>; Nogal, Veronica<sup>1</sup>; Muñoz, Maria Jesus<sup>1</sup>

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**Key words:** red squirrel, coccidiosis, pathology

**Background:** A decline of red squirrel populations from some public parks of Madrid has been observed during the last two decads. To reinforce these populations a captivity breeding program has been established since 2004. Since December 2008 to May 2009, nine of 20 squirrels died.

**Methods:** Nine red squirrels were submitted for necropsy. Intestinal contents were analyzed for parasite testing. Tissues from seven squirrels were collected in 10% buffered formaline for histopathological diagnosis.

**Results:** Gross pathology reveals emaciation (9/9) with a severe mite infestation (8/9). The perineal region was spotted with faeces (8/9), suggesting the presence of diarrhea. Several portions of intestinal tract were distended and fulfilled with liquid (9/9), especially the ceca, large intestine and distal portions of small intestine. Presence of *Enterobius* sp. adults was also noted (6/9). Parasite testing revealed a high degree of coccidia infestation belonging to *Eimeria* sp. (9/9), affecting mainly the distal intestinal portions. Adults and eggs of *Enterobius* sp. were also observed (7/9).

Main histopathological findings were observed at intestinal level. A high degree of coccidia infestation in the enterocytes was observed in all the individuals analyzed (7/7). Destruction of the villi was evident in some segments of the intestinal tract (5/7), with mild to moderate lymphoplasmacytic infiltrates in the lamina propria (5/7). The gross pathology and histopathological findings were similar to coccidiosis for other mammal species. *Eimeria* spp. has been considered as low pathogenic in red squirrels, acting mostly as asymptomatic carriers. In this sense, the reason of the outbreak is not known. However, the high level of stress associated to captivity and weather conditions might play an important role in the high degree of parasitism observed.

**Conclusion:** Due to its great pathogenic potential under captivity conditions, coccidia should be taken into account for health status control in red squirrel breeding programs.

## POSTER PRESENTATION 42

### Presence of *Trichinella britovi* in a red fox (*Vulpes vulpes*) in Brescia Province (Italy)

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**Key words:** fox, *Trichinella* sp, Trichineasy, surveillance

**Background:** In Italy foxes (*Vulpes vulpes*) are present and abundant everywhere and hold a high position in the animal food chain. Since they represent the main indicator species of the local presence of *Trichinella* spp., especially *T. britovi*, dead or hunted foxes are often used in monitoring programmes.

**Methods:** In Brescia province, the agreement among official veterinary service, public administrators and hunter associations, made possible sampling the carcasses of 228 foxes found dead or hunted, between June 2009-May 2010. Around 50% were coming from areas of > 400 mtr altitude. The presence of rabies was firstly excluded; then, a pool of 10 gr of muscles (diaphragm, masseter, lower hind limb) was analysed for the presence of *Trichinella* spp larvae. According to the EC 2075/2005 directives, Trichineasy (Syntec International) method was used. Such machine grinds, digests and filters the samples and deposits the material on a membrane filter, which is stained with a fluorescent reagent to detect trichina larvae. The species identification was performed by the National Reference Laboratory for *Trichinella* using a specific multiplex-PCR.

**Results:** The prevalence was very low: larvae of *Trichinella* spp. were found in just one animal. This was a young male, with characteristic lesions of sarcoptic mange, found dead in Sellero, a town in Vallecamonica at 600 meters on sea level. The load was 45 larvae/gr of muscle analyzed and they were further characterized as *T. britovi*.

**Conclusions:** Whereas foxes have a low importance for meat consumption, the absence of *T. spiralis* in wild carnivores is an essential step in the monitoring programs of pigs for acquiring the status of free area at regional level.

The presence of *T. britovi* is indicative of the circulation of this zoonotic parasite in the wild circle even if both the prevalence (0.5%) and the age of infected animals (young) were different than expected.

## POSTER PRESENTATION 43

### Prevalence of *Leishmania infantum* in wild and free-roaming domestic carnivores in Mallorca Island, Spain

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**Key words:** Leishmaniosis, mustelidae, viverridae, zoonosis

**Background:** The role of wild and free-roaming domestic carnivores as reservoir of *Leishmania infantum* was investigated in the endemic, Mediterranean island of Mallorca (Balearic Islands, Spain).

**Methods:** Serum and blood and/or spleen samples were obtained from 170 animals: 48 dogs from a kennel, 86 wild-caught feral cats, 23 pine martens (*Martes martes*), 10 common genet (*Genetta genetta*) and 2 weasels (*Mustela nivalis*).

**Results:** Seroprevalence by Western Blotting was 38% in dog and 16% in feral cat. Prevalence of infection by PCR was 44% in dog, 26% in cat, 39% in pine marten and 10% in genet. Lesions compatible with leishmaniasis were only observed in 24% of infected dogs and in no other species. Restriction fragment length polymorphism (RFLP) analyses showed that some patterns were shared by different species.

**Conclusions:** The prevalence detected, absence of apparent disease and the population size of feral cat and pine marten make these species potential primary or secondary hosts for *L. infantum* in Mallorca.

## POSTER PRESENTATION 44

### Prevalence of *Toxoplasma gondii* and *Neospora caninum* antibodies in Spanish ibex (*Capra pyrenaica hispanica*)

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**Key words:** *Toxoplasma gondii*, *Neospora caninum*, *Capra pyrenaica*, Seroprevalence, Spain

*Toxoplasma gondii* and *Neospora caninum* are two closely related intracellular apicomplexan protozoan of worldwide distribution. Both parasites have been associated to abortion and perinatal mortality, causing significant economic losses in the livestock industry. The aims of the present study were: to analyze seroprevalence against *T. gondii* and *N. caninum* in Spanish ibex (*Capra pyrenaica hispanica*) populations from southern Spain and to provide information on risk factors associated with these infections.

Antibodies against *T. gondii* and *N. caninum* were determined in serum samples from 531 Spanish ibexes from southern Spain. Seroprevalence to *T. gondii* was 27.5% (146/531; CI95%: 1:25). Seroprevalence to  $\geq 23.7$ -31.3) using the modified agglutination test (MAT *T. gondii* significantly increased with age ( $P < 0.001$ ). Statistically significant differences were also observed among regions and sampling year. Significantly lower seropositivity was found in Granada (17.6%) compared to Málaga (35.5%) and Jaén (41.8%). Seroprevalence during the period 2006-2007 was significantly higher compared to the period 2008-2009. There were not statistically significant differences between sex and habitat conditions.

Thirty of 531 (5.6%) analysed Spanish ibexes presented antibodies against *N. caninum* using cELISA and 27 of them (5.1%; CI95%: 3.1-7.1) were confirmed by indirect fluorescent antibody test. No statistically significant differences were observed in the seroprevalence to *N. caninum* between ages, sexes, locations, year of sample collection and habitat conditions. Cross-reactivity between *T. gondii* and *N. caninum* was not found.

The results obtained indicate widespread exposure to *T. gondii* in Spanish ibex populations. To our knowledge, this is the first report of *N. caninum* circulation in Spanish ibex. The seroprevalence levels suggest that the Spanish ibexes are more exposure in the natural environment to *T. gondii* than to *N. caninum*. The presence of both parasites in this species might have important ecological implications.

## POSTER PRESENTATION 45

### Reindeer Warble Fly Larvae (*Hypoderma tarandi*) in a Roedeer (*Capreolus capreolus*) in Sweden

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<sup>1</sup>National Veterinary Institute

**Key words:** *Hypoderma tarandi*, warble fly, *Capreolus capreolus*, Sweden

Warble flies are generally host-specific and the distribution of *Hypoderma tarandi* coincides with the area of reindeer and reindeer herding. However, there are a few reports of reindeer warble flies parasitizing novel hosts such as moose (*Alces alces*), red deer (*Cervus elaphus*), canids and man.

About thirty second- and third instar larvae of *Hypoderma tarandi* (L.) (syn. *Oedemagena tarandi*), (Diptera, Oestridae) , were found (developing) in the subcutaneous tissue over the back of a pregnant roedeer E) situated within the°N 14,15°killed in a traffic accident in Jämtland (63,21 Swedish reindeer herding area.

## POSTER PRESENTATION 46

### Roe deer (*Capreolus capreolus*) mortality and the role of *Haemonchus contortus* (Nematoda, Trichostrongylidae)

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**Key words:** *Haemonchus contortus*, roe deer, mouflon, mortality, the Netherlands

In May 2009, the Dutch Wildlife Health Centre was asked to investigate increased mortality and wasting in roe deer (*Capreolus capreolus*) in a Dutch wildlife area. An important factor for instigation of the investigation was to ensure the well-being of the unique 200-head herd of mouflon (*Ovis gmelini musimon*) in the park. The park is an enclosure consisting chiefly of heather and forest, measuring approximately 5000 ha. In 2009, 306 roe deer were counted, at same time of year in 2010 only 170 remained.

Twenty-three roe deer were submitted for post-mortem examination between May 2009 and June 2010. Roe deer were either found dead or shot in a moribund state. Necropsy was performed and samples were taken for histology with additional parasitological, virological and bacteriological investigation when appropriate. Parasite load was estimated as mild, moderate, or severe. In addition, the park submitted six mouflons for post-mortem examination.

Eighteen roe deer (18/23, 78%) showed *Haemonchus contortus* infestation in the abomasum. Two-thirds of these (12/18, 67%) had severe infestation without other significant pathological findings. In contrast, only one third (6/18, 33%) had significant, additional unrelated, lesions. The remaining uninfested five deer (5/23, 22%) were submitted in spring 2009 and in winter 2009-2010 and showed different and unrelated other lesions. *H. contortus* infestation was also found in three of the six mouflons, only one of which showed clinical signs.

These findings suggest that *H. contortus* plays an important role in the ongoing roe deer mortality event. Though *H. contortus* has been previously found in roe deer and has been associated with mortality in domestic ruminants, to the best of our knowledge it has not previously been associated with herd mortality of this magnitude. Mouflons were shown to be susceptible, but infestations were less severe and the herd continues to appear clinically unaffected.

## POSTER PRESENTATION 47

### Investigation of pathogenic infections in raccoon dog from northern Sweden

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<sup>1</sup>National Veterinary Institute

**Key words:** Raccoon dog, pathology, virology, bacteriology, parasitology

The raccoon dog is an invasive species in Sweden. They are coming in to the northern part of the country along the Finish border. In the period 2008-2009 45 raccoon dogs were shot and 1 was euthanized, in an ongoing eradication project, and sent to The National Veterinary Institute for necropsy and examination for pathogenic agents. In addition, 5 animals were killed in traffic accidents and they were also included in the study.

All 51 animals underwent a post mortem examination and in case of pathological findings samples for histopathology were collected.

Intestinal content from 49 animals were submitted for cultivation for *Salmonella* spp.

Samples from cerebrum and medulla oblongata from 39 animals were investigated on the incidence of rabies virus with immunofluorescence technique.

The gastro-intestinal tract and the lungs of 49 animals were opened and complete parasitological examinations were performed for isolation and identification of endoparasites. Muscle samples were also used for the detection of *Trichinella* spp.

The only pathological change found macroscopically was dermatitis secondary to *Sarcoptes* sp. in 2 animals. The mange was detected in one of the animals.

All animals tested negative for rabies virus, *Salmonella* spp., *Trichinella* spp. and *Echinococcus* spp.

The incidence of helminths in stomach and intestine were 88% including nematodes (*Uncinaria stenocephala* (67%), *Toxocara canis* (14%), *Molineus* sp. (12%), *Capillaria putorii* (8%), *Crenosoma vulpis* (4%) and *Physaloptena* sp. (2%)), one trematod (*Alaria alata* (6%)) and one cestod (*Mesocestoides* sp. (3%)). Two species of parasites were detected in the lungs (*Crenosoma vulpis* (12%) and *Capillaria* sp. (6%)).

The disease incidence in raccoon dogs in Sweden appears to be low. The only pathological changes recorded were skin infection caused by *Sarcoptes* mange in 2 individuals. None of the raccoon dogs showed pathological lesions associated with the reported parasite infestations.

## POSTER PRESENTATION 48

### Seroprevalence of *Toxoplasma gondii* and *Neospora caninum* in north-eastern Atlantic harbor seal (*Phoca vitulina vitulina*) and grey seal (*Halichoerus grypus*)

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**Key words:** Grey seal; Harbor seal; *Neospora caninum*; *Toxoplasma gondii*

Antibodies to *Toxoplasma gondii* and *Neospora caninum* were determined in serum samples from 47 grey seals (*Halichoerus grypus*) and 56 harbor seals (*Phoca vitulina vitulina*) from the Atlantic coasts of United Kingdom and France. Antibodies to *T. gondii* assayed by the modified agglutination test (MAT) were found in 14 (13.6%; IC95%: 7.0-20.2) of 103 seals tested, with titres of 1:25 in 13 seals and 1:50 in 1 seal. Seroprevalence against *T. gondii* (MAT 1:25 or higher) was significantly higher in grey seals (23.4%) compared to harbor seals (5.4%). No significant differences were found between seroprevalence against *T. gondii* and sex, age or geographical locations. Seroprevalence to *N. caninum* assayed by cELISA was 7.8% (IC95%: 2.6-13.0) and no statistically significant differences were observed between species, age, sex or location. These results show natural exposure of European harbor and grey seals to *T. gondii* and *N. caninum* oocysts in the Atlantic Ocean. To our knowledge, this is the first serological survey of *T. gondii* in European grey and harbor seals and of *N. caninum* in grey seals worldwide.

## POSTER PRESENTATION 49

### Serum biochemistry values in free ranging Red deer population infected with *Fascioloides magna*

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**Key words:** *Fascioloides magna*, Croatia, Red deer, serum biochemistry

**Background:** In two last decades, *Fascioloides magna* caused significant production losses in open hunting grounds in several endemic areas in Croatia. In order to improve diagnostic measures we investigated the effects of *Fascioloides magna* infection on the serum biochemistry values in naturally infected Red deer population in the northern Croatia.

**Methods:** Blood samples from 22 *Fascioloides magna* infected deer and from 19 non-infected were taken from the heart immediately after they were shot. The liver from each animal was sliced into 1.0 to 2.0 cm wide, parallel slices, and carefully examined. Fecal samples were collected from each deer, and fecal examination was performed using the sedimentation method.

**Results:** Significantly higher values for aspartate aminotransferase (AST), lactate dehydrogenase (LDH), glutamate dehydrogenase (GDH) and globulin were recorded in *Fascioloides magna* infected deer, whereas glucose value and albumine/globuline ratio were significantly higher in deer without *Fascioloides magna*.

**Conclusions:** It has been concluded that serum biochemistry could be used in diagnosis of *Fascioloides magna* infections in Red deer population.

## POSTER PRESENTATION 50

### Study of the circulation of *Trichinella* spp. in wildlife in France

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**Key words:** *Trichinella*, *Sus scrofa*, *Vulpes vulpes*, risk assessment

In France, human trichinellosis from infected pork had declined markedly since the 1970's, due to better practices in pig production. Besides, swine carcasses are tested at slaughterhouse in accordance with the European regulation 2075/2005, which requires systematic testing for *Trichinella* on swine but allows this control to be restricted to breeding sows and boars and pigs from non-controlled housed if a wildlife survey is carried out.

The last human outbreaks were caused by wild boar meat consumption and controls on hunted wild boars have revealed a prevalence of 1/10 000, proving that the sylvatic cycle still occurs. Wild boars (*Sus scrofa*) and foxes (*Vulpes vulpes*) are regarded as the main reservoirs of *Trichinella* spp. in wildlife and can thus be considered as good indicators for this parasite in the wild.

The aim of this study is to provide epidemiological data on the circulation of the parasite in wildlife in regions with large pig populations.

The study took place in 5 French "départements" characterised by an important indoor and/or outdoor pig production. From August 2009 up to June 2010, a total of 2411 wild boars and 1200 foxes were analysed by artificial digestion. Five grammes of diaphragm or tongue were sampled in each wild boar whereas 10 grammes of diaphragm or foreleg were sampled in each fox.

So far (300 more foxes are expected to be analysed), all samples are negative in both wild boars and foxes.

Given the number of animals sampled, we can conclude that the apparent prevalence of trichinellosis is comprised between 0 and 0,25% in foxes and between 0 and 0,12% in wild boars (95% confidence interval).

In addition to open-air pig surveillance and compulsory controls on marketed wild boars, these results may contribute to the risk assessment of *Trichinella* transmission from wild species to domestic pigs.

## POSTER PRESENTATION 51

### *Toxoplasma gondii*, *neospora caninum* and *encephalitozoon cuniculi* in cotton-tail rabbits from Piedmont (North West Italy)

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**Key words:** *Toxoplasma gondii*, *neospora caninum*, *encephalitozoon cuniculi*, cotton-tail rabbits

**Background:** Cottontail rabbit (*Sylvilagus floridanus*) was introduced in north west Italy in the '60 and is nowadays widespread in this part of the country where it is managed as a pest. Even if serological analysis have shown that European Brown Hare can be infected by *Neospora caninum* and *Toxoplasma gondii*, no data are available, as well as for *Encephalitozoon cuniculi*, for cottontail rabbits. These Protozoa and Microsporidia can occur in a wide range of animals even if diseases due to them are often asymptomatic while the parasites persist in several tissues including brain, muscles and kidney. Considering that no data are available on the presence of the above mentioned agents, we deemed it interesting to evaluate by PCR the presence of these parasites in tissues of cottontail rabbit culled in Piedmont (NW Italy).

**Methods:** from 2008 to 2009 144 cottontail rabbits were necropsied using sterile scalpels for every animals and tissues (brain, kidney, spleen, liver and skeletal muscle) were collected and stored at -20°C. Total genomic DNA was extracted from these tissues using the commercial kit GenomeElute™ and extracted were tested by PCR using the primers Nc5Nc21 for *N. caninum*, Tox4-Tox5 for *T. gondii* and ECUNIF and ECUNIR for *E. cuniculi*.

**Results:** Fourteen out of 144 animals (9.7%) were positive for *E. cuniculi*, 4 (2.8%) for *N. caninum* and 3 (2.1%) for *T. gondii*.

**Discussion:** Our results evidence, for the first time, the presence of *E. cuniculi*, *T. gondii* and *N. caninum* in cottontail rabbit. Prevalence is high for *E. cuniculi* and this suggests that this parasite can be maintained in cottontail rabbit population, while the low prevalence for both *N. caninum* and *T. gondii* is due to the low exposure in sylvatic areas to oocysts shedded by dogs and cats respectively. This research has been supported by a grant of the Regione Piemonte Assessorato Agricoltura.

## POSTER PRESENTATION 52

### **Toxoplasma gondii in wild boar and roe deer in Northern Italy: serosurvey and PCR-RFLP**

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**Key words:** Toxoplasmosis, wild ungulates, serosurvey and PCR-RFLP

*Toxoplasma gondii* infects all warm-blooded animals; in Europe several studies carried out in wildlife show seropositivity towards this parasite, in particular in wild ungulates.

In Northern Italy in the last years the culling of wild boar and roe deer is significantly increased and then the game meat consumption. As eating of raw or undercooked meat is a risk factor for Toxoplasmosis transmission to humans, we performed a serosurvey for this protozoan and its research in the muscular tissue.

The samples were collected during the 2008 and 2009 hunting seasons; wild boar sera were tested by IFIT (Toxo-spot \*IF, bio-Meriaux) while roe deer by a commercial Elisa kit (ID Screen\* Toxoplasmosis Indirect ELISA, IDVET, Montpellier, France); we analysed respectively 281 and 505 sera. 63 wild boar (22.4%, I.C. 95% 17.77-27.84) and 110 roe deer sera were positive (21.78%, I.C. 95% 18.31-25.69).

We further examined the muscular tissues of the seropositive animals for directly detecting the parasite by a PCR-RFLP assay targeting the 18S small-subunit ribosomal gene of *T. gondii*. The PCR was carried out on samples of muscular tissue (heart, diaphragm and masseter) of 53 seropositive wild boar and from 49 hearts of seropositive roe deer. All the samples tested negative. By the restriction enzyme analysis of the amplified products we detected positive samples for *Sarcocystis* spp., that by sequencing analysis has been identified as *S. miescheriana* in wild boar and as *S. cruzi* and *S. gracilis* in roe deer. Although we couldn't detect the parasite in muscular tissue, the serological results show a remarkable exposure to *T. gondii* in both host species and recommend a correct information and public health implication, also considering that consumption of undercooked or cured game is a widespread habit.

## 4. BACTERIAL WILDLIFE DISEASES

### POSTER PRESENTATION 53

### **Brucellosis in a live stranded harbor porpoise (*Phocoena phocoena*)**

Jauniaux, Thierry<sup>1</sup>; Brenez, Cecile<sup>2</sup>; Fretin, David<sup>3</sup>; Godfroid, Jacques<sup>4</sup>; Haelters, Jan<sup>5</sup>; Jacques, Thierry<sup>5</sup>; Kerckhof, Francis<sup>5</sup>; Mast, Jan<sup>2</sup>; Sarlet, Michael<sup>1</sup>; Coignoul, Freddy<sup>1</sup>

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**Key words:** brucella ceti, porpoise, brucellosis, pathology

*Brucella ceti* infection in cetaceans is described in striped dolphins (*Stenella coeruleoalba*), Atlantic white-sided dolphins (*Lagenorhynchus acutus*), in bottlenose dolphins (*Tursiops truncatus*), harbor porpoises (*Phocoena phocoena*) and mink whale (*Balaenoptera acurostrata*). The present communication describes the first confirmed case of *B. ceti* infection and associated lesions in a live-stranded harbor porpoise along the Belgian coast (Marine Animals Research and Intervention Network program-MARIN). The animal was necropsied and histology, immunohistochemistry (IHC), transmission electron microscopy (TEM) as well as bacteriology were performed. The animal, a female of 41 kg and 152 cm was severely emaciated. Relevant lesions were skin ulcers, severe nematode infestation (airways and pulmonary blood vessels) and severe necrotizing pneumonia. The IHC for the detection of *Brucella* spp. revealed intracytoplasmic positive staining in mononuclear cells in skin ulcers, spleen, lymph nodes, lung, uterus, mammary gland (parenchyma and milk) and brain. By TEM, very large numbers of relatively small, coccoid bacteria were observed intra- and intercellularly in the genital ulcer. A *Brucella* isolate was obtained from brain and lung. The isolates showed catalase, oxidase and urease activity, did grow in the absence of CO<sub>2</sub> and agglutinated anti-A monospecific antiserum. The variable number tandem repeat (VNTR) profile of the strain was typical of *B. ceti*, in agreement with the biochemical typing. The present study suggests that the stranded animal suffered from bacteraemia associated with *B. ceti* and is the first case described for the Belgian and northern French coastline. Many similarities appear between gross-lesions and microscopical findings between this case, and other cases of cetacean brucellosis described elsewhere in Europe. The presence of *Brucella* sp. antigens in mammary ducts and in skin ulcers may indicate ways of bacterial transmission between individuals. It raises the question of a risk of zoonosis when a cetacean is handled on the beach or in rehabilitation center.



## POSTER PRESENTATION 54

### **Clostridium botulinum type c outbreak in wild mammals in Italy**

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<sup>1</sup>Ausl Bologna; <sup>2</sup>Ausl Reggio Emilia; <sup>3</sup>Izslar; <sup>4</sup>Istituto Superiore Di Sanita'; <sup>5</sup>Izsve

**Key words:** Clostridium botulinum type C – nutria (*Myocastor coypus*) – rat (*Rattus norvegicus*) – avian botulism

During the late summer of 2008 an uncommon increase of the mortality rate of different avian species was observed along Crostolo and Rodano rivers in Emilia Romagna region, Italy (lat. 44°67'64"N, long. 10°62'56"E). The largest part of the affected birds was represented by mallards (*Anas platyrhynchos*). Other species involved were little egret (*Egretta garzetta*), common kingfisher (*Alcedo atthis*), great cormorant (*Phalacrocorax carbo*), common moorhen (*Gallinula chloropus*), yellow wagtail (*Motacilla flava*), rock pigeon (*Columba livia*), European magpie (*Pica pica*), hooded crow (*Corvus corone cornix*) and common pheasant (*Phasianus colchicus*). In addition to avian species, 21 dead coypus (*Myocastor coypus*) and 4 Norway rats (*Rattus norvegicus*) were retrieved in the areas of the epizootic. Overall 26 birds, 4 coypus and 1 rat were submitted for necropsy, bacteriological, toxicological and virological examinations. Sera collected from 3 moribund mallards and sera obtained from heart clots of dead mammals were filtered through a 0.22 µm filter and tested for Clostridium botulinum neurotoxins by mouse test. Intestinal and liver samples collected from birds and mammals carcasses were tested for the presence of *C. botulinum* by bacteriological procedures and PCR for type A, B, C, D, E, F. All tested sera resulted positive for *C. botulinum* type C neurotoxin and *C. botulinum* type C was isolated and detected by PCR. The remaining investigations resulted negative. *C. botulinum* type C has been occasionally observed in mammals such as cattle, cats, dogs and horses but, to the authors' knowledge, this is the first report of natural acquired botulism in coypus and rats connected with an avian botulism outbreak. Water and plant contamination from infected carcasses or maggots could have lead to mammal species intoxication. This is especially true for coypus that are strictly vegetarian, while rats intoxication could also have derived from the ingestion of maggots or from scavenging toxin-laden carcasses.

## POSTER PRESENTATION 55

### **Comparative study of different antigenic preparations in the development and validation of an enzyme-linked immunosorbent assay for antibodies against Mycobacterium bovis in red deer**

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**Key words:** *M. bovis*, ELISA, validation

Tuberculosis caused by *Mycobacterium bovis* (bTB) is a worldwide distributed disease that affects humans, livestock and wildlife. bTB diagnosis in wildlife is usually made by post mortem study of tissue samples. Deer have been frequently found infected in endemic areas where sometimes it is necessary to determine their in vivo bTB status. Serologic tests like ELISA could be very useful for ante mortem diagnosis in wild and captive deer in contrast with other techniques that require either repeated animal handling as skin test, or immediate laboratory processing as INF-γ ELISA. In the present work, 4 captive red deer were immunized with inactive *M. bovis*, *M. paratuberculosis* or *M. avium* in order to obtain hiperimmune sera to be used as control. PPD<sub>b</sub>, ESAT 6, MPB70, MPB83 and CFP10 antigens were evaluated individually or grouped in an indirect enzyme-linked immunosorbent assay (ELISA). The ELISA was optimized using different serum dilutions and conjugate concentrations to better detect antibodies against *Mycobacterium bovis* in control red deer serum. The ELISA test was validated on 120 sera from culture and lesion confirmed TB positive and negative red deer. The selected test yielded a sensitivity of 76,19% and an specificity of 86,87% for the best balanced cut-off using a combination of MPB70, MPB83 and CFP10 antigens, 1/200 serum dilution and 0,05 µg/ml of Protein G as conjugate. The moderate specificity could be interpreted as the result of the test detecting bTB cases with non-visible lesions and non-viable bacteria. On the other hand there were some confirmed TB animals that were ELISA negative. They might represent anergic animals with limited immune responses or recently infected animals not having developed yet a humoral response. These results indicate that this assay might have a potential for easier in vivo diagnosis of TB in red deer if used alone or for broader immunopathological coverage if combined with cell-mediated tests.

This work was supported by the Department of Environment, Spatial Planning, Agriculture and Fisheries of the Basque Government.

## POSTER PRESENTATION 56

### Detection of 'Mycoplasma aquilae' in an Eurasian buzzard (*Buteo buteo*) in Austria

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**Key words:** buzzard, mycoplasma

Mycoplasmas are intra- or extracellular bacteria that belong to the order of Mollicutes. In birds of prey few Mycoplasma species have been described so far. Discussion whether these are commensal rather than pathogenic is still ongoing.

An adult, male European buzzard (*Buteo buteo*) weighing 660g was presented dead at the Pathology department of the Research Institute of Wildlife Ecology, Vienna (FIWI). The animal showed signs of massive trauma as it had an open fracture of the femur. Furthermore the surrounding area of the right eye appeared profoundly swollen and discolored. In the right infra-orbital sinus a severe fibrinopurulent to necrotizing inflammation was present forming an abscess extending to the beak. Also, moderate fibrin exudation into the right orbit completely surrounding the eye, as well as a severe, diffuse lung bleeding was seen. Samples from the abscess and the lung were submitted for bacterial examination. In the lung tissue moderate to high amounts of *Escherichia coli* as well as moderate amounts of *Staphylococcus aureus* were present. Swabs taken from the abscess yielded high numbers of *S. aureus*, but also a moderate number of 'Mycoplasma aquilae' identified by 16S rRNA gene sequencing.

'M. aquilae' is a non-validly published mycoplasma species that was first isolated from a Spanish imperial eagle (*Aquila adalberti*) in 2004. In addition, it has been recovered from lung samples from Saker and Gyrfalcons with chronic respiratory disease. However, the pathogenic potential of this organism has to be defined in further studies. In the described case the causative role of the different bacterial species from tissue and swab samples for the development of severe sinusitis is difficult to determine. This case is the first report of 'M. aquilae' in an Eurasian buzzard and the first association of this bacterial species with sinusitis.

## POSTER PRESENTATION 57

### Detection of *Brachyspira intermedia* in wild boar (*Sus scrofa*) in Spain

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**Key words:** *Brachyspira* spp.; wild boar; Spain

The wild boar, *Sus scrofa*, is one of the most common wild animals in Spain. Since some years ago, a lot of hunting grounds have changed their wild boar management in the middle-South of Spain, population rates have increased, mainly due to changes in farming techniques, to levels similar to ones presented by Iberian swine.

Spirochaetes of genus *Brachyspira* colonize the large intestine of some mammals and birds and are responsible for production losses in pigs and chickens since this microorganism is a cause of dysentery. To the best of our knowledge it has not been reported any isolation of this bacteria genus from wild boar.

Forty samples originated from hunted wild boars large intestine were analyzed. Faecal samples and rectal swabs from 200 farming animals, either with diarrhoea or healthy ones, with ages between two and three months old were studied as well. Samples were transported to laboratory in Amies medium and cultured in agar supplemented with sheep blood (5 and 10%) and containing the antimicrobials spectinomycin, spiramycin, vancomycin, colistin and rifampicin. Samples were incubated anaerobically at 42° C for 6-7 days. Microscopical examination and biochemical tests for *Brachyspira* spp. identification were performed. Furthermore, PCR of *nox* gene was used for identification among *B. hyodysenteriae*, *B. pilosicoli* and *B. intermedia*. A spirochaete strain was isolated from a faecal sample of a farmed three month old *Sus scrofa* with dysentery. The isolation presented weak  $\beta$ -haemolysis and was indole positive,  $\alpha$ -galactosidase negative,  $\alpha$ -glucosidase positive and  $\beta$ -glucosidase positive. Through PCR and sequencing 16S rRNA, the strain was identified as *B. intermedia*. Although this isolation could mean that this spirochaete constitute part of the wild boar commensal gut microbiota, no more positive samples were found.

## POSTER PRESENTATION 58

### Antimicrobial resistance profiles in *E. coli* and *Salmonella* spp. strains isolated of yellow-legged gulls from the Chafarinas Islands (Spain)

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<sup>1</sup>CISA-INIA

**Key words:** gull, antibiotic resistance, Spain, salmonella, *e. coli*

**Background:** The Chafarinas Islands are located at the south western Mediterranean Sea. They host the second largest breeding colony of the endangered Audouin's gull (*Larus audouinii*). The yellow-legged gull (*Larus michahellis*) has also a breeding colony that has increased during the last years. Predation of Audouin's gull chicks by yellow-legged gulls during the breeding season is well documented. This interaction and the fact that the yellow-legged gulls feed on dumps may increase the risk of transmission of anthropogenic pathogens from this species to Audouin's gulls. The objective of this work is to evaluate the antimicrobial resistances in *E. coli* and *Salmonella* spp. obtained from yellow-legged gulls as an indicator of the anthropogenic degree of their microbiota.

**Methods:** A total of 32 antimicrobials and combinations were tested for resistance of 18 *E. coli* strains and 2 of *Salmonella* spp. Isolated from yellow-legged gulls from the Chafarinas islands.

**Results:** Ten of the 18 *E. coli* strains presented at least one antimicrobial resistance whereas the two *Salmonella* spp. were resistant to at least seven antibiotics. The most frequent antimicrobial resistance was against ampicillin (8/18 for *E. coli* and 2/2 for *Salmonella* spp.), followed by sulphametoxazole (7/18 for *E. coli* and 2/2 for *Salmonella* spp.), ticarcillin (5/18 for *E. coli* and 2/2 for *Salmonella* spp.), tetracycline (5/18 for *E. coli* and 1/2 for *Salmonella* spp.), amoxicillin-clavulanic acid ticarcillin-clavulanic acid, (3/18 for *E. coli* and 2/2 for *Salmonella* spp.), trimethoprim-sulphametoxazole (3/18 for *E. coli*), chloramphenicol (2/18 for *E. coli* and 1/2 for *Salmonella* spp.), piperacillin (1/18 for *E. coli* and 2/2 for *Salmonella* spp.), cephalothin (2/18 for *E. coli*), and nalidixic acid (1/18 for *E. coli* and 1/2 for *Salmonella* spp.).

**Conclusion:** The results obtained indicate the presence of antimicrobial resistances in Chafarinas Islands, and may pose a potential health risk for the Audouin's gull.

## POSTER PRESENTATION 59

### Impact of group size and external sources of infection on the efficacy of vaccination for reducing bovine tuberculosis in badgers

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**Key words:** group size, efficacy, vaccination, badgers

**Background:** The use of vaccination to reduce bovine tuberculosis (bTB) infection in wildlife offers a potential alternative to culling, in those situations where perturbation induced by culling can exacerbate bTB problems locally or where culling may be either socially or politically unacceptable. In Britain, culling-induced perturbation of badgers has been shown to increase bTB infection in cattle, and the development and effective deployment of a bTB vaccine for badgers are therefore high priorities for research. However, as with any culling-based disease management programme, the effectiveness of vaccination focused on a single species will depend on the level of other, external sources of infection in the ecosystem.

**Methods:** We use a spatial stochastic simulation model to investigate the impact of different vaccination strategies on bTB persistence in badger populations in Britain, in relation to badger population density and different levels of external infection.

**Results:** The greatest reductions in bTB prevalence were obtained in smaller badger group sizes, and through higher efficacy and longer duration of vaccination. Vaccination was less effective in controlling bTB in larger badger groups. Recovery of bTB in the badger population was facilitated by higher levels of external infection, although recovery was slowed by higher efficacy and longer duration of vaccination. The lower efficacy vaccine applied for ten years was significantly better at reducing disease than a higher efficacy vaccine applied once.

**Conclusions:** The results show that vaccination is likely to be an effective disease control measure in lower-density badger populations. However, for vaccination to be effective for controlling disease in higher-density badger populations, a long-term vaccination policy combined with effective control of external sources of infection will be required.

## POSTER PRESENTATION 60

### **Campylobacter spp. isolation from scavenging seabirds at Gough Island, South Atlantic Ocean**

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**Key words:** thermophilic *Campylobacter*, scavenging seabirds, Gough Is.

**Background:** Remote places with little human presence can be particularly sensitive to the introduction of infectious agents, such as human enterobacteria. Gough Island is one of the most remote islands lying in the South Atlantic Ocean, about 2,700 Km from Cape Town. It is uninhabited, but the South African National Antarctic Program has continually maintained the personnel of a weather station on the island since 1956. Although human waste on Gough is currently not made available to the fauna of the island, enterobacteria may have been spread by scavenging seabirds in the past. Therefore, we surveyed the prevalence of thermophilic *Campylobacter* in the seabird community on Gough, including the two major scavenging species: the Southern giant petrels (*Macronectes giganteus*) and the subantarctic skuas (*Catharacta antarctica*).

**Methods:** Cloacal swabs were obtained from 138 subantarctic seabirds from 9 species from September to October 2009 on Gough Island: Soft plumage petrels (*Pterodroma mollis*; N=32), Atlantic petrels (*Pterodroma incerta*; N=21), Southern giant petrels (N=9), Yellow nosed albatrosses (*Thalassarche chlororhynchus*; N=13), Sooty albatrosses (*Phoebastria fusca*; N=5), Subantarctic skuas (N=15), Great shearwaters (*Puffinus gravis*; N=16), Northern rockhopper penguin (*Eudyptes moseleyi*; N=24) and Broad billed prion (*Pachyptila vittata*; N=3). *Campylobacter* was isolated using conventional culture methods and was confirmed by PCR.

**Results:** *Campylobacter* was only isolated from Subantarctic skuas (73.3% prevalence). Ten out of 11 positive birds carried *Campylobacter lari* (66.7%). Skuas feed mainly on carrion and other seabirds, and scavenge to different extent on human waste.

**Conclusions:** We did not detect *Campylobacter* species with likely human origin, such as *C.jejuni* and *C.coli*. However, this is the first study reporting a high prevalence of *C. lari* in a subantarctic seabird species. *C.lari* may have been introduced by humans on Gough but may also have wild origin. Therefore, further genotyping studies are needed to distinguish between the two possibilities.

## POSTER PRESENTATION 61

### **Preliminary results of the prevalence and distribution of mycobacterial infections in eurasian badgers (meles meles) in Spain**

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Dalley, Deanna J<sup>6</sup>; Lesellier, Sandrine<sup>6</sup>; Casais, Rosa<sup>1</sup>; Espí, Alberto<sup>1</sup>; Gortázar, Christian<sup>2</sup>; Prieto, José M<sup>1</sup>

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<sup>4</sup>Laboratorio de Sanidad Animal; <sup>5</sup>Neiker-Tecnalia;

<sup>6</sup>Veterinary Laboratories Agency

**Key words:** Badger, *Meles meles*, mycobacterial infections, Spain

**Background:** The eradication of bovine tuberculosis from cattle may be compromised, particularly in areas where there is a relatively low incidence of disease in domestic animals, if infected wildlife species, such as Eurasian badgers (*Meles meles*), share the same environment and contribute to transfer the infection, as it happens in Great Britain and Ireland.

**Methods:** To study the situation in Spain, as part of wildlife surveillance for bovine tuberculosis, carcasses of 85 road killed badgers were examined. Thirty additional badgers were sampled during trapping operations. Whole blood, serum and tissue samples were taken for immunological (Interferon-Gamma, ELISPOT, DDPVet Test, Brock Stat-Pack Test and paratuberculosis ELISA), histopathological, immunohistochemical, bacteriological (supplemented Middlebrook 7H9 broth and MGIT liquid culture systems, and Coletsos, Herrold and Löwenstein-Jensen solid media) and molecular (PCR, RT-PCR and Spoligotyping) examination. **Results:** Data on prevalence, pathology, genotyping and spoligotyping are presented in this study. *Mycobacterium bovis* and *Mycobacterium avium* Complex (MAC) organisms were isolated and identified by RT-PCR from six (7,05%) and five (5,88%) of the road-killed badgers, respectively. These had no visible lesions consistent with tuberculosis. Microscopically, focal, small granulomas, formed mainly by macrophages and lymphocytes, were observed in the retropharyngeal, sub-mandibular, tracheo-bronchial, mediastinal and mesenteric lymph nodes and in the lungs. Both macrophages and lymphocytes showed positive immunolabelling for mycobacterial antigens.

**Conclusions:** We have found evidence of both *M. bovis* and MAC infection in badgers in Spain. However, the preliminary results seem to show that the prevalence of bovine tuberculosis in badgers in Spain is quite lower than in Great Britain or Ireland. Currently, the pattern of infection in the population of Spanish badgers and their potential risks to cattle is unknown. Questions to answer in the future will be: "Are badgers tuberculosis maintenance hosts in Spain?", "Do badgers play any role in the epidemiology of MAC infections in Spain?"

## POSTER PRESENTATION 62

### **Pseudomonas species isolated from a Harbour Porpoise (*Phocoena phocoena*) with a granulomatous epididymo-orchitis: a case report**

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**Key words:** epididymo-orchitis, *Phocoena phocoena*, *pseudomonas*

This case report describes an epididymo-orchitis in a stranded adult sexually mature free-living harbour porpoise (*Phocoena phocoena*). Orchitis, with or without epididymitis, is a rare finding in harbour porpoises and is most commonly associated with infections with *Brucella* species or (systemic) mycotic infections. *Pseudomonas* species are Gram-negative, non-fermenting bacteria, and are known to be facultative pathogens that most commonly cause pathological changes in immunocompromised animals and humans. *Pseudomonas aeruginosa* has been associated with orchitis in humans and several animal species including goats, cattle and dogs.

The animal was examined as part of an ongoing investigation into causes of death in harbour porpoises stranded on the Dutch coast. This project is a joint venture between Royal Netherlands Institute for Sea Research (NIOZ), Institute for Marine Resources and Ecosystem Studies (IMARES), National Museum of Natural History (Naturalis), Seal Rehabilitation Centre (Lenie 't Hart) and Utrecht University funded by the Ministry of Agriculture, Nature and Food Quality (LNV). Necropsy was performed using an adaptation of an internationally recognized cetacean necropsy protocol. The animal showed severe depletion of fat reserves and atrophy of musculature (cachexia) and the most significant pathological change encountered was a severe unilateral chronic granulomatous epididymo-orchitis. Seminiferous tubules were multifocally distended by large quantities of (necrotic and degenerate) macrophages and fewer neutrophils, frequently with central necrosis. Intra- and extracellular rod-shaped bacteria were visible using light microscopy. Bacteria stained negative with Ziehl-Neelsen, Fite-Faraco, Gram and PAS stains and a *Pseudomonas* species was successfully cultured. With the exception of the very poor bodily condition, no significant underlying pathology was demonstrated in this animal.

To the best of our knowledge this is the first report of *Pseudomonas* species-associated epididymo-orchitis in any species of cetacean. At the time of writing, molecular typing of the bacterium is being carried out using 16s rRNA gene sequencing techniques.

## POSTER PRESENTATION 63

### **Restriction endonuclease analysis (REA) of isolated strains of Ballum serogroup from Northern Portugal rodents**

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**Key words:** Leptospirosis, *Leptospira*, rodents, REA, Portugal

**Background:** In the 90's, Collares-Pereira and collaborators reported the molecular typing of a high number of strains belonging to serovar Arborea (serogroup Ballum) from *Mus musculus* and *Rattus rattus* of Azores islands, and from *Mus musculus* and *Rattus norvegicus* of mainland Portugal. In both surveyed regions, rodents showed an Arborea-like profile, clearly different from type Castellon 3 (serovar Castellonis) and Ballum strain (serovar Ballum), with an exception of one Ballum serovar isolated from a *Mus spretus* in the Centre of Portugal. The purpose of this study is to report the first identification of Ballum serogroup rodent isolates from a northern Portuguese region.

**Methods:** Eleven strains isolated from Northern Portugal rodents were identified by restriction endonuclease analysis (REA), after being serotyped as serogroup Ballum by the microscopic agglutination technique. Of these, eight isolates were obtained from *Mus musculus*, two from *M. spretus* and one from *Rattus norvegicus*. REA profiles of field cultures were compared with those of the following reference strains of *L. borgpetersenii* serogroup Ballum: Mus 127, S102, Arborea and Castellon 3.

**Results:** All the strains showed ClaI restriction patterns compatible with strains from serovar Ballum and distinct from Arborea or Castellonis serovars.

**Conclusions:** The molecular analysis by REA suggests clear epidemiological differences between Northern Portugal rodent isolates and the rest of the country (including the Azores Islands), as regards the serovar-type of Ballum serogroup strains maintained by rodents in the ecosystem. In addition, Ballum-type strains in the north of Portugal confirmed to have three different rodent species as reservoirs: *Mus musculus*, *M. spretus*, and *Rattus norvegicus*. Therefore, this is the first report of the isolation of Ballum serovar strains from *Mus musculus* and *Rattus norvegicus* in Portugal.

## POSTER PRESENTATION 64

### Risk-based surveillance of tuberculosis in red deer, wild boar and domestic cows in Switzerland and principality of Liechtenstein

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**Key words:** *Mycobacterium bovis*, *Mycobacterium caprae*, Principality of Liechtenstein, Surveillance, Switzerland

Wildlife populations are a known reservoir for *Mycobacterium bovis* and *M. caprae*. They represent a source of infection for domestic animals and a threat to public health. Switzerland and Liechtenstein are officially free of bovine tuberculosis (TB) in livestock and no case are known to occur in wildlife. As neighbouring countries (especially Austria and Germany) reported increasing numbers of cases in wildlife and livestock, risk-based investigations have been initiated in Switzerland and Liechtenstein.

Risk areas for wildlife were identified along the Swiss border. Sample size for active surveillance was calculated to detect disease at a prevalence of >5%. Blood, lymph nodes and tonsils were collected from 137 hunted red deer (*Cervus elaphus*) and 12 wild boar (*Sus scrofa*) in autumn 2009. Additional 231 deer and 287 wild boar will be sampled in autumn 2010. Furthermore, hunters and game-wardens were requested to send organs with TB-like lesions for pathological examination including histology (H&E and Ziehl Neelson stains). Organs of all animals are screened for *Mycobacterium* spp. by culture and PCR. In addition, 581 cows from Liechtenstein and Switzerland, which spent the summer 2009 on Austrian pastures, were tested with the Comparative Cervical Tuberculin Test (CCT). In case of questionable or positive results, animals were re-tested with CCT and Interferon gamma (IFN-gamma) assay.

TB-like lesions were reported in two deer. No acid-fast bacteria were identified at histology. Culture of all wildlife organs is on-going. 21/581 cows reacted inconclusively with the CCT. 19 were re-tested (CCT, IFN-gamma assay) but did not show positive reactions. 2 animals were slaughtered. They were negative at meat inspection, by culture and PCR.

Preliminary results are not suggestive of TB presence in Switzerland and Liechtenstein, neither in wildlife nor in livestock. However, results from pending analysis and from the sampling round 2010 are needed to draw final conclusions.

## POSTER PRESENTATION 65

### Salmonella Hessarek in starling (*Sturnus vulgaris*): diagnosis and molecular study of outbreak and collection isolates

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**Key words:** starling, salmonella hessarek

**Background:** *Salmonella enterica* subsp. *enterica* serovar Hessarek (*S. Hessarek*) belongs to serogroup B and is considered a rare serovar. Firstly identified as a new serovar following isolation from a dead crow (*Corvus corax*) in Iran, it has been mostly isolated from starling (*Sturnus vulgaris*) since, in this species it exhibits high virulence giving rise to outbreaks sometimes characterized by significant mortality. Therefore, it is considered a serovar with high host specificity. In this report we describe an outbreak of *S. Hessarek* infection in starlings occurred in October 2009 in the city of Modena, Emilia-Romagna Region (Italy).

**Methods:** Thirty-two dead starlings were subjected to a diagnostic protocol including necropsy, bacteriological, virological and parasitological examination. *S. Hessarek* was isolated from thirty-one birds and ten isolates were genotyped by PFGE with XbaI and AvrII endonucleases. Further fourteen collection-isolates originating from diverse places and time-periods were genotyped with the same method to assess the genetic variability of this serovar and to elucidate possible epidemiological relationships among isolates.

**Results:** Hepatomegaly with small foci of necrosis in the liver, splenomegaly, focal haemorrhages of the pericardium and lungs and intestinal congestion were observed at necropsy. *S. Hessarek* was isolated in pure culture from all tested organs. Virological tests for Newcastle Disease, West-Nile Disease Flavivirus and type A avian influenza were negative. Parasitological tests were negative for all animals but one. All ten outbreak isolates showed the same XbaI and AvrII PFGE profiles. The fourteen collection strains were grouped into three different profiles with both enzymes, among them six belonged to the same genotype as the outbreak isolates.

**Conclusions:** Our report confirms that *S. Hessarek* is a pathogen capable of causing an acute and deadly disease in starling. Considerations regarding the genetic variability of tested isolates are presented in the poster

## POSTER PRESENTATION 66

### Septicaemia associated with a new *Streptococcus* species in a Pyrenean chamois (*Rupicapra pyrenaica*)

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**Key words:** Septicaemia, *Streptococcus*, chamois, *Rupicapra pyrenaica*

In October 2007, a free-ranging six-year-old male Pyrenean chamois (*Rupicapra pyrenaica*) was found ill and weak at the National Hunting Reserve of Freser-Setcases in the Pyrenees (NE Spain) and died a few hours after being captured. Necropsy and histological examination were performed following standard procedures. Samples of liver and spleen were cultured on Columbia blood agar plates and incubated for 24 hours at 37°C under both aerobic and anaerobic conditions. Biochemical identification was performed by using the commercial Rapid ID32 Strep system. Molecular identification was attempted by 16S rRNA gene sequencing. Because outbreaks of a pestivirus-associated disease have been affecting the chamois population in the Pyrenees for the last 8 years, RT-PCR was performed from a spleen sample to discard a viral infection.

At postmortem examination, the animal was in good body condition and the main gross findings included multiple liver abscesses and lung congestion. Microscopically, multifocal suppurative splenitis was observed. An acute bacterial septicaemia was suspected to be the cause of death. RT-PCR assay was negative. A Gram-positive catalase-negative coccus-shaped organism was isolated from spleen and liver. Tentative biochemical identification was not successful. Comparative sequence analysis revealed that the unknown cocci were member of the genus *Streptococcus*, being phylogenetically most closely related, but distinct, to *Streptococcus ovis*. The results of the phylogenetic analysis suggest that the chamois isolates represent a novel *Streptococcus* species. We are currently doing a polyphasic taxonomic study of these isolates in order to formally describe a new species of the genus *Streptococcus*, for which the name *Streptococcus rupicaprae* sp. nov. will be proposed. The description of a new *Streptococcus* species associated with a septicemia in a Pyrenean chamois will contribute to improve the knowledge about the range streptococci potentially affecting this species, as well as evaluate the impact of the streptococcal infections in this population.

## POSTER PRESENTATION 67

### Tuberculosis and other mycobacterial infections of wildlife in Hungary

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**Key words:** tuberculosis, *Mycobacterium caprae*, wildlife, cattle

Infrequent sporadic outbreaks of bovine tuberculosis (TB) in some region of Hungary led to introduce a systematic, countrywide wildlife TB-monitoring (based on pathology and bacteriology). In the last two hunting seasons more than 700 wild animals (including free living, fenced and farmed wildboars, red deers and fallow deers as well as roe deers, red foxes and badgers in lower number) were submitted to the laboratory. Samples of lymphonodes with or without visible lesions and other lesioned organs were used for cultivation of mycobacteria. Approx. 70 animals (wildboars, red deers and fallow deers) were infected by *Mycobacterium tuberculosis* complex strains which were identified exclusively as *M. caprae*. All these TB-infected animals originated from the two regions where most of the TB outbreaks in grazing cattle were observed. We considered these areas with expanded wildboar and red deer populations as endemic TB regions where wildboar and red deer can be natural reservoirs of bovine TB. River Danube and certain artificial barrier (like motorways) seem to be important borders preventing spread of wildlife TB. Up to now no TB-infected population of wildlife has been identified from the eastern part of the country, although we isolated several non-tuberculous mycobacteria (NTM) (including *M. avium* ssp., *M. avium* ssp. paratuberculosis, *M. fortuitum*, *M. goodii*, *M. goodii*, *M. goodii*, *M. goodii* and several non-typable species) from wildboars and deers killed there. We found higher prevalence of NTM infections of wildboar in overpopulated but TB free regions confirming the high impact of ecological factors (e.g. environmental conditions, population density, social behavior) on the spread of mycobacterial infections. Based on our investigations we conclude that dense populations of wildboars and maybe red deers supported with feeding and drinking and disturbed by intensive hunting can maintain bovine TB and other mycobacterial infections and serve as source of infection for wild and domestic animals.

## POSTER PRESENTATION 68

### Vaccination of Eurasian wild boar with inactivated *Mycobacterium bovis* results in immune responses and protection after challenge similar to BCG: Preliminary results

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<sup>1</sup>IREC

**Key words:** *M. bovis*, BCG, Tuberculosis, *Sus scrofa*, Vaccine

**Background:** Wild boar (*Sus scrofa*) oral BCG vaccination results in the reduction of infection and clinical disease and the upregulation of immunoregulatory genes that may be associated with protective response to *M. bovis* infection in this species. More studies are needed to further characterize BCG vaccine efficacy and the immune response of wild boar to vaccination and challenge as well as on alternative formulations.

**Methods:** Twenty wild boar piglets were randomly assigned to 4 treatment groups of 5 animals each: (a) control, (b) parenteral inactivated *M. bovis*, (c) oral inactivated *M. bovis*, (d) oral BCG. Oral vaccination was done using baits developed for wild boar. All animals were challenged with 106 cfu of an *M. bovis* field strain 2 months post-immunization (p.i.), and euthanized at 7 months p.i. Serum antibody responses were monitored by ELISA. The PBMC mRNA levels of IL4, RANTES, C3, IFN-gamma and methylmalonyl-CoA mutase (MUT) were analyzed at the same times. Tissues were examined for gross and histopathological lesions and cultured for mycobacteria.

**Results:** Preliminary results suggested that (1) a significant reduction in lesion scores and a reduction in culture scores occurred in all vaccinated groups when compared to the controls; (2) the protection was similar between BCG vaccinated wild boar and those vaccinated with inactivated *M. bovis*; (3) serum antibodies against *M. bovis* allowed differentiating vaccinated not challenged from vaccinated challenged wild boar, in group b; (4) the expression of MUT was similar between BCG and inactivated *M. bovis*-immunized wild boar. The expression of immune response genes IL4, RANTES, C3, and IFN-gamma probably reflected differences in host immune response to live and inactivated mycobacteria.

**Conclusions:** Protection after challenge was similar between BCG vaccinated wild boar and those vaccinated with inactivated *M. bovis*, suggesting a possibility of using inactivated vaccines to control bTB in wild boar.

## 5. VIRAL WILDLIFE DISEASES

### POSTER PRESENTATION 69

#### Bluetongue virus spreading and impact in wild ungulate in France

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**Key words:** bluetongue, ungulate, persistence, reproduction success, transmission

Since the 2000's, the serological BTV conversion of wild ruminant species has been confirmed in Europe. Previous studies suggest that wild populations of red deer (*Cervus elaphus*) are potential reservoirs. But the capacity of persistence of BTV in wild populations independently of the domestic livestock is still questionable. In addition, the impact of BTV on the reproduction success of wild red deer has not yet been explored. We present here a study conducted in France in wild ruminant species from March 2008 up to March 2010, i.e. before and after an exhaustive vaccination had been conducted in livestock. Shot dead and captured animals were blood and/or spleen sampled. The infectious status of individuals was explored by performing a competitive ELISA test (against VP7 BTV protein) on sera and a real time RT-PCR multiplex on the organs of seropositive individuals. Genital tracts of the does were examined and foeti analysed using RT-PCR. Pregnancy was confirmed by observing foeti or detecting the bovine foetal hormone in the serum of does. We sampled more than 2500 ungulates from 2008 up to 2010. Positive results were mainly observed in the Red Deer. In 2008-2009, 41% of red deer were seropositive, 84% of these being also positive to RT-PCR but negative to viral isolations. In 2009-2010, the proportion of positive results was lower, but positive results in fawns suggested the persistence of BTV in wild populations. We found neither an evidence of transmission from does to foeti nor a correlation between pregnancy and infection. We discuss these results with respect to game management and livestock prophylaxis.



## POSTER PRESENTATION 70

### Canine distemper virus (CDV) infection in free-ranging Iberian lynxes (*Lynx pardinus*)

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**Key words:** *Lynx pardinus*; canine distemper virus; real-time RT-PCR; phylogenetic analysis; serosurvey

Canine distemper virus (CDV) is a morbillivirus that is the etiological agent of one of the most important viral diseases affecting canids and an expanding range of other carnivores. Using real-time RT-PCR, CDV RNA was detected in organs of an Iberian lynx (*Lynx pardinus*) found dead in the Doñana National Park, Southwestern Andalusia, Spain. This finding may be of great importance for the conservation of the species; at present the Iberian lynx is the most critically endangered wild felid.

The aim of the present study was to elucidate the significance of CDV for the Iberian lynx population. High viral loads were evident in the dead lynx, suggesting an etiological involvement of CDV in its death. When carnivores from the same region were analyzed by CDV RT-PCR, a stone marten (*Martes foina*) was positive. Phylogenetic analyses demonstrated high identity of the two detected CDVs and a close relationship to the European dog lineage of CDV. Antibodies to CDV were detected in 14.8% of 88 tested free-ranging Iberian lynxes. The sample seroprevalence was significantly higher in lynxes from the Doñana Natural Space (22.9%) than Sierra Morena (5%). The stone marten and a red fox (*Vulpes vulpes*) also tested seropositive.

In conclusion, CDV is present in the Iberian lynx population, especially in the Doñana region, with sporadic cases of disease. To reduce the infectious pressure of CDV on this endangered population, a mass dog vaccination in the surrounding areas should be considered.

## POSTER PRESENTATION 71

### Canine distemper virus outbreak in a beech marten population in Flanders

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**Key words:** CDV

Canine distemper virus (CDV) causes a serious viral disease in domestic dogs and wild carnivores. It is a single-stranded RNA morbillivirus of the family paramyxoviridae. Other well known morbilliviruses are measles, rinderpest virus and phocine distemper virus.

In the spring of 2009, 25 beech martens (*Martes foina*) from places all over Limburg were brought to the “Wildlife Rescue Centre” of Opglabbeek. The diseased martens showed signs of lateral decubitus, tremor, convulsions, respiratory distress and conjunctivitis. They all died after a while. Due to the high number of sick animals and the mortality rate, five beech martens were necropsied following standard procedures. The animals were in a poor condition and showed signs of starvation. In most cases the lungs showed macroscopic and histological lesions of interstitial pneumonia. Histological research showed multiple acidophilic intracytoplasmic viral inclusions in pneumocytes and in lung, bladder and gastric epithelium. Additionally a non-purulent meningitis was found. Immunohistochemistry confirmed the presence of CDV in lung, brain and gastric tissue. Distemper virus N-protein RNA was amplified from brain, spleen and lung tissue with real time PCR. The morbillivirus was genetically identified as a CDV and phylogenetic analysis showed that it was 100% identical to an isolate from a beech marten in Germany.

Life history of beech martens in Flanders shows a particular evolution. From the end of the 19th century, extermination on a large scale caused a dramatic population decline, and the species disappeared almost completely. However, after WWII, a small bulwark was able to develop in the eastern part of Brabant and the southern part of Limburg. From the 1990's onwards, the population in Flanders is characterised by the start of a remarkable increase of density in the ‘historical’ bulwark region, and a steady ongoing area expansion. Actually, population density is still the highest in the south-eastern part of Flanders. To which degree this outbreak affects the growth of the beech marten population in Flanders remains an open question.

## POSTER PRESENTATION 72

### Cetacean morbillivirus tissue distribution using RT-PCR in striped dolphins (*Stenella coeruleoalba*) and long-finned pilot whales (*Globicephala melas*) stranded during the CeMV outbreak of 2006-2007

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**Key words:** morbillivirus, tissue distribution, striped dolphins, long-finned pilot whales

**Background:** Cetacean morbillivirus (CeMV) is considered the most pathogenic virus for cetaceans. Since CeMV was implicated in the bottlenose dolphin mass stranding along the Northwestern Atlantic during 1987-88, several epizootics in different odontocetes have been described worldwide. Main known target tissues for morbilliviral infections in dolphins concern nervous, respiratory and immunological systems. In the present study, a CeMV tissue distribution using RT-PCR was made for the first time in two species of cetaceans: striped dolphins (*Stenella coeruleoalba*) and long-finned pilot whales (*Globicephala melas*).

**Methods:** A novel RT-PCR detection of CeMV targeting the fusion protein gene was performed on 126 samples of different tissues from 26 cetaceans: striped dolphins (n=14) and long-finned pilot whales (n=12) stranded along Spanish coasts during the recent 2006-07 morbilliviral epizootics.

**Results:** The fusion protein gene of CeMV was detected in 14 individuals (7 pilot whale and 7 striped dolphins). Presence of CeMV tissues vary between the two species. For long-finned pilot whales, percentages were: lymph node, kidney and nervous system (100%), lung (85.7%), spleen (80%) and liver (66.7%). For striped dolphins, percentages were lymph nodes and spleen (100%), lung (83.3%), kidney (80%), nervous system (66.7%), and liver (25%).

Similar positives percentages were observed in lymph node and lung. Striped dolphins showed more percentage of positive spleen samples than long finned pilot whales, whereas the last presented more positive kidney, nervous system and liver samples.

**Conclusions:** The results obtained may indicate a different pattern of viral distribution between striped dolphins and long finned pilot whales. Further histopathological studies are required to establish if the observed differences by RT-PCR detection are also correlated to different patterns of lesions between these species.

## POSTER PRESENTATION 73

### Data supporting the active role of cottontail (*Sylvilagus floridanus*) in the epidemiology of EBHS

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**Key words:** eastern cottontail, serological surveys, EBHS, molecular characterization

**Background:** The eastern cottontail (*Sylvilagus floridanus*), an American lagomorph, was illegally introduced in Piemonte on 1966 and is currently widespread in several areas of North-Central Italy. A seroepidemiological survey was conducted in Alessandria province on 2000 to determine the role of cottontail as host or reservoir of hares' pathogens. As main outcome it was found that cottontails could have been naturally infected with EBHSV, developing a specific immunity. Thereafter, the infection of seronegative cottontails proved their susceptibility to EBHSV in experimental conditions.

**Aim of this work is a)** to report the results of serological surveys for confirming the previous data in a more wide territory; **b)** to report the first natural EBHS outbreak in a mixed population of hares and cottontail and **c)** the molecular characterization of the virus identified in dead animals.

**Methods:** From 2003 to 2009, 148 serum samples and 37 organs of cottontails, captured or found dead in North-Central Italy were analyzed. Serological (cELISA) and virological (sandwich ELISA, western blot and PCR) tests were performed using methods developed at the OIE Reference Laboratory. On late 2009 an EBHS outbreak occurred in a fenced area near Milano where a high density of both hares and cottontails was present. Animals found dead were examined and the EBHSV identified strains from both species were amplified, sequenced and compared.

**Results:** Serological investigations confirmed the presence of positive anti-EBHS titres in naturally infected cottontails. Moreover, during the EBHS outbreak, at least one cottontail found dead show typical gross lesions and tested virologically positive for EBHS (liver and spleen). The viral strains from hares and cottontail were amplified by PCR and VP60 products were sequenced, showing 100% identity.

**Conclusions:** It is even more evident that cottontail could be a natural host of EBHSV and may transmit it to hares.

## POSTER PRESENTATION 74

### Discovery and monitoring of an EBLV-1 infected serotine reproduction colony in France

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Key words: bat, rabies

The first bat rabies case has been identified in *Eptesicus serotinus* in 1989 in North East of France. The passive surveillance of bat rabies has been improved since 2000 under the request of veterinary services. The active one started in 2007 when a close collaboration of bat workers was established with AFSSA Nancy.

Since the improvement of the bat network, 45 rabid *Eptesicus serotinus* have been reported in the country infected by EBLV-1 (with respectively 10 and 35 cases of isoform a and b).

On 28 June 2009, bat specialists alerted AFSSA Nancy and local veterinary services about massive mortality in a serotine colony located in the roof of an old house in Ancy-Moselle (department of Moselle). 40 serotines had already been found dead by the owner of the house and were not analysed. A group of 9 juvenile serotines were received on the 29 June, 4 out of the 5 that could be analysed were shown infected by EBLV-1b.

This reproduction colony of 135 bats at the end of June was then monitored both passively and actively. This active surveillance consisted of micro-samples of oral swabs and blood for serology and virology taken from trapped/released bats. This surveillance lasted from the beginning of July to the end of October, when the colony left the house for hibernation. This survey respected all the legislative aspects of bat protection and conservation on one hand and all human health aspects on the other one.

The surveillance has shown that 6 serotines of the colony were diagnosed positive with traditional rabies tests. RT-PCR confirmed all the 6 cases and detected 2 more cases. 80 serotines were trapped/released during the active survey. The global results of active and passive surveillance will be presented with the monitoring of the colony (radio-tracking, counting of animals).

## POSTER PRESENTATION 75

### Epidemiologic study of pestivirus infection in both wild and domestic ruminants: a survey in the Ubaye Valley (Alpine mountains, France)

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Key words: pestivirus, ruminants, France

Since several years, Pestivirus infections have been widely documented among wild ruminants. Earlier epidemiologic studies often incriminated interspecies transmission between wild and domestic ruminants. In order to assess this statement, this study was carried out to investigate the apparent prevalence of pestivirus in both wild and domestic ruminants in a determined alpine valley. Five areas were identified for a relevant contact rate between wild and domestic animals.

In wild animals, samples (serum and spleen) were performed on hunted animals. Serum samples collected for brucellosis prophylaxis were used for the monitoring of domestic ruminants older than 2 years. Screening of pestivirus antibodies against p80 protein (named also NS3), common to all Bovine Viral Diarrhea Virus (BVDV) and Border Disease Virus (BDV), was achieved in all wild animal samples and in 10% of sera taken from domestic herds by blocking enzyme linked immunosorbent assay (ELISA) (Synbiotics, Lyon, France). Moreover, virus investigation was carried out in all samples collected from hunted animals and in association with local veterinarians for domestic ruminants (investigation based on clinical suspicion of persistently infected animals), using a conventional reverse transcription-polymerase chain reaction (RT-PCR).

For domestic ruminants, a total of 24 herds were screened. All herds were positive for pestivirus-specific antibodies. Individual sero-prevalence reached 76.5 % (95% confidence interval [CI95%]: [74.2 – 79.4 %]) of the 1039 animals tested. For wild ruminants, 29.7 % (CI95%: [19.6 – 39.8 %]) of the 79 chamois tested and 25.9% (CI95%: [9.4 – 42.4 %]) of the 27 roe deer were antibody positive. The results of the last virus investigations will be also presented.

These first results indicate a very high and unexpected seroprevalence in domestic ruminants, and an important one in wild ruminants. Epidemiological study has to be completed in order to establish the relationship between wild and domestic animals for the transmission of pestivirus.

## POSTER PRESENTATION 76

### Epidemiological surveillance of bluetongue virus serotypes 1, 4 and 8 in Spanish ibex (*Capra pyrenaica hispanica*) from southern Spain

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**Key words:** Bluetongue; Spanish ibex; Serotypes; Spain

Bluetongue (BT) is an arthropod-borne disease caused by a virus (BTV) belonging to the genus Orbivirus. BT is a reportable disease of socioeconomic impact in the international trade of ruminants. To date, 24 distinct BTV serotypes have been identified. In Andalusia (southern Spain), BTV-4 was detected in October 2004, BTV-1 was registered from July 2007, while BTV-8 appeared in October 2008 from northern regions. The aim of the present study was to evaluate the presence and circulation of BTV serotypes 1, 4 and 8 in Spanish ibex (*Capra pyrenaica hispanica*) between 2006 and 2009 in Andalusia, a region with a wide circulation of BTV in livestock.

Thirty-one out of 770 (4.0%; CI95%: 2.6-5.4) Spanish ibexes analyzed by ELISA showed antibodies against BTV. Twenty-four out of 31 seropositive samples were tested using virus neutralization test. Neutralizing antibodies against BTV-1 and BTV-4 were detected in seven and ten animals, respectively, four of them showed neutralizing antibodies to both serotypes. Unfortunately, seven sera could not be analyzed by VNT due to cytotoxicity of the sample. Seropositive animals to BTV-4 were found during the period 2006-2008, while BTV-1 circulation was confirmed from 2007 to 2009. None of the ibexes presented neutralizing antibodies against BTV-8. BTV RNA was not found in any of the 380 blood samples tested. However, BTV-1 RNA was detected in one out of 34 spleen samples analyzed.

Although BTV circulation was limited in Spanish ibex populations, the results of the present study demonstrate that Spanish ibexes were exposed and responded serologically to BTV-1 and BTV-4. The detection of BTV-1 RNA and the presence of seropositive Spanish ibexes in areas where BT outbreaks have not been detected in livestock confirms the susceptibility of this species and supports the idea that Spanish ibex could act as potential BTV reservoir in certain areas.

## POSTER PRESENTATION 77

### European brown hare syndrome: past, present and future

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**Key words:** lagovirus, hare, hepatitis, pathology

**Background:** European Brown Hare Syndrome (EBHS) is an acute viral hepatitis caused by a Lagovirus in the family Caliciviridae. It affects free-living and farmed European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*). The objectives of this review were: i) to provide a compilation of the current knowledge of EBHS, ii) to identify knowledge gaps which preclude the understanding of the epidemiology, and iii) to propose priority lines of research. **Methods:** In this study we review the accumulated, Europe-wide knowledge on the following aspects of the disease: history of the initial detection and emergence of the infection, clinical manifestations, pathology, pathogenesis, organ and cellular distribution of virus, immune response and diagnostics.

**Results:** EBHS was first described as a new epidemic disease of unknown cause in the early 1980's in Sweden. Its viral etiology was deciphered in 1989. In the 20 years that followed, EBHS was recognized in many European countries. Most hares die acutely within 48-72 hours after infection without showing signs of disease. In sub-acute forms hares show abnormal behavior, anorexia and depression. Hares which recover may develop chronic forms of disease with persistence of the virus in the liver. The most frequent pathological presentation is acute periportal or massive liver necrosis. The virus is localized in hepatocytes during early stages of infection and it persists in macrophages during later stages. An antibody response develops early and apparently confers life-long immunity. Little information is available about latent infections, pathogenicity of EBHS virus strains, length and route of virus shedding and other factors.

**Conclusions:** EBHS continues to be an important disease of hares in many countries of Europe. A small proportion of the hares develop chronic hepatitis and harbor virus in the liver for a period of unknown length. For diagnostics and epidemiological studies it is essential to understand the various manifestations of EBHS. Research on EBHS is part of a project from the European Union Seventh Framework Programme (2007-2013) under grant agreement n° 222633 (WildTech).

## POSTER PRESENTATION 78

### Experimental infection studies with chamois border disease virus

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**Key words:** Border disease virus chamois pig sheep

Severe outbreaks of disease in Pyrenean chamois (*Rupicapra pyrenaica*) associated with a new Border Disease Virus (BDV) have been reported in the Pyrenees (Spain and France) since 2001. The aim of this study was to investigate the disease under experimental conditions in three animal models: pig, sheep and Pyrenean chamois.

All animals were inoculated by oro-nasal route with a BDV isolated from a diseased chamois. We used 36 seronegative pigs for the first trial, 16 seronegative lambs for the second and for the third, we captured 11 chamois, infected 7 of them (5 seronegative and 2 seropositive) and 4 were maintained as controls. Whole blood, nasal, oral, rectal and urine swabs were collected. Necropsies, routine histopathological studies and immunohistochemistry were performed. RT-PCR, virus isolation and virus neutralization tests were used to detect BDV and seroconversion.

Neither clinical signs nor significant lesions were observed in any pig or sheep and BDV was detected between days 3 and 14 postinfection (pi) when neutralising antibodies appeared until the end of the experiment. Two infected seronegative chamois died of haemorrhagic diarrhoea and a third one died of bronchopneumonia. All but one of the 5 seronegative infected animals presented a persistent viraemia from day 2 pi until they died or were euthanized (day 34 pi) and neutralizing antibodies were observed in all infected chamois from day 11 pi until death. The major pathological findings were observed in brain and they were positive for BDV antigen by immunohistochemistry.

Chamois BDV is infectious in swine and sheep, and although the infection was subclinical, it can represent a major concern for pestivirus diagnosis in these species. In chamois, we conclude that BDV isolated from naturally-infected animals is the primary agent of the disease described for the first time in 2001.

## POSTER PRESENTATION 79

### Humoral and viral responses in red deer (*Cervus elaphus*) following vaccination and experimental infection with Bluetongue virus serotypes 1 and 8

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**Key words:** Bluetongue; Red deer; Vaccination; Infection; Serotypes

Bluetongue virus (BTV; Orbivirus, Reoviridae) is transmitted by biting midges belonging to *Culicoides* genus (Diptera: Ceratopogonidae) causing Bluetongue (BT), a reportable disease of domestic and wild ruminants. BT is a hemorrhagic, infectious and non contagious disease associated to climate change. Since BTV challenged Europe in 1998, vaccination and movement restriction of domestic ruminants are used as control methods.

In the present study, a susceptible species such as red deer (*Cervus elaphus*) was vaccinated and challenged against BTV. Four out of 12 deer were vaccinated against BTV serotype 1 (BTV-1) and four against serotype 8 (BTV-8). Four more deer were kept as unvaccinated controls, two per each serotype. Forty days after vaccination (dpv), all deer were challenged against the corresponding serotype. Serological and virological responses were analyzed from vaccination until 28 days post infection (dpi).

Results demonstrated that immunized deer reached higher specific antibody levels ( $p < 0.05$ ) from 40 and 34 dpv, for BTV-1 and BTV-8 vaccinated deer respectively, compared to unvaccinated animals. Vaccinated deer maintained stable neutralizing antibody titres until the end of the study (28 dpi). Unvaccinated deer remained seronegative until challenge and showed neutralizing antibodies from 7 dpi. Viral RNA was detected and isolated in the blood of non-vaccinated deer from 2 days after the BTV challenge until the end of study, whereas no BTV RNA was found in vaccinated deer. No statistically significant differences ( $p < 0.05$ ) in body temperature were observed. Clinical signs compatible with BT were very mild and no lesions were found at necropsy. In conclusion, this study has provided evidence indicating that red deer is BTV-1 and BTV-8 sensitive and that the administration of monovalent vaccines was safe and effective to prevent virus infection in red deer.

## POSTER PRESENTATION 80

### Isolation, characterization and associated-pathology of a canine morbillivirus with enhanced neuronal tropism

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**Key words:** Canine Morbillivirus, fox, neuronal tropism, Switzerland

An ongoing outbreak of canine morbillivirus (CM) infection spreading from the eastern borders of Switzerland and affecting red foxes (*Vulpes vulpes*), Eurasian badgers (*Meles meles*) and other mustelids, was first observed in 2009. In order to characterize the virus-associated pathology and to assess some of the features of the CM strain/s infecting the wild carnivore population, a thorough investigation was started.

From January 2009 to mid-April 2010, 126 animals either found dead or killed by game-wardens because of obvious respiratory and/or neurological clinical signs consistent with CM infection, including 101 red foxes, 18 badgers, 6 stone marten (*Martes foina*) and 1 pine marten (*Martes martes*), were sent to the Centre of Fish and Wildlife Health (FIWI) of the University of Bern for post-mortem examination. Full necropsy was performed on all animals, while immunohistochemistry, reverse transcription polymerase chain reaction (RT-PCR) and virus isolation attempts were performed on selected cases.

CM infection was confirmed by histopathology in 43 red foxes, 10 badgers, and 1 pine marten. Common lesions were broncho-interstitial pneumonia with syncytia and eosinophilic intracytoplasmic/nuclear inclusions; minimal to mild lymphohistiocytic meningitis to meningo-encephalitis with intranuclear inclusions mostly within neurons (also necrotic) and less frequently in glial cells. Lesions in the white matter were mild with demyelination and vacuolization. Inclusions were also seen in the pelvis and urinary bladder with minimal lymphocytic infiltrate, in the spleen with lymphoid depletion and in the gastro-intestinal epithelium with no obvious inflammatory response. Characteristic syncytial cells were observed in cell cultures. Partial amplification and sequencing of the viral nucleoprotein confirmed the identity of the infectious organism.

The histological findings are consistent with an unusual infection pattern of a Canine Morbillivirus strain characterized by systemic spread, marginal central nervous system inflammation and presumptive enhanced neuronal tropism. The ongoing investigation aims to shed lights on this hypothesis.

## POSTER PRESENTATION 81

### Management of an EBHS outbreak in captive European brown hares (*Lepus europaeus*)

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**Key words:** European Brown Hare Syndrome; haemorrhages; necrotising hepatitis; antibody development

The European Brown Hare Syndrome (EBHS) is a viral disease caused by a Calicivirus which affects only European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*). It is known to occur since the 1980's and was detected so far in most European countries. Outbreaks usually arise in autumn and show a rapid course of illness characterised by neurological disturbances, severe necrotic hepatitis, and haemorrhages. More often lack of clinical signs but peracute death is described especially in the wild. Here we described an outbreak which occurred in spring and lasted for 15 days in a captive hare colony in the field research station of the IZW in Niederfinow, Brandenburg. Routine necropsy, histology, and transmission electronmicroscopy were performed as well as PCR for virus detection and ELISA for antibody monitoring.

Overall pathological findings were severe necrotising hepatitis and splenitis. Mortality rate was 55 %. A significantly higher mortality rate was seen in animals younger than 12 months. Pregnant females either aborted their foetuses or died pregnant. Only one offspring born during the outbreak survived. Shortly after the outbreak, the surviving hares developed a specific anti-EBHS titre which decreased within nine months following the outbreak. Hares between one to three years of age developed a significantly higher titre than younger or older hares. Offspring born after the outbreak showed lower titres. After two months, that titre was not detectable any longer.

So far not much was known about the relationship between an acute outbreak and subsequent titre development. Though the outbreak described here was catastrophic for our breeding colony we had the opportunity to monitor the whole spectrum of pathological lesions and antibody development from the beginning which might help to close the gap of knowledge in EBHS.

## POSTER PRESENTATION 82

### Occurrence of infections with the bluetongue virus and the bovine viral diarrhoea virus in free-ranging wild ruminants in Switzerland

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**Key words:** bluetongue virus, bovine viral diarrhoea virus, survey, Switzerland, wild ruminants

In 2006, an outbreak of Bluetongue (BT) caused by the BT-virus serotype 8 occurred in central Europe. Switzerland participated in the vaccination campaign of domestic ruminants, following recommendation of the European veterinary authorities. In parallel, the Swiss veterinary authorities began an eradication programme for Bovine Viral Diarrhea (BVD) in 2008, aimed at eliminating persistently infected animals. The goal of the present study is to determine if wild ruminants may represent a reservoir for BTV and BVDV, and therefore interfere with the BVD eradication program, respectively contribute to the spread of BTV in Switzerland.

During the hunting season 2009-2010, blood samples were collected from 236 roe deer (*Capreolus capreolus*), 248 red deer (*Cervus elaphus*), 335 chamois (*Rupicapra rupicapra*) and 298 ibex (*Capra ibex*) from Switzerland (total: 1117 animals). Until now, 999 samples were screened for BTV antibodies with an ELISA (VMRD). Positive samples were subsequently tested with two other ELISAs (BDSL and INGEZIM) and by RT-qPCR. 509 samples of chamois and ibex were tested for BVDV antibodies with an ELISA (in-house). Seronegative samples were analysed by Real-Time RT-PCR and seropositive samples were additionally tested by SNT.

The presence of antibodies against BTV was confirmed in 7/999 animals (0.7%), which were PCR-negative. In contrast, 3/105 selected seronegative animals were PCR-positive for BTV-8. Concerning BVDV, 11/509 samples were seropositive (2.2 %) and all seronegative samples were PCR-negative. Preliminary results indicate that infections with BTV are rare in Swiss wild ruminants, in line with the low observed prevalence in livestock. Regarding BVDV, prevalence in chamois and ibex is low, but samples from cervids remain to be tested to draw further conclusions. A second sampling round will take place during the hunting season of 2010/2011, thus increasing the sample size and providing further information on the trend of BTV and BVDV infections in wildlife.

## POSTER PRESENTATION 83

### Pathological finding in Bluetongue virus experimental infected red deer

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**Key words:** pathology bluetongue experimental deer

**Background:** Pathology of bluetongue virus (BTV) infection has been studied in domestic ruminants, but also in white-tailed deer (*Odocoileus virginianus*). Clinical signs and lesion patterns in bluetongue virus (BTV) infection are associated with vascular disorders.

**Methods:** Four female red deer were inoculated with BTV-1; four with BTV-8; and three were controls. Skin biopsies were taken at 14 and 50 days post infection (dpi); and detailed necropsy were doing at 98-122 dpi. All samples were analyzed by histopathology and RT-PCR and immunohistochemical study were doing in selected tissues.

**Results:** The skin biopsies at 14 dpi were PCR positive in 5 of 6 infected animals, but those taken at 50 dpi yielded negative results. No macroscopic and microscopic lesions were found. At necropsy, only two deer showed mild and unspecific macroscopic lesions compatible with. No microscopic bluetongue-compatible lesions were found. Immunohistochemistry of the tissue samples obtained at necropsy revealed no stain with the protocols applied, and RT-PCR was positive for 13 selected post-mortem tissue samples.

**Conclusions:** Red deer can be infected with BTV serotypes 1 and 8 and develop an intense and long-lasting viraemia, although clinical signs or specific lesion could not be detected. Detection of BTV RNA in skin at 14 dpi, but not later, suggests that transmission of the virus to *Culicoides* vectors may be more efficient during the peak of the viraemia. In deer, immunohistochemical studies could be better results in frozen or bouin-fixed tissues than in formalin-fixed ones.

## POSTER PRESENTATION 84

### Recurrent local mortalities of eurasian collared doves (*Streptopelia decaocto*) in Southcentral Spain due to aPMV-1

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**Key words:** avian Paramyxovirus 1, Eurasian collared dove, reovirus

**Background:** The Eurasian collared dove (*Streptopelia decaocto*) is a small gregarious sedentary columbiform species that since the 1960s has greatly expanded in numbers and distribution range throughout most of Europe. In South central Spain the species is generally found in small to large colonies near human settlements. Since 2005 recurrent mortality events have been reported for this species from different localities situated in a radius of approximately 100km in a cerealistic area spiked with sporadic shallow wetlands. Mortalities are generally observed in winter and only on two occasions live individuals could be observed displaying central nervous signs. Gross lesions are nonspecific consisting mainly of generalised congestion and enlarged kidneys. No other avian species were affected during the outbreaks.

**Methods:** Tissue samples of the most recently dead individuals were collected during necropsy and submitted for RTPCR, viral culture in chicken embryo fibroblast and liver cells and processed for histopathologic examination.

**Results:** Avian Paramyxovirus 1 (aPMV-1) was detected by both, culture and real time RTPCR in brain, spleen and lung tissue of individuals of three of the five mortality events and was identified as highly pathogenic pigeon type aPMV-1. Additionally another virus was isolated from the kidneys of doves from the first (largest) outbreak in 2005 and identified as avian Reovirus. Histologically nonpurulent encephalitis and severe tubular necrosis in the kidneys were the most important findings.

**Conclusions:** Infection with pathogenic pigeon type aPMV-1 is thought to be the cause of the mortality events in Eurasian collared doves in the region. Concurrent factors that lead to the outbreaks such as potential infections with other pathogens (reovirus) or debilitating conditions (cold, starvation) are under investigation.

## 6. ECOLOGY

### POSTER PRESENTATION 85

#### Association of Roe deer mortality with *Anaplasma phagocytophilum* infection – a region-wide study in contrasted landscapes

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**Key words:** *Anaplasma phagocytophilum*, Roe deer, mortality, spatial distribution, landscape

Several causal factors have been suspected in recent episodes of grouped mortalities in Roe deer (*Capreolus capreolus*) in Europe. To investigate the role of infection with *Anaplasma phagocytophilum*, a region-wide case-control study was designed (region of Franche-Comté, eastern France). From september 2007 to May 2008, 36 diseased and dead Roe deer were collected in 12 biogeographical units, in a diversity of landscapes. Controls were 114 healthy animals killed by hunting, representative on age, sex and spatial distribution criteria. Serological testing and PCR detection of *Anaplasma phagocytophilum* were performed in both groups. Necropsy findings were available for 25 cases. Mixed generalized models were used to assess the association between tests results and health status (diseased or dead vs. healthy) of the Roe deer, after adjustment for the sex and age of the animal, and the geographical unit where the sampling was performed. A vast majority of animals from diseased and healthy categories (88% and 90 % respectively) were positive for anti-*A. phagocytophilum* antibodies, but no statistically significant ( $P=0.68$ ) difference was evidenced between categories. The nested-PCR detection of 16S rRNA gene on blood samples was positive in respectively 88 % and 69 % of diseased and healthy animals. The risk for a Roe deer of being diseased or dead was significantly higher ( $OR=4.19$ ,  $P=0.034$ ) if tested PCR positive, than if being tested negative. None of the putative causes of death that were identified at necropsy (trauma, polyparasitism, respiratory pasteurellosis...) matched the hypothesis of acute anaplasmosis. Incidence of mortality was lower than preceding years, and no clustering in space or time was detected during this sampling period. Thus, infection with *Anaplasma phagocytophilum* appears widespread throughout the entire region. The positive association of disease or death with PCR detection in blood may be explained by indirect effects of the infection on Roe deer health.



## POSTER PRESENTATION 86

### Babesiosis survey in roe deer (*Capreolus capreolus*), and wild boars (*Sus scrofa*), from North West and Central Italy

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**Key words:** *Babesia bigemina*, *B. microti*, *B. divergens*, roe deer, wild boar

**Background:** Babesiosis are tick-borne disease due to an intraerythrocytic parasite belonging to the genus *Babesia* (Apicomplexa, Piroplasmida). Infection is one of the most common among free-living animals worldwide and is gaining increasing attention as an emerging tick-borne zoonosis in humans. Considering that no data are available from wildlife in the North West of Italy, we decide to investigate Roe deer and wild boar collected from North-West of Italy and from Isola d'Elba.

**Methods:** Total genomic DNA was extracted on spleens collected from 134 roe deer and 99 wild boar, using the commercial kit GenomeEluteTM. The samples were tested with the PCR *Babesia*/*Theileria* catch-all according to the protocol described in Gubbels et al. (1999, J Clin Microbiol, 37: 1782-1789). PCR positive samples were sequenced after cloning and aligned with ClustalX v1.83, and analysed with Phylo Win 2.0 software. We performed Neighbour-Joining with 1000 bootstrap replicates to obtain a high confidence degree.

**Results:** Forty-five (33.5%) out of 134 roe deer and 21 (21.2%) of 99 wild boar were PCR positive. The results after sequencing show that roe deer, regardless of their origin, and wild boars from Piedmont were infected by *B. bigemina*. On the contrary in wild boars from the Isola d'Elba the *Babesia* species involved are: *B. bigemina*, *B. microti* and *B. divergens*. The phylogenetic tree shows that there are 4 different clusters of *B. bigemina*, and that those from the Isola d'Elba are separated from the ones found in Piedmont.

**Conclusions:** Our results highlight the abundance of a potential zoonotic Babesian species in wild animals. We are now investigating the similitude of the strains we found with *Babesia* spp. genotype EU1, that have in single cases also been identified in splenectomized humans. This research was financed by the Regione Piemonte Assessorato Agricoltura and the Parco Nazionale Arcipelago Toscano.

## POSTER PRESENTATION 87

### Cerebral coenurosis in mountain ungulates in the French Alps: an interaction between domestic life and wildlife or an evidence of a sylvatic cycle?

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**Key words:** coenurosis, *Taenia multiceps*, wild ungulates, France

The SAGIR network investigates and records the causes of mortality amongst wild animals in France since 1986. When wild animals are found dead or sick by hunters, hunting federations or public technicians of the ONCFS they are collected and sent to the local veterinary diagnostic laboratory. Post-mortem examination and subsequent laboratory analyses are then carried out for every wild bird and mammal collected through the SAGIR network. All the data are summarized at a national level (AFSSA Nancy) for epidemiological statistics.

In 25 years, we report 7 clinical cases of cerebral coenurosis in free-ranging mountain ungulates in France. Four were diagnosed in chamois (*Rupicapra rupicapra*), 1 in ibex (*Capra ibex ibex*) and 2 in mouflon (*Ovis gmelini musimon*), all located in the Alps where pastures are widely shared between domestic flocks and wild ungulates during summer. All these animals were found alive or seen alive a few moments before death: they showed neurologic symptoms (amaurosis, bruxism, ataxia, loss of fear against humans). Gross pathological findings are detailed. A coenurus occupying until 60% of the brain was found in all animals.

The larval stage of *Taenia multiceps* is commonly found in the brain of sheep, whereas the adult form of the tapeworm can be found in dogs. The finding of this parasite in wild ungulates is probably due to the presence of sheepdogs in the mountain pastures, as this parasitological disease is commonly seen in domestic herds. However, the potential role of wild carnivores like foxes in the parasitic cycle should also be considered.

People should be aware of the potential zoonotic risk from the dog as some rare but severe zoonotic cases of cerebral coenurosis have been reported in human medicine. Particular attention should be given to the contamination of dogs feeding on dead sheep.

## POSTER PRESENTATION 88

### Chlordecone Contamination of the French Island of Martinique: Landbirds are not spared

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**Key words:** chlordecone, birds, ecotoxicology, contamination

Chlordecone is an organochlorine insecticide used until 1993 in the banana plantations of the French Antilles. Like other organochlorine pesticides such as DDT, Chlordecone is extremely persistent in soils (estimated half-life of 10 years), accumulates in the fatty tissue of living organisms and is toxic to humans and wildlife. Even though the use of Chlordecone was banned in 1993, surveys conducted by the French Department of Environment and the French Department of Health in 2001 revealed its wide presence in soils and rivers in Martinique.

According to the short-term and long-term toxic effects of chlordecone on animals, a study is performed to measure the level of contamination of wildlife in Martinique. Some sedentary species were selected to compare the levels of contamination in soils and water with the levels in animals. Zenaida Dove (*Zenaida aurita*) and Snowy Egret (*Egretta thula*) are the two selected bird species according to their ecology. The first one is granivorous and is hunting in Martinique. The results will be important for the risk assessment for the consumers. The second species is piscivorous and will give some informations about the biomagnification of chlordecone in an aquatic ecosystem. Biologic samples (liver, blood and eggs) are submitted to the toxicology laboratory at the Veterinary College (Lyon, France). They are analysed according to a published technique for the analysis of chlordecone in animal tissues. Up to now, 120 samples were analysed. The results showed a high level of contamination in Zenaida Dove caught in contaminated fields. Sampling in Snowy Egret is under way.

## POSTER PRESENTATION 89

### Detection of botulism toxins in serum compared to liver in waterfowl

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**Key words:** Botulism, toxins, waterfowl, comparison

**Background:** Botulism (western duck disease) is causing many deaths in waterfowl in the Netherlands each year. Botulism in waterfowl is caused by the C type botulism neurotoxin (BoNT C) produced by the bacterium *Clostridium botulinum*. To distinguish botulism from other differential diagnosis like intoxication by cyanotoxins, testing on botulism toxins is performed. The standard diagnostic sample to detect botulism toxins is the liver, after post mortem examination.

The aim of this study is to investigate the diagnostic value of serum samples compared to liver samples in the detection of botulism in waterfowl

**Methods:** We screened 35 (water)birds from different locations suffering from symptoms of botulism and analyzed bloodserum samples and liver samples simultaneously for the presence of BoNT's by the mice bioassay according to the CDC protocol.

**Results:** In 26 birds BoNT C could be detected in the serum but not in the liver, in one bird we found BoNT C in the serum as well as in the liver and 8 birds appeared to be negative for BoNT's in the serum as well as in the liver.

**Conclusions:** In conclusion BoNT C could be detected more sensitive in serum samples compared to liver samples. This will improve laboratory diagnosis and opens the way to an earlier and easier detection of botulism toxins in waterfowl as transport of the birds to the laboratory is not needed.

## POSTER PRESENTATION 90

### Detection of *Leishmania infantum* in red foxes (*Vulpes vulpes*) from south-east France using real-time quantitative PCR

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**Key words:** Leishmaniosis, Fox, Epidemiology, France, Reservoir

**Background:** Zoonotic leishmaniosis caused by *Leishmania infantum* is endemic on the French Riviera. Dogs serve as the main reservoir of the parasite. The potential role of wild carnivores as sentinels or reservoirs of *Leishmania infantum* is still a matter of debate. We conducted a longitudinal epidemiological survey in order to assess the carriage of *L. infantum* in red foxes from the department of Var, in south-east France, using a real-time quantitative PCR.

**Methods:** From 2006 through 2009, we collected samples of spleen (and other organs whenever possible) from 86 red foxes, 84 from the military camp of Canjuers and 2 others no far from the city of Hyères. In the laboratory, a real-time quantitative PCR was run for the detection and quantification of *Leishmania infantum* DNA in biological samples. The kinetoplast DNA was chosen as the molecular target.

**Results:** Seven of the 84 red foxes were found positive, 5 from the camp of Canjuers (5/84 - 6%) and 2 from Hyères (2/2).

The number of *Leishmania* varied from 0.03 to 12000 per 106 nucleate cells. We found *Leishmania* DNA in spleen (6/86), liver (2/38) but not in kidney (0/23), skin (0/14) or blood (0/15) samples. Kidney samples were available from 6 of the 7 positive animals and all proved negative. No suspect lesion of leishmaniosis was observed at necropsy.

**Conclusions:** The endemic area for leishmaniosis of the French Riviera is only a few kilometres south of the camp of Canjuers. Hyères is inside the endemic area. All the parasitic loads were low except for one red fox from Hyères. The results of our study highlight the role of red foxes as relevant sentinels for leishmaniosis expansion northwards. In the endemic area, our observations reinforce the assumption that red foxes can serve as feral reservoir for *Leishmania*.

## POSTER PRESENTATION 91

### Detection of *Rickettsia massiliae* in *Rhipicephalus turanicus* ticks collected from red foxes in Marseille (France)

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**Key words:** *Rickettsia massiliae*, *Rhipicephalus turanicus*, tick, fox, rickettsiosis, reservoir

**Background:** Recently, there has been an increase in the population of red foxes (*Vulpes vulpes*) in the suburbs of Marseille (France). Foxes are often reported to be infested by ticks, especially of *Rhipicephalus* sp. The ticks of this genus are notorious vectors of Mediterranean spotted fever (*Rickettsia conorii*). Another species of *Rickettsia*, *Rickettsia massiliae*, was first isolated from *Rhipicephalus* ticks in Marseille in 1993. The first isolation from a patient was reported in 2006 in Sicily.

**Methods:** Two foxes killed by hunters in the suburbs of Marseille in 2008 were examined for the presence of ticks. We have found 50 and 23 hard ticks, on each fox. All ticks were identified as *Rhipicephalus turanicus*. Total DNA was extracted from whole ticks. We have tested tick DNA by *gltA*-based qPCR system for the detection of all rickettsial species, internal transcribed spacer-based qPCR for *Bartonella* species and *Rickettsia massiliae*-specific qPCR system.

**Results:** *Rickettsia* genus-specific qPCR was found positive in 33 of 73 ticks (45.2%). qPCR specific for *R. massiliae* showed that all 33 positive ticks contained DNA of this species. DNA of *Bartonella* was not found in any of ticks.

**Conclusions:** This study reports the first detection of humanpathogenic *Rickettsia massiliae* in ticks from red foxes. Our data confirm the role of the fox as the host for *R. turanicus* ticks and provides the background for further studies on the possible role of foxes as a reservoir for rickettsiae.

## POSTER PRESENTATION 92

### Effect of blood lead levels on the constitutive immune response in white-tailed sea eagle (*Haliaeetus albicilla*) nestlings

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**Key words:** lead toxicity, innate immune response, white-tailed sea eagle, nestling

**Background:** Lead intoxication from hunting ammunition is the most important mortality factor of white-tailed sea eagles (*Haliaeetus albicilla*), however little is known about the effects of lead exposure on the avian innate immune system.

**Methods:** Here we investigated the effect of chronic lead toxicity on the constitutive immune response in white-tailed sea eagle nestlings from Northern Germany. Besides measuring lead concentration in the blood, we quantified different effectors of the innate immunity. We measured the levels of the natural antibodies and complement using a hemolysis-hemagglutination assay (n = 26, 13 females and 13 males) and leukocyte concentration and differential leukocyte counts (n = 19, 10 females and 9 males) of eagle nestlings sampled in spring 2004 and 2005.

**Results:** The level of the accumulated lead was highly variable in white-tailed sea eagle nestlings (range: 0.086 µg/L – 619,6 µg/L). Eagles exposed to high lead concentrations are stressed, which is reflected in the increased heterophil to lymphocyte ratio (F1,15 = 5.506, P = 0.033). After controlling the nestling sex and capture year, increased lead levels were negatively correlated with levels of the complement and the total white blood cell count (F1,22 = 6.906, P = 0.015; F1,15 = 4.32, P = 0.0552, respectively), while the blood's lead concentration was not significantly related to natural antibody concentration (F1,22 = 0.14, P = 0.71).

**Conclusion:** The results of our preliminary study suggest that lead toxicity can cause immunosuppression in white-tailed sea eagle nestlings, however further studies are needed to describe (1) consequences on the animals' body condition and survival and (2) pathogen susceptibility in lead-associated immunosuppressed individuals.

## POSTER PRESENTATION 93

### Identification and characterization of *Mycoplasma* spp. and *Salmonella* spp. in griffon vultures and wild tortoises housed in a Wildlife recovery centre in Sardinia (Italy)

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**Key words:** Vultures, Tortoises, *Mycoplasma*, *Salmonella*, Wildlife centres

Wildlife recovery centres are essential infrastructure for the care, management, breeding and conservation of many wildlife species. On the other hand, they represent a reservoir of different pathogens, as often animals originating from different localities are kept together, housed in captive and/or crowded conditions, sometimes for long recovery times. We selectively screened the birds of prey and the tortoise populations at the wildlife centre Centro Fauna Bonassai in Sardinia (Italy), by taking tracheal and cloacal swabs, and by applying molecular and immunoblotting techniques, apart from standard microbiological isolation methods. Two novel *Mycoplasma* spp. were isolated from the upper respiratory tract of four Eurasian griffon vultures (*Gyps fulvus*) and phylogenetically classified within the *Mycoplasma* taxonomy at the group and cluster levels. We detected the pathogenic *Mycoplasma agassizii* in *Testudo marginata* and *T. hermanni* individuals recovered in the centre, and also *Salmonella enterica* serotype Abony, with relevant zoonotic implications, in *T. marginata*. Different levels of pathogenicity and prevalence of these strains have different implications for the management and reintroduction of vultures and tortoises.

## POSTER PRESENTATION 94

### Impacts of climate change on the life cycle, distribution, and host range of the winter tick (*Dermacentor albipictus*) in the Arctic and Subarctic

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**Key words:** *Dermacentor albipictus*, winter tick, climate change, distribution range, serology

Fluctuations in climate directly affect the ecology and phenology of animals and plants, including ticks. The winter tick (WT) *Dermacentor albipictus* is a parasite of deer, elk, woodland caribou and moose. During the 1980s its northern distribution was limited to southern Yukon, however, in recent years, it has been detected further north. This expansion may be associated with climate change driven shifts in WT life cycle and host distribution, and may pose threats to the barren-ground caribou (BGC) population in the Canadian north that are facing a significant decline. The objectives of this research are: (1) to develop a serological assay to detect eventual exposure to WT in BGC; (2) to determine historical and current WT host range and geographic distribution, and (3) to evaluate climate factors linked to WT distribution. Nineteen engorged female ticks are being raised in laboratory conditions to lay eggs until become larvae and captive reindeer will be experimentally infested and used as model species to validate the test. The serological assay will be developed extracting a protein from WT saliva using Western blot, and this protein, used as antigen in ELISA test to detect WT exposure on BGC serum. Chemical digestion of hides from hunted moose and BGC together will also be used to delineate current WT distribution in Northwest Territories and Yukon. Data from 27 weather stations across Northern Canada were used to estimate the change in suitable range for the WT using previously developed weather indicator (degree days above - 5.6°C) from the late sixties (1965-1969) until now (2005-2009) showing an impressive expansion of WT potential range, confirmed by current data (WT found 700 Km northern than in the 60s). Serology and chemical digestion will allow to assess the current WT distribution, its determinants, and predict its future distribution under different climate change scenarios.

## POSTER PRESENTATION 95

### Influence of tick (Acari: Ixodidae) hosts on the epidemiology of zoonotic tick-borne pathogens in natural foci

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**Key words:** *Anaplasma phagocytophilum*; *Borrelia burgdorferi*; *Ixodes*; *Haemaphysalis*; Host

Tick-borne diseases are currently of great concern worldwide because of their increasing geographic range and emergence in naive areas. Tick hosts may drive tick ecology and therefore tick-borne pathogen epidemiology. The complex scenario in which tick-borne pathogens have evolved makes it necessary to analyse the influence of local environmental factors in the different distribution areas of pathogens. This would allow for a global understanding of influential factors as well as for the consideration of local peculiarities influencing the epidemiology of tick-borne pathogens. We aimed to study the role of adult tick hosts (i.e. wild/domestic big mammals) on ticks and on the dynamics of two zoonotic tick-borne pathogens, *Borrelia burgdorferi* s.l. and *Anaplasma phagocytophilum*, in natural foci of Atlantic climatic areas of the Iberian Peninsula.

To test our hypotheses we surveyed 9 sites in northern Iberian Peninsula. We estimated tick abundance by dragging the vegetation with a blanket, and host abundance by means of dung counts. We tested the influence of host abundance on tick abundance and pathogen prevalence by means of multivariate statistical models.

Captures consisted in 5771 ticks of the genera *Ixodes* and *Haemaphysalis*. Mean prevalences of *A. phagocytophilum* and *B. burgdorferi* s.l. in *I. ricinus* nymphs were  $20.5 \pm 3.7\%$  and  $4.0 \pm 1.8\%$ , respectively. We found a positive influence of host abundance on larvae and nymph abundance. Models highlighted the negative effect of big mammals' abundance on *B. burgdorferi* s.l. prevalence and its positive influence on *A. phagocytophilum* prevalence.

Understanding how current environmental conditions drive vector population abundance and pathogen ecology may provide knowledge to be used as a control tool to prevent the expansion of already present vector-borne diseases at regional or local scales. We herein provide a first approach to understanding the influence of hosts and other environmental factors on the population dynamics of several relevant tick species and on the most relevant tick-borne pathogens in Atlantic areas of the Iberian Peninsula.

## POSTER PRESENTATION 96

### The geographical distribution of *Ixodes ricinus* in Norway

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**Key words:** *Ixodes ricinus*, tick, geographical distribution, GIS

The geographical distribution of *Ixodes ricinus* in Norway

**Abstract:** The objective of this study was to determine the distribution of the tick *Ixodes ricinus* in Norway. In Scandinavia seasonal climatic conditions are limiting factors restricting the northern distribution. Possible climate effects are most noticeable close to the vectors geographical distribution limits as represented in the north by Norway.

**Methods:** Historical and current data ranging from Tambs-Lyche (1943) and Mehls (1983) published maps, notifications of human and animal tick-borne diseases from 1990-2008, a survey by the national newspaper "Aftenposten" in 2009 and a tick- questionnaire survey performed amongst clinical veterinarians in 2009 were investigated by descriptive and explorative methods, as disease-maps and localization of observed ticks in a geographical information system (GIS).

**Results:** There was a high consistency between the different datasources. *Ixodes ricinus* has now established itself in municipalities where it previously was absent and the study also revealed municipalities with a well established population of ticks but with no presence of tick-borne diseases as Lyme borreliose in humans and Babesiosis in cattle.

**Conclusion:** The different maps show shift in latitude and altitude distribution of *Ixodes ricinus* in Norway, compared to previously published maps in 1943 and 1983. This indicates that the proportion of the human population in Norway potentially at risk for being exposed to tick-borne pathogens may be increasing.

## POSTER PRESENTATION 97

### The role of wildlife in the ecology of human African trypanosomiasis

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**Key words:** wildlife, trypanosome, vector-borne, ecology

Human African trypanosomiasis (HAT) is an important neglected and re-emerging zoonosis. Caused by *Trypanosoma brucei rhodesiense* and transmitted by a tsetse vector, HAT is fatal without treatment and represents a significant public health threat in sub-Saharan Africa. Wildlife are frequently implicated as HAT reservoirs but the ecology of the disease is complex and not well understood. To explore the role of wildlife in the ecology of HAT in Serengeti National Park, Tanzania, over 600 samples were collected from a wide range of wildlife species and analysed for trypanosomes using PCR, the first substantial study on trypanosomes in wildlife using molecular techniques. Prevalence and risk factors for *T. brucei* s.l. and *T. brucei rhodesiense* infection were assessed. In addition clonal sequence analysis was used to investigate the other trypanosome species circulating in wildlife. *T. brucei* s.l. was found in 14 species, with prevalence varying with species, age and location. *T. brucei rhodesiense* was identified in lion (*Panthera leo*), hyaena (*Crocuta crocuta*) and reedbuck (*Redunca redunca*). Sequence analysis identified several other trypanosome species circulating in wildlife, including potential identifications of some trypanosome species not previously identified in these species. The significance of these results for the role of wildlife in the ecology of HAT will be discussed.

## POSTER PRESENTATION 98

### Ticks infesting wild animals in Northern Italy

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**Key words:** Ticks, wild animals, Northern Italy

**Background:** The aim of this study was to provide more data on the Ixodid fauna of wildlife collected in Emilia Romagna and Lombardia in order to better understand their ecology.

**Methods:** Ixodid ticks were collected from roe deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*), red fox (*Vulpes vulpes*), wolf (*Canis lupus*), hedgehog (*Erinaceus europaeus*), shrew (genus *Sorex*), badger (*Meles meles*) and european brown hare (*Lepus europaeus*). Ticks were removed and identified following taxonomic standard keys. Prevalence differences among host species, tick species and collection period were tested by  $\chi^2$  test.

**Results:** Ticks were collected from August 2008 until December 2009. A total of 3,225 ticks removed from 491 animals were identified. Ticks belonged to nine species: *Ixodes ricinus* (n=2,222; 68.9%), *Rhipicephalus sanguineus* (n=647; 20%), *Dermacentor marginatus* (n=172; 5.3%), *Ixodes canisuga* (n=119; 3.7%), *I. hexagonus* (n=53; 1.64%), *Hyalomma marginatum* (n=6; 0.18%), *Haemaphysalis punctata* (n=3), *I. acuminatus* (n=2), *Hae. concinna* (n=1). *I. ricinus* tick represents the most frequently detected species in our habitats. Also, we found that *D. marginatus* is often found on wild boar (34/44); *I. canisuga* parasites fox only and, interestingly, we found some specimens of the brown dog tick (*Rh. sanguineus*) on foxes, roe deer, wild boars and hares. Significant difference (p<0.01) was found in seasonality for *I. ricinus*, *Rh. sanguineus* and *D. marginatus*, which were the more frequently sampled species.

**Conclusions:** Our data confirm that *I. ricinus* is the dominant species in roe deer, red deer and hares in Emilia Romagna and Lombardia regions, while *D. marginatus* is strongly associated with wild boar. Passive surveillance on hunted wild fauna could provide a useful and economic tool to collect data on ticks and to achieve a better understanding of tick host preference for wild vertebrate species.

## 7. WILDLIFE MANAGEMENT

### POSTER PRESENTATION 99

#### Assessment of factors influencing the occurrence and pathological picture of sarcoptic mange in red foxes (*Vulpes vulpes*)

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**Key words:** Epidemiological pattern, influencing factors, *Sarcoptes scabiei*, Switzerland, *Vulpes vulpes*

Sarcoptic mange can have devastating effects in wild populations. However, the clinical and pathological pictures of mange show notable variations, both between animal species and between individuals. The aim of the present study was to assess the importance of potential influencing factors, namely host age and gender, concomitant diseases and infections, chronic intoxication, season, geographical origin (including epidemiological pattern, hunting management and climate), and mite genetic on the occurrence and severity of mange in the red fox (*Vulpes vulpes*). 153 mangy, 124 healthy and 14 non-mangy but diseased foxes were collected in 2004-2006 from several geographic areas of Switzerland with different epidemiological patterns of mange. All foxes were necropsied and selected cases were submitted to histological, parasitological, bacteriological and toxicological (chloralosis) examination. Mange cases were classified into type A (early lesions), B (chronic, fatal lesions) and C (healing stage). Specimens of *Sarcoptes scabiei* were genetically analysed. Information on hunting management was collected by telephone interviews, and data on climate (temperature, precipitations) were provided by Atlas of Switzerland ([www.atlasderschweiz.ch](http://www.atlasderschweiz.ch)).

Mange occurred significantly more frequently in mild-humid than both cold-humid and cold-dry climate zones. Significant differences between types of mange lesions were detected depending on the geographical origin of the foxes and the presence of a severe infestation with endoparasites. Type B was more often observed in relatively recently infected areas compared to those where mange has existed for a longer period of time. All other considered factors did not reveal significant differences. Our results suggest that climate influences are relevant for the occurrence of mange but not for its severity. Association between mange and endoparasitosis may indicate decreased fitness of the affected animal. Most importantly, animals from an area where mange has been endemic for a long time seem more likely to survive the disease, which possibly indicates a selection process.

## POSTER PRESENTATION 100

### Capture, chemical immobilization and post-release monitoring of wild boar (*Sus scrofa*) in South Central Spain

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**Key words:** wild boar, anaesthesia, post-release monitoring, mortality, tiletamine-zolazepam-medetomidine

**Background:** We aimed testing an anaesthetic protocol (tiletamine-zolazepam in combination with medetomidine hydrochloride) to handle wild boar reducing stress and risk to animal in order to perform scientific studies. For this purposes, post-release monitoring of previously immobilized animals is also essential.

**Methods:** During 2009 and 2010 we captured wild boar in South-central Spain (twenty up to date, but captures will continue throughout summer 2010) using iron mesh portable box traps and corrals baited with maize. Once trapped, the wild boar were anaesthetised by either IM hand injection with syringe or a blow gun according to the difficulty of approach. Anaesthesia procedure was assessed using anaesthetic, clinical (blink reflex, blood oxygen saturation, heart and respiration rates) and blood (haematology and serum biochemistry) variables. At the end of the anaesthesia, the wild boar were released at the point of capture and post-capture survival and monitored using GPS collars.

**Results:** Our average error of body estimation weight was 10.9 %. Mean time until incoordination was 2.6 min, induction time was 4.8 min, and recovery time lasted until 71.7 min (mean hypnosis time was 66.9 min). No heart or respiratory failure due to added stress occurred. Post-release monitoring by GPS-devices revealed no mortality due to anaesthesia. Corrals conveyed additional difficulty of approach for chemical restraint.

**Conclusions:** Post release monitoring in the wild is crucial to evaluate any adverse effect of anaesthetic drugs in wild animals. The use of our immobilizing agents at the describe dosages for wild boar allowed for manipulating them safely, apparently without risk for the animal. In summary, with a dosage rate 3 mg/kg tiletamine-zolazepam and 0.05 mg/kg medetomidine, all animals slept for more than one hour in average (minimum 23 min), with the advantage of being reversible with atipemazol. The very agitated recovery phase previously described for tiletamine-zolazepam alone was not evidenced in this study.

## POSTER PRESENTATION 101

### Contrasting health status in two populations of roe deer

Gilot-Fromont, Emmanuelle<sup>1</sup>; Benoit, Etienne<sup>2</sup>; Gaillard, Jean-Michel<sup>1</sup>; Gibert, Philippe<sup>3</sup>; Bonenfant, Christophe<sup>1</sup>; Mastain, Olivier<sup>3</sup>; Klein, François<sup>3</sup>

Université de Lyon<sup>1</sup>; VetAgro-Sup<sup>2</sup>; Office National de la Chasse<sup>3</sup>

**Key words:** hematology; immunocompetence; roe deer;

**Background:** Possible determinants of disease spread and emergence include host, parasite and environmental causes. While pathogen characteristics and transmission patterns have been largely investigated, host susceptibility and its variation among hosts have received less attention as possible causes of disease spread.

**Methods:** We compared two populations of roe deer *Capreolus capreolus*, located in areas with contrasted climatic conditions and resource availability, and showing opposite demographic regimes. The Trois-Fontaines population faces with favorable habitat in a highly productive mixed forest, whereas the Chizé population is limited by frequent summer droughts in a less productive forest. As a result, the roe deer population at Trois-Fontaines has a colonizing demographic regime, whereas the population at Chizé is no longer growing. Using blood samples obtained during the winter 2009-2010 capture sessions, we measured hematological, biochemical and immunological parameters in 73 and 113 roe deer at Chizé and Trois-Fontaines, respectively.

**Results:** As expected from the marked differences of demographic performance between populations, many parameters strongly differed between Chizé and Trois Fontaines. The most striking differences involved red blood cell lineage and biochemical parameters. At Chizé, roe deer had lower hemoglobin levels, associated to a trend to hypochrome macrocytic anemia. Other clear differences included lower levels of albumin and creatinine and higher levels of globulin and haptoglobin at Chizé compared to Trois-Fontaines. Overall, these results suggest a lower health status at Chizé, with more frequent nutritional deficiencies and inflammatory processes than in Trois-Fontaines.

**Conclusions:** Physiological parameters indicated a better health condition at Trois-Fontaines than at Chizé, matching the difference previously reported in demographic performance and phenotypic quality between these populations. Future works will aim to test whether this difference of health status is related to different levels of disease spread or other stressing factors.



## POSTER PRESENTATION 102

### Do populations of wild dogs threaten human health in Indigenous communities of the Wet Tropics?

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<sup>1</sup>James Cook University

**Key words:** Indigenous community, dingo, wild dogs

**Background:** Wild dogs pose a threat to biodiversity conservation, the health of domestic animals and humans in the Wet Tropics. Indigenous communities are a particular high risk group due to their general lack of dog management. Previously the link between dog health and human health in Indigenous communities has been lacking in scientific data support. By using a multidisciplinary approach to the project and integrating results, a better understanding of the nature of the wild dog problem in the Wet Tropics can be determined.

**Methods:** Camera traps are set to capture vision of wild dog movements and faecal scats will be collected for disease analysis. Dogs in the community will be given a veterinary health check and samples (faeces, blood, skin scrapings) will be taken for further disease analysis.

Additional samples will be collected from dogs trapped in control operations in the area. These dogs will be humanely killed and necropsied to provide more informative samples for disease analysis and parasite prevalence. Environmental samples will be collected from public recreational areas. Analysis of samples will be undertaken using conventional and molecular diagnostic tools.

**Results:** None at this stage.

**Conclusions:** Control and management models can be developed with these problems and biosecurity in mind. Models will aim to be transferable to other regions, particularly in northern Australia thus enabling these dog control strategies to be implemented in remote Indigenous communities.

This project will enable capacity building at community level in order to increase responsibility and ownership of a dog management solution. Integration of the ecological, veterinary and human health and social aspects of the wild dog problem will contribute to the development of more targeted and cost effective control programs for wild dogs and their diseases. It has the potential for significant impact because of its “one health” approach.

## POSTER PRESENTATION 103

### Does roe deer disease contribute to traffic accidents with roe deer in Utrecht?

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**Key words:** roe deer pathology traffic accidents

This six month project intends to gain insight into the health status of roe deer (*Capreolus capreolus*) involved in traffic accidents in the Province of Utrecht. It is part of a larger project by the Province and its fauna management unit, which aims at understanding the underlying causes of the gradual increase in traffic accidents involving roe deer. Presented are the midterm pathology results of the first three months.

All roe deer assumed to be involved in traffic accidents in the Province, found dead or euthanized between mid-January and March 2010, and with a closed thoracic cavity and abdomen, were submitted for complete necropsy to the Faculty of Veterinary Medicine, Utrecht University, NL. Based on the post-mortem findings, the cases were classified into three categories: cases with lesions likely to have contributed to increasing the chance of being hit by traffic (“Debilitating Condition”, DC); cases with lesions that are unlikely to have contributed to this (“Incidental Finding”, IF); and cases without lesions other than traumatic (“Healthy”). Twenty-three (23) roe deer were investigated, 8 females and 15 males. All animals had signs of severe external trauma. Five (22%) cases were classified as DC, nine (39%) as IF, and 9 (39%) as “healthy”. The DC cases had an arterioportal hepatic fistula, extensive pleuritis and abomasitis, an old non-closed legfracture, uveitis or myocarditis. The most common findings in the IF cases were focal, chronic, mild to moderate respiratory tract lesions.

The variability of the lesions in the DC group strongly suggests that there is no single health problem enhancing the chance of roe deer being hit by traffic in the Province. Future investigations into the background health and a higher number of animals are needed.

## POSTER PRESENTATION 104

### Effect of hemoparasites *Trypanosoma cruzi*, *T. evansi* and microfilariae on health of free-ranging coatis from the Pantanal wetlands, Brazil

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Key words: *Trypanosoma*, health, *Nasua*, Pantanal, Brazil

*Trypanosoma cruzi* and *T. evansi* (Kinetoplastida: Trypanosomatidae) are protozoans of health and economic importance since they cause Chagas disease in humans and “Mal de Cadeiras” disease in horses, respectively. Coatis (Procyonidae: *Nasua nasua*) have been suggested as important reservoirs for these parasites in the Brazilian Pantanal wetlands. Here we examined the relative importance of high parasitemias by *T. cruzi*, *T. evansi*, and abundance of microfilariae on the health of coatis captured in the Pantanal from 2006 to 2009 (N = 72). We used coati body condition and hematological parameters as response variables in general/generalized linear models that were compared using Akaike criteria. Forty-three percent of the coatis showed high parasitemias by *T. cruzi*, 28% for *T. evansi* and 93% for microfilariae. Body condition decreased in coatis with high parasitemias by *T. evansi*, especially during the reproductive season. Total red blood cells (RBCs) and packed cell volume (PCV) decreased in males showing high parasitemias by *T. evansi*, while females showed variable response. Total white blood cell counts (WBCs) and neutrophils decreased in males with high parasitemias by both *Trypanosoma* species, but again females showed variable response. Monocytes decreased with *T. cruzi*, while eosinophils decreased with *T. evansi* high parasitemias. High abundances of microfilariae contributed to decreased coati health, but its effect size was comparatively small. While *T. evansi* is known to cause anemia in coatis, *T. cruzi* causes variable changes in RBCs, PCV and WBCs of hosts. We observed a synergistic effect of high *Trypanosoma* parasitemias and microfilariae on decreasing coati health. However, females apparently handle acute infection better than males. In addition, an overall decrease in health condition occurred during the breeding season, when coatis are under reproductive stress and may be more prone to infection.

## POSTER PRESENTATION 105

### Estimation of the society's willingness to pay to avoid that *Echinococcus multilocularis* becomes endemic in Sweden

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Key words: *Echinococcus multilocularis*; Willingness to pay

**Background:** *Echinococcus multilocularis* (EM) is considered to be an emerging parasite. The EU Commission has indicated that due to the cost and inconvenience the present requirements to prevent introduction of EM to the five EU member states, including Sweden, that consider themselves free from EM are considered disproportionate. The regulation (EC) No 998/2003 allows these countries, over a transitional period, to maintain their national requirements for dogs and cats to be treated against EM by anthelmintics before entering the country.

**Methods:** As a basis for discussion with the EU commission, a contingent valuation study to investigate people's willingness to pay to avoid introduction of the parasite was initiated. To minimize potential problems of non-responses, protest answers, strategic bias, and anchoring bias, the study was performed as a dichotomous-choice study. That is, the randomly selected respondents were divided into groups where members of each group were given one bid only (are you willing to pay XX Euro to prevent that EM becomes endemic in Sweden) but where the amount of the bid differed between the groups. Respondents were also given information on the epidemiology of the parasite including the zoonotic aspects. Data on the number of persons accepting a certain bid were used in a random utility model (non-parametric Turnbull lower bound model) to estimate society's willingness to pay to avoid that EM becomes endemic in Sweden. The effects of individual characteristics of the respondents, such as age, gender, education, income, marital status, number of children/grand children, pet ownership (dogs), and out-door activities, on their willingness to pay were analysed using a logit (spike) model.

**Results and Conclusion:** Results and conclusion will be presented

## POSTER PRESENTATION 106

### Genetic diversity in Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) from Catalonia, NE Spain

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**Key words:** Pyrenean chamois, major histocompatibility complex (MHC), genetic diversity, immune response, disease resistance

**Background:** The major histocompatibility complex (MHC) is involved in the immune response. MHC class II DRB1 gene is likely the most polymorphic locus in the genome, much of its variation located in the exon 2. This high polymorphism is associated with the response to infectious agents, low MHC diversity related to higher vulnerability to infectious diseases. The aim of this study is to analyse genetic variability at this locus in the Pyrenean chamois (*Rupicapra pyrenaica pyrenaica*) in relation to geographical origin and infectious diseases.

**Methods:** Muscle samples of 81 Pyrenean chamois from four populations from Catalonia, north-east Spain (Alt Pallars-Aran, Cadí, Freser, and Cerdanya) were collected and stored frozen. Genomic DNA was isolated using the Wizard® Genomic DNA purification kit (Promega). PCR amplification of the MHC class II DRB gene (230bp fragment of exon 2) was performed using two cattle primers HL030-HL031 and semi-nested primers HL032. Amplicons were purified and sequenced. The sequences were analysed by electropherograms, to check out heterozygote individuals; aligned with MEGA 4.1 and manually adjusted, after checking for misalignments. Haplotypes of heterozygote samples were inferred using either HAP, phasing method or the Bayesian approach.

**Results:** We analysed 162 MHC-DRB exon2 nucleotide and aminoacid sequences. Twenty-nine different sequences were identified. When compared with previously published sequences in GenBank, 55 samples corresponded to three sequences identical to previous reported alleles, namely Rupy-DRB\*02 (n=20), Rupy-DRB\*04 (n=5), and Rupy-DRB\*11 (n=30); in the remaining 107 samples 26 new haplotypes, which have not been previously described, were found and named as Rupy-DRB\*14 to Rupy-DRB\*39.

**Conclusions:** Allele distribution is discussed according to geographical and health status. Translated sequences exhibited high aminoacid polymorphism, most variations occurring within the PBR (peptide binding region). The significantly higher rate of non-synonymous (dN) than synonymous (dS) substitutions and positive Tajima's D value indicate positive selection.

## POSTER PRESENTATION 107

### Investigation and prevention of mortality associated with immunosuppression and management in reintroduced ciril buntings (*Emberiza cirilus*) in England

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**Key words:** reintroduction, mortality, ciril buntings, immunosuppression

During a trial translocation of ciril buntings in 2005, four out of 17 birds died (24%) and isosporosis was diagnosed. Capture and captive rearing of free-living ciril bunting chicks from Devon with subsequent reintroduction into Cornwall commenced in 2006, with 72 of 75 reared ciril bunting chicks successfully released. In 2007, however, 26 out of 73 chicks died whilst in captivity and six shortly after their release (44%).

All birds that had died were submitted for full post-mortem examination, including microbiological examination and histology. Tissues were subsequently submitted for virological examination (passage through embryonated eggs and tissue culture, and examination by electron microscopy (EM)). Seventy-five percent (N=24) of these chicks appeared to have either inadequate amounts of thymic tissue present on gross post-mortem examination and/or a smaller Bursa of Fabricius than would be expected at this age. Enteritis was found grossly in ten ciril buntings, with *Campylobacter jejuni* phage type UT identified by bacteriology in four cases. At this time, *Campylobacter* sp. had also been detected in a member of the bird capture and transport staff. Protozoal coccidia typical for *Isospora* sp. were identified in the intestinal content by microscopic examination in four of the cases with enteritis and in one bird without enteritis. Additionally, there was evidence of systemic infection with *Isospora* sp (atxoplasmosis) (N=2). Further organisms that were isolated and associated with pathological changes included non-haemolytic *Staphylococcus* sp., *Aeromonas hydrophilia/caviae*, *Serratia odorifera*, *Escherichia coli* and *Aspergillus* sp. No viruses could be isolated by culture or detected by EM.

The pathogens found were considered secondary causes of morbidity and mortality, and no primary aetiological agent or environmental risk factors could be identified. Husbandry conditions were thought to be the stressors. Once management factors such as overcrowding and inadequate hygiene were addressed in 2008, the mortality rate due to immunosuppression dropped to 4% (N=3).

## POSTER PRESENTATION 108

### Is the long-term monitoring of *E. cervi* L1 excretion in red deer a sensitive indicator of changes in population and management?

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**Key words:** red deer, *elaphosrongylus cervi*, health indicator, population dynamics, management

**Background:** Measures of performance in wild ungulate populations must consider condition and health of individuals, which relate to habitat, diet quality, management and population demography. We monitored the *Elaphostrongylus cervi* (Nematoda) L1 excretion in a number of red deer populations from 2000 to 2010 in South Central Spain and evaluated changes over that period to estimate whether parasite counts may be sensitive indicators of annual changes in population and management factors.

**Methods:** We chose different populations where a range of deer densities is present. Faecal samples were collected from carcasses recovered during the normal hunting seasons (Nov–Feb) and larvae were extracted and counted. Precise age was determined. We estimated body condition, body biometry and spleen mass. We visited the sampling sites in September every year to obtain field estimates of red deer density. A number of habitat and management variables were considered on the basis of their ecological value to explain individual and population levels of parasite excretion.

**Results:** We found a statistically significant association between parasite abundance changes and variations in individual life traits and demographic parameters.

**Conclusions:** Our results are consistent with variations in extrinsic factors being evident in intrinsic homeostasis, namely the immune response to parasites. Also these factors could well determine changes in the exposure to parasitic forms. *E. cervi* L1 excretion may result a sensitive indicator of changes in population and management factors since at the individual level, this extrapulmonary parasite has a life span comparable to those of their host, and an immunologically determined threshold number of adult parasites maintain a close relationship with host immune defence. Any change resulting in declines in nutritional intake could, in turn, result in poorer body condition and body development, increased parasite excretion and reductions in reproductive rates.

## POSTER PRESENTATION 109

### Is veterinary treatment of casualty and diseased wildlife appropriate?

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**Key words:** badger, casualty, treatment, veterinary

There are many reasons why veterinary clinicians become involved in the care and treatment of wildlife casualties, although these often have more to do with public demand than welfare and conservation concerns. The desire to provide more than just first aid and a lack of ecological training can result in veterinary treatment creating more problems for wildlife than it solves. Clinical cases can however, provide unique access to indigenous wildlife and this may yield information not encountered in other fields of study.

123 adult badger (*Meles meles*) casualties were presented to a veterinary surgery and wildlife rescue centre over a three years period mostly from situations of high badger-human interface including at the roadside (37%) or in buildings (33%). A full clinical examination included blood profiles and radiography. Common clinical findings were conspecific bite wounds (55%), acute trauma usually as a result of road traffic accidents (RTA) (40%) and chronic debility as a result of previous trauma (6%) or disease (15%). Clinical signs consistent with tuberculosis were identified, including radiographic lesions (15%) that have not previously been reported in this species.

Several ethical questions were raised when treating injured badgers that are equally applicable to other wildlife species:

- How to deal with injury directly caused by man e.g. RTA
- How to deal with injury that is part of normal species ecology e.g. bite wounds
- How to deal with disease with zoonotic, livestock and wildlife risks e.g. tuberculosis

The answers require the multidisciplinary input of pathologists, ecologists, epidemiologists, and veterinary surgeons. The continued dialogue between these parties ensures that the increasing public desire to assist injured and debilitated wildlife is managed appropriately based upon sound evidence base.

## POSTER PRESENTATION 110

### Pedicle Fly-Strike in Roe Deer in Sweden

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**Key words:** myiasis, *Lucilia illustris*, antler, roe deer, *Capreolus capreolus*

This report presents four cases of myiasis, fly-strike, in roe deer (*Capreolus capreolus*) in Sweden in August-September 2009. The heads of four roe deer bucks were submitted to the National Veterinary Institute in Uppsala, Sweden. Three bucks were found within a limited area in the south of Sweden (Skåne), one was found dead, one was euthanized when found terminally ill, and the third was shot during hunting. The fourth buck was found terminally ill and euthanized in west-central Sweden (Dalsland). All four bucks were mature animals in their prime, with large pedicles and trophy size antlers. They were infested with myriads of maggots in circumferential skin ulcerations at the base of the antlers. In all four cases the ulcerations were so deep that the frontal bone was exposed and the bone sutures had been partly dissolved. The maggots were identified as greenbottle blow-flies (family Calliphoridae, species *Lucilia illustris*).

During the same period a further four bucks and a reindeer (*Rangifer tarandus*) with the same condition were reported from other parts of Sweden.

Greenbottle blow-flies normally only initiate fly-strikes in tissues with existing lesions. In these advanced cases it was not possible to determine whether the lesions had been preceded by traumatic injuries. Blow-fly strike in roe deer has not been reported previously in Sweden and it is possible that hot, humid weather conditions in parts of southern Sweden contributed to these incidents.

## POSTER PRESENTATION 111

### Reintroduction of the Eurasian Crane (*Grus grus*) to England: health considerations during rearing and release phases

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**Key words:** Eurasian Crane, Reintroduction, disease risk

The Great Crane Project (a partnership venture between WWT, RSPB, Pensthorpe Conservation Trust and Viridor Credits Environmental Company) aims to re-establish a breeding population of Eurasian Cranes *Grus grus* in the UK. Eggs from wild German cranes were imported to the UK where they were hatched and chicks reared at WWT Slimbridge for release onto the wetlands of the Somerset Levels some 100 miles away.

Following a Disease Risk Analysis conducted by the Zoological Society of London (including both published and unpublished WWT data), a number of risk factors were identified, disease protocols written, and mitigation measures and controls put in place. These included a combination of the following:

1. Strict biosecurity measures were enforced at the rearing facility to reduce risk of introduction of pathogens and spread between birds.
2. Environmental management at the rearing site, and to some extent at the release site, was used to reduce risk from traumatic injury, avian botulinum toxin, *Aspergillus fumigatus* spores, foreign body ingestion and to reduce environmental disease vectors and pests.
3. Human 'crane parents' in crane costumes provided daily physical exercise, careful dietary and weight management and reduced intraspecific aggression.
4. Veterinary drug treatments were used to reduce risks from coccidia, *Ancanthocephala* sp., *Capillaria* sp., tracheal helminths and *Tetrameres* sp.
5. Regular targeted screening was used to inform the above veterinary treatments and to test for avian influenza viruses, West Nile virus, paramyxovirus (including Newcastle disease virus) and inclusion body disease of cranes virus (IBDCV).

IBDCV posed the most serious potential disease risk to the reintroduction project. A risk algorithm for the disease was drawn up and results from wild German cranes (source population), existing UK captive cranes, UK wild cranes and the cranes for release were used to assess risk.

## POSTER PRESENTATION 112

### Sanitary evaluation of crab-eating fox populations (*Cerdocyon thous*, Linnaeus 1766) and domestic dogs in the Private Reserve Engenheiro Eliezer Batista and its surrounding, Pantanal, Brazil

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**Key words:** *Cerdocyon thous*, epidemiology, conservation, Pantanal

*Cerdocyon thous* is an omnivorous canid species endemic from South America that contributes to the balance of small animal's populations, besides acting in the seed dispersal of tropical plants. The main threat to the species is the deforestation with the loss of natural habitats that reduces the prey base, and takes the populations to get in touch with domestic animals. With this contact the crab-eating foxes can be infected by diseases or disseminate others, causing serious problems to the public health and conservation. Our goal is to identify in *C. thous* populations of the EEB Private Reserve located in Pantanal (57°28'24" W/ 18°05'25"S), the antibodies against *Leishmania* sp., the rabies virus and canine distemper, besides identifying the ectoparasites from captured individuals. Likewise, we look to identify in domestic dogs of the surrounding reserve the same antibodies and ectoparasites to check possible epidemiologic relations. We carried out a mammal survey in the reserve through the use of camera traps with the intention of checking the wild species that possibly can be in contact with the domestic animals, and a census of the resident domestic dogs around the reserve. With an effort of 540 days/cameras we identified 25 species of wild mammals, besides the crab-eating fox. Some species are considered vulnerable as the tapir or near threatened like the jaguar. We also identified the presence of nearly 50 domestic dogs around the reserve. We are going to collect blood samples of these dogs during February 2011, and to apply a questionnaire turned to the owners regarding to their animal's behavior. During March to June 2011 we are going to capture the foxes for blood collection, and begin to process the laboratory analyses of all collected samples. With our results we hope to contribute with the wildlife conservation in the area as well as with health quality for the communities involved.

## POSTER PRESENTATION 113

### Sarcoptic mange in wild European rabbit (*Oryctolagus cuniculus*) is related to restocking

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**Key words:** sarcoptic mange, wild rabbit, introduction, restocking

**Background:** Although sarcoptic mange is widely known to affect many mammal species worldwide, it has just recently been reported for the first time in some free-ranging European wild rabbit (*Oryctolagus cuniculus*) populations. This species of mammal has been recently considered as Near Threatened in the IUCN's Red List, thus we found highly interesting to explore which factors derived from game management (mainly restocking rates and local population abundance) influenced the presence of the disease. Additionally we evaluated whether the parasite affected the local populations by exploring the population trend in the affected hunting estates.

**Methods:** Our study area is located in Tarragona (NE Spain) and consists of 50 hunting estates. Combining the studied variables (rabbit abundance and restocking rate in the first case, and presence of mange and year effect in the latter), we obtained several models with biological meaning. Then, we performed a model selection procedure following the theoretic information approach based on Akaike's Information Criterion corrected for small sample sizes.

**Results:** Presence of mange depends on animal abundance and on restocking numbers (Explained Deviance= 22.09%). Rabbit abundance (2001-2007) depends on the presence of mange and on the effect of the year (ED= 23.86%), and clearly declined in the hunting estates with mange, whereas abundance is maintained in mange-free zones.

**Conclusions:** Wild rabbit restocking is related to the presence of sarcoptic mange in our study area, thus, this measure should be avoided if an efficient sanitary control of the released animals is not carried out. The presence of this disease is also coincident with a population decline in hunting estates, which can be a hinder in rabbit conservation programmes. This case highlights the importance of sanitary control of wild populations. Further research is needed to clear out other aspects such as the origin of the parasite.

## POSTER PRESENTATION 114

### Serosurvey of sarcoptic mange, canine distemper virus and canine parvovirus and their possible interactions in the Iberian wolf

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Key words: serosurvey, wolf, mange, distemper, parvovirus

(Poster)

1. An increase in the number of wolves affected by sarcoptic mange has been detected in Asturias (Northern Spain) during recent years. A long-term (2004-2010) serological study was done in order to elucidate whether this increase is related to the presence of other diseases such as canine parvovirus (CPV) and canine distemper virus (CDV).
2. Blood samples were collected from 88 wolves, divided into three geographical sectors (East, Centre and West) and three-age groups: adults (>2 years old, n=40), sub-adults (between 1 and 2, n=24) and juveniles (< 1 year old, n=21), with 3 animals of unknown age.
3. Serological data concerning sarcoptic mange demonstrated the continuous presence of specific antibodies in the population, showing peaks in 2006 (25,00% seroprevalence), 2008 (21.01%) and 2010 (37.50%), whereas no animal showed lesions before 2008. The average value was 20.45%, with 94.40% of the seropositive cases appearing from January to June. Although seroprevalence appears equally distributed between both sexes, the percentage of males with lesions and isolated mites doubled that of females. Seroprevalence was similar in the three age groups, but lesions or mites were not detected in sub-adults.  
With regard to CPV, annual seroprevalence was always ≥50%, while for CDV it ranged from 7.14 to 28.57%. The average values for the population sample (n=88) were 60.22 and 19.31% for CPV and CDV, respectively. The prevalence for CPV and CDV were 82.5 and 35.00% in adults, 58.33 and 4.17% in sub-adults and 23.81 and 4.76% in juveniles, respectively. A higher percentage of seropositive animals for the three diseases was observed in the west sector (where there is a higher density of wolves).
4. Although CDV could be related to the death of juvenile wolves, none of these three diseases seem to threaten the long-term population survival of Asturian wolves.

## 8. SURVEILLANCE

### POSTER PRESENTATION 115

#### Absence of brucellosis in lama of zoos in Albania

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<sup>3</sup>Zoo of Tirana

Key words: brucellosis, serologic, infection, blood, zoo

During the period September 2009 – April 2010 was applied the study for monitoring the presence of brucellosis in all Albania Zoo-s. The monitoring was applied in collaboration between the staff of Veterinary Faculty and Food Safety and Veterinary Institute of Albania. Lamas were taken blood in jugulars arteries were from Zoo of Tirana, Zoo of Fier, Zoo of Durres and some lamas that keep on the around restaurants in some city of Albania. The number of lama which was taken in study was 36 and the analysis for presence or no presence for brucellosis was applied with serologic method and method alergen. For better confirmation we took blood samples in two seasons of the year, in autumn and the spring. In the two periods there was a control in total 36 blood samples and from two controls don't confirmed presence of brucellosis. This is the first control for presence of brucellosis in lama at Albania's Zoos. The consideration for application this monitoring was induced from high incidence of brucellosis in domestic animals and the presence of brucellosis for some cases in human. The presence of some abortion in zoos of Albania and entrance of the zoo animals in Albania uncontrollable were other reason for realization this study. Even though from this monitoring there isn't proved any presence of brucellosis in lama, this is not reason, that other controls are not necessary, because the dangerousness from this infection is permanent.

## POSTER PRESENTATION 116

### Contact of livestock with an invasive species, the sacred Ibis (*Threskiornis aethiopicus*) - a multidisciplinary approach for risk assessment

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**Key words:** *Threskiornis aethiopicus*, feeding behaviour, ducks, *Salmonella* spp., *Chlamydomydia* spp.

Feral sacred Ibis (*Threskiornis aethiopicus*) from zoological parks in western and southern France have settled in wetland habitats and proliferated in the last 20 years. In 2006, France has decided to take measures to regulate these populations. Major concerns arise from their possible impact on biodiversity, by predation or ecological niche occupation, and their potential role as a reservoir and transmitter of pathogens. Indeed, they habitually feed on wetland invertebrates and small preys in marshland pastures, but also frequently on landfills, manure, as well as open-air duck breeding premises.

In a preliminary study in 2008 and 2009 in western France, their feeding behaviour was studied on landfills, marshland pastures and duck farms. Furthermore, on a hundred animals killed during regulation operations, necropsy was performed as well as coproscopy for the detection of intestinal parasites. A subset of the animals was submitted to detection of avian influenza, *Salmonella* spp. and *Chlamydiae*.

The study showed that the animals fed in groups from 2 to 150 birds. They were in contact with many other wild species on landfills, with grazing cattle on marshlands and they mingled closely with open-air bred ducks. Their frequentation of feeding sources in a 6-10 km vicinity of resting or breeding locations seemed regular, with seasonal differences in overall distribution. Carriage of parasites and bacteria was moderate, and no macroscopic lesions were detected at necropsy. For avian influenza, anti-H5 seroprevalence was 60 % in 2008. Molecular analysis of *Chlamydiae* suggests carriage of novel, potentially zoonotic genotypes.

To date the risk appears moderate, except in case of an epizootic avian disease, because of the close contact with domestic birds. Risks should be regularly

## POSTER PRESENTATION 117

### Digitalizing historic wildlife pathology records

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**Key words:** Archives, digital, pathology, wildlife

**Background:** Historic wildlife surveillance records can be useful for back-tracking disease introductions, doing temporal comparisons and other research. Wildlife disease investigation has a long history in Sweden, reaching back to the late 19th century, but all historic material is presently stored as paper journals in different and often distant archives. Today, most computer searches will not be helpful in retrieving records or data from the pre-computer age, and manual searches are very time consuming for an individual researcher. The objective of the project is to have all historic records computerized.

**Methods:** We have initiated a project to digitalize all available archived wildlife pathology records. The first step is to make a register with date, case number, species and diagnosis. The next step is to scan handwritten journals to build an electronic archive.

**Results:** The electronic historic archive has been initiated, but will continue to be added on during the coming years, as over 60 years of accumulated handwritten records are to be digitalized.

**Conclusions:** When completed, a researcher on a specific wildlife species or wildlife disease will have easy access to all preserved historic files, and details on any further materials that may have been archived, e.g. tissues in paraffin blocks, macerated skeletal specimens etc. Several issues concerning electronic archiving formats, uniformity of spelling, use of species names and multiple synonyms for diagnoses etc, need to be discussed when planning and building this kind of database.



## POSTER PRESENTATION 118

### Emerging diseases, wildlife rehabilitation and disease surveillance

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**Key words:** Emerging diseases, wildlife rehabilitation, surveillance, west nile virus, avian influenza

**Background:** Disease emergence from the wildlife reservoir has become a global problem and a relatively frequent event with examples such as high pathogenic avian influenza H5N1, severe acute respiratory syndrome SARS or West nile fever. On the other hand threatened wildlife is, mostly for social reasons, treated in privately or public owned rehabilitation centres, especially in developed countries. These centres are frequently understaffed and deficient in biosecurity and can thus be a focus for transmission of pathogens between animals and animals and humans. The emergence of zoonotic pathogens from the wildlife reservoir has caused reallocation of funds to hitherto underfunded fields such as adaptation of laboratory test to wildlife hosts. Also some wildlife species, especially avian scavengers have been recognised as useful sentinel species for some pathogens such as AIV.

**Methods:** Between 1995 and 2008, blood samples were obtained upon admission of wild birds, mostly raptors to a rehabilitation centre in central Spain and a plasma bank was established. Samples of this plasma bank have been employed in numerous ongoing retrospective studies on seroprevalence of avian viruses (aPMV-1, WNV, AIV, hepatitis B, etc.), protozoa (Neospora, Toxoplasma) and exposure to residues of antimicrobials.

**Results:** Species distribution, spatial and temporal variation patterns detected during these retrospective studies for example for AIV and WNV reflect the results obtained by large scale surveillance programs in the same area (Europe/Iberian Peninsula). Positive samples have been used for validation/evaluation of serological tests applicable to wildlife.

**Conclusions:** Samples from wildlife admitted to rehabilitation centres can be of great interest for use in surveillance programs, on the other hand funds allocated to surveillance programs can help to improve diagnostics and biosecurity at rehabilitation facilities.

## POSTER PRESENTATION 119

### Emerging wildlife disease management: Acute action funding scheme in Sweden, exemplified by investigations on emerging infectious frog diseases

Agren, Erik<sup>1</sup>; Olofson, Ann-Sophie<sup>1</sup>

<sup>1</sup>National Veterinary Institute

**Key words:** emerging diseases, management, wildlife, chytridiomycosis, rana virus

**Background:** Basic passive wildlife disease surveillance in Sweden is funded by the state and by hunter license fees. Additional state funding is reserved for acute action projects to deal with emerging diseases. The infectious amphibian diseases chytridiomycosis, diagnosed in imported *Xenopus* sp. research frogs in Sweden, and rana virus outbreaks in free-living frogs in neighboring Denmark are examples resulting in acute action fund initiatives.

**Methods:** When the wildlife disease reporting system received information on increased risk of the infectious frog diseases affecting free-living frogs in Sweden, a specific project plan was compiled and submitted to the wildlife disease advisory board to secure funding. A project to secure national diagnostic capacity, disseminate information, and start screening free-living amphibian populations was initiated.

International networking was used to obtain PCR-protocols and positive control samples for diagnostic purposes. National networking was used to identify local knowledge and field personnel to start sampling for screening. Internet-based searches were used to collate information and produce an information folder for distribution to the public. Information to herpetologists and conservation professionals was given at various seminars.

**Results:** PCR-methodology was tested with positive controls. Initial and ongoing screening of at-risk sites and future relocation sites has so far been negative for chytrid fungus or rana virus in Swedish free-living amphibians. There is now increased awareness regarding the diseases amongst people handling amphibians.

**Conclusions:** With promptly accessible reserve funds for acute action initiatives, the passive wildlife disease surveillance can quickly be enhanced with targeted active surveillance or suitable activities. A difficulty is the unpredictability of the number of acute actions that may arise during a year, and a limiting factor is the number of qualified personnel resources available to run the acute action projects, with simultaneous and continuous routine passive wildlife disease surveillance.

## POSTER PRESENTATION 120

### Fox population counting during an epizootic of rabies in North-Eastern Italy

Citterio, Carlo Vittorio<sup>1</sup>; Obber, Federica<sup>1</sup>; Scremin, Mara<sup>1</sup>; Capello, Katia<sup>1</sup>

<sup>1</sup>Ist. Zooprofilattico Sperimentale delle Venezie

**Key words:** rabies, fox, fox counting

An epizootic of rabies, spreading from the eastern Italian border, has been occurring in the fox population of North-Eastern Italy since October 2008. Before 2008, the Italian territory had been officially rabies free since 1997. The spread of the disease in North-Eastern Italy appeared faster than previously reported, considering that from October 2008 to November 2009 rabies covered a distance of more than 100 km. From the index case in 2008 to 26 May 2010, 265 rabies cases were recorded in the Friuli Venezia Giulia, Veneto and Trentino Alto Adige regions. These data suggest that the distribution and population dynamics of the reservoir species, namely the red fox (*Vulpes vulpes*), have changed with respect to the past, probably in parallel with environmental modifications. In this context, a reliable evaluation of fox distribution and dynamics should be very useful, as a tool to support the eradication measures (in particular the oral vaccination campaigns).

A first check of the available data evidenced scarce information. Actually, specific counts were available only in one area, while hunting statistics were not standardised and not representative of the entire territory, due to the high variability of the hunting interest for the fox.

With the aim of estimating the fox population trend, we proposed a simple method, in addition to the hunting season statistics, based on two main topics: i) daily recording of each campaign for monitoring the oral vaccination effectiveness, covering different periods during the year; and ii) spring night counting indices, exploiting the censuses for red deer, in order to reach a higher standardisation in collecting data. A first feedback was available from the Veneto region, where 40 cards of vaccination campaign monitoring and 250 cards of night counts were obtained in 2 months (March-April 2010). A specific Access Database was developed for these data.

## POSTER PRESENTATION 121

### Monitoring wildlife diseases: syndromic surveillance for the detection of unusual events

Warns-Petit, Eva<sup>1</sup>; Morignat, Eric<sup>2</sup>; Calavas, Didier<sup>2</sup>; Artois, Marc<sup>1</sup>

<sup>1</sup>VetAgro Sup, Veterinary campus of Lyon; <sup>2</sup>French Food Safety Agency

**Key words:** mortality, syndromes, disease, surveillance, detection

**Background:** Recent studies have shown that amongst emerging infectious disease events in humans, which have been increasing since 1940, 60% were zoonoses, and 70% of these were linked to wildlife. Disease surveillance of wildlife should help to improve health protection of these animals and also of domestic animals and humans that are exposed to these pathogenic agents. Our aim was to develop tools capable of detecting unusual disease events in free ranging wildlife, by adopting a syndromic approach, as it is used for human health surveillance.

**Methods:** We propose to use the information registered by a national network monitoring causes of death in wildlife in France since 1986, called SAGIR. More than 50.000 cases of mortality in wildlife were recorded up to 2007, representing 244 species of terrestrial mammals and birds, and were attributed to 220 different causes of death. Facing the multiplicity of species and causes of death, syndromic classes were defined by a statistical typology of the lesions observed on the carcasses. We then carried out time series analyses on overall mortality and syndromes, using two complementary methods of detection, one robust detection algorithm developed by Farrington and another generalized linear model with periodic terms.

**Results:** We established the historical trends of occurrence of these syndromes and identified greater-than-expected counts. The revealed signals were then characterized by species and diagnoses of causes of death. Reporting of unusual mortality events in the network bulletin was used to interpret the identified signals.

**Conclusion:** The study analyses the relevance of the use of syndromic surveillance on this type of data and gives arguments in favour of its ability to detect some unusual disease events. It is part of a European project on the development of novel technologies for the surveillance of emerging and re-emerging infections in wildlife (WildTech).

## POSTER PRESENTATION 122

### New Flubird Database - Platform for Data Exchange and Knowledge Building in Avian Influenza Surveillance

Staubach, C<sup>1</sup>; Mathey, A<sup>1</sup>; Kowalczyk, S<sup>1</sup>; Kranz, P<sup>1</sup>; Globig, A<sup>1</sup>; Tubbs, N<sup>2</sup>; Harder, T<sup>1</sup>; Conraths, FJ<sup>1</sup>; Osterhaus, A<sup>3</sup>  
<sup>1</sup>Friedrich-Loeffler-Institut; <sup>2</sup>Wetlands International; <sup>3</sup>Erasmus MC

**Key words:** New FluBird, avian influenza, surveillance, database

To tackle shortcomings in the current understanding of the epidemiology of avian influenza viruses in migratory wild birds, a network of virologists, ornithologists and epidemiologists was built on related initiatives and co-operations. As a central instrument for this purpose, a database system was developed to store, manage and analyse data from the different disciplines, as well as flanking environmental data. Data access rights can be configured independently for different users, and different data types, respectively. Interaction by project participants is possible via a secured internet connection and a web interface.

Emphasis is placed on the integrative process of combining the interdisciplinary data for analysis, which is realized on different levels. Interactive software modules allow for the creation of database queries and target parameters which are shared by the different types of data. For example, the integration of "International Waterbird Census"- and Flyway-data provided by Wetlands International allows searches for the occurrence of AIV in conjunction with a specific distribution of host species. The resulting subsets of interest can be ordered, stratified and visualized in form of tables and diagrams, as well as in thematic maps created by means of a linked map server.

In respect of the project orientation towards an Early Warning System for incursions of highly pathogenic avian influenza viruses (HPAIV), an automated Email notification function was implemented. Using the aforementioned query modules, users can define certain conditions or events, the occurrence of which will trigger the automated sending of a (notification) mail.

An exchange of data and information with related initiatives, for example, the wild-bird monitoring campaigns in the European Union or the Global Avian Influenza Network for Surveillance (GAINS) of the World Conservation Society (WCS) is easily achieved, since data structures and coding systems were implemented and designed to preserve compatibility.

## POSTER PRESENTATION 123

### Preliminary analysis of causes of mortality in common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) stranded on the Dutch coast, 1975-2008

Osinga, Nynke<sup>1</sup>; Shahi Ferdous, M. Mostafa<sup>1</sup>; Morick, Danny<sup>1</sup>; Udo de Haes, Helias A.<sup>2</sup>; Brakefield, Paul M.<sup>2</sup>; Kuiken, Thijs<sup>3</sup>

<sup>1</sup>Seal Rehabilitation and Research Centre; <sup>2</sup>Leiden University; <sup>3</sup>Erasmus MC

**Key words:** pathology, common seal, grey seal, the Netherlands

Pathological examination of stranded marine mammals provides unique information on the health status of their populations. Our goal was to analyse strandings of seals on the Dutch coast between 1975 and 2008.

Stranding data (n = 2521) and necropsy data (n = 379) of common seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) found dead or that died before admission were obtained from the Seal Rehabilitation and Research Centre (SRRC) database. Necropsy data of common seal strandings during the phocine distemper virus epidemics in 1988 and 2002 were excluded.

Common seal strandings increased from 0 to about 100 per year over the investigated period, with an outlier of more than 2000 in the epidemic year 2002. This increase corresponds with the growth of the common seal population in the Netherlands. Cumulative monthly stranding rate of common seals, excluding epidemic years 1988 and 2002, peaked at more than 100 in June and July, which coincides with the pupping period. Grey seal strandings increased gradually from 0 to about 40 per year over the investigated period, which corresponds with their recolonisation of Dutch waters. Cumulative monthly stranding rate of grey seals peaked at nearly 60 in December, again coinciding with the pupping period.

The most common causes of death in common seals were drowning and bycatch (55 of 185), starvation (20), intestinal volvulus (20), and parasitic bronchopneumonia (17). The most common cause of death in grey seals was drowning and by-catch (15 of 51). Infectious diseases were significantly more frequent in common seals than in grey seals. The preliminary results of our analysis suggest that by-catch is an important mortality factor for both common and grey seals along the Dutch coast, and that infectious diseases are more important as a cause of death for common seals than for grey seals.

## POSTER PRESENTATION 124

### Serological survey for viral infections in European wildcats *Felis silvestris* in Navarre, Spain

Revilla, Miguel<sup>1</sup>; Urra, Fermin<sup>2</sup>; Martínez, David<sup>1</sup>; Bañeres, Alfonso<sup>3</sup>; Ara, Paula<sup>1</sup>; Arnal, MariCruz<sup>1</sup>;

Peribáñez, Miguel Angel<sup>1</sup>; Fernández de Luco, Daniel<sup>1</sup>

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**Key words:** *Felis silvestris*, serological survey, Navarre

Several viral diseases are well studied in domestic cats but there are limited information about occurrence and prevalence in the European wildcat *Felis silvestris*. The aim of this study is to determine whether there is contact among feline viruses and European wildcats in Navarre.

Twenty-nine carcasses of European wildcats were collected by the rangers between 2000 and 2007 and stored frozen at -18°C. All the animals were found dead on the road or very close and had the typical skin and head phenotype of wildcat. At the necropsy, 7 animals did not have a good body condition, all wildcats had multiple traumatic injuries. Thoracic fluid samples were possible to obtain from 28 animals and were analyzed using commercial ELISAs (Enzyme-linked immunosorbent assay) to detect antibodies to feline calicivirus (FCV), feline coronavirus (FeCoV), feline immunodeficiency virus (FIV), feline leukaemia virus (FeLV) and feline parvovirus (FPV).

Positive results were obtained for FeLV with 64.28% (18/28) and FPV with 21.40% (6/28). The rest of analyzes was negative.

## POSTER PRESENTATION 125

### The SAGIR network in France: a 40-year toxicovigilance scheme in the field.

Decors, Anouk<sup>1</sup>; Berny, Philippe<sup>2</sup>; Moinet, Marie<sup>3</sup>; Dunoyer, Charlotte<sup>4</sup>; Mastain, Olivier<sup>1</sup>

<sup>1</sup>National Hunting and Wildlife Agency; <sup>2</sup>VetAgro Sup;

<sup>3</sup>French Food Safety Agency; <sup>4</sup>Fédération nationale des chasseurs

**Key words:** toxicovigilance, sagir, wildlife, France

SAGIR is a wildlife network founded in 1968 but so-called only in 1986 ([www.oncfs.gouv.fr/recherche/reseaux/sagir.php](http://www.oncfs.gouv.fr/recherche/reseaux/sagir.php)). It was created by the ONCFS which is the French public agency responsible for wildlife, its habitats and hunting in France. SAGIR is a nationwide network. It even covers some overseas departments. Its main aim is to check, record, and report wild animal mortality incidents and to alert authorities in case of abnormal mortality. In 1968, the first aim was to report the side effects of pesticides on wild birds and mammals. A few years later, in 1972, it became a general wildlife health monitoring system in France, interested in all causes of wild birds and mammals mortality.

Hunters and public technicians are responsible for the collection of wild animals found dead or sick and for their transport to the local veterinary diagnostic laboratory. Post-mortem examination is carried out for every wild bird and mammal collected within the SAGIR network. Whenever acute poisoning is suspected, biologic samples and their corresponding necropsy findings are submitted to the toxicology laboratory at the Veterinary College (Lyon, France). Poisoning is confirmed when the exposure information, clinical and necropsy findings, and the presence and amount of a given poison are gathered. The information for each case is entered into a database for forthcoming investigations. Up to now, over 58,000 records including 119 different species, nearly 10,000 toxicological tests and a few more 2,000 confirmed poisoning events have been diagnosed and registered in the national database. For each case, SAGIR tries to identify if it was a misuse, abuse or approved use of pesticides, according to the observers' informations and the e-phy database (<http://e-phy.agriculture.gouv.fr>).

The 40-year toxicovigilance results will be detailed.

Due to its experience, SAGIR has been involved in post-registration studies on thiamethoxam, tefluthrin, methiocarb and mercaptodimethur in 2009. Specific protocols have been set up to survey wildlife mortality at the time of corn and rape seeding. The results will be detailed.

The 40-year toxicovigilance and post-registration studies results show that SAGIR network is a competitive system for detecting wildlife mortality due to high acute toxicity molecules in France. However, SAGIR has some limits in relation with its sampling protocol and the limiting existing factors such as detecting dead animals in the field. These limits are important for the results' interpretation.

## POSTER PRESENTATION 126

### Veterinary risk of deer in robust natural corridors in the Netherlands

de Vos, Clazien<sup>1</sup>; Groot Bruinderink, Geert<sup>2</sup>

<sup>1</sup>Central Veterinary Institute of Wageningen UR; <sup>2</sup>Alterra, Wageningen UR

**Key words:** disease transmission, qualitative risk assessment, fallow deer, red deer, roe deer

**Background:** In the Netherlands robust natural corridors will be created to connect natural areas and to enlarge the habitat of plant and animal species. Some of these corridors will allow for migration of wild ungulates. Extension of the range of red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) might, however, facilitate spread of animal diseases, both within these populations and to domesticated animals. Roe deer (*Capreolus capreolus*), on the contrary, already present a potential veterinary risk for the livestock sector since they live throughout the country. The objective of this study was to assess the additional risk posed by the future presence of red deer and fallow deer in robust nature links.

**Methods:** For this purpose a qualitative risk assessment was conducted taking into account

- (a) expected number of deer in future robust natural corridors;
- (b) prevalence of specific diseases in deer populations in the Netherlands;
- (c) differences in susceptibility to and excretion of pathogens in wild ungulates;
- (d) disease transmission routes from wild deer to livestock.

**Results:** Main results of the risk assessment are:

- Overall deer densities will increase by approximately 50%.
- Only the presence of bluetongue, paratuberculosis, and possibly IBR have been confirmed in red deer, whereas no diseases have been observed in fallow deer and roe deer.
- Red deer and fallow deer live in larger groups than roe deer increasing the probability of intraspecific transmission by direct contact.
- Red deer and fallow deer have a larger niche overlap with cattle than roe deer increasing the probability of interspecific transmission via indirect contact and vectors.

**Conclusions:** Migration of red deer and fallow deer in robust natural corridors will result in a slight increase of the general veterinary risk for the Dutch livestock sector.

## POSTER PRESENTATION 127

### Wild boar population monitoring program in emilia-romagna region (Italy): results of years 2006-2009

Rugna, Gianluca<sup>1</sup>; Spaggiari, Brunella<sup>2</sup>; Grazioli, Santina<sup>1</sup>; Licata, Elio<sup>3</sup>; Sozzi, Enrica<sup>1</sup>; Tamba, Marco<sup>1</sup>; Merialdi, Giuseppe<sup>1</sup>

Izslar<sup>1</sup>; Ausl Bologna<sup>2</sup>; Regione Emilia-Romagna<sup>3</sup>

**Key words:** Wild boar - Swine Vesicular Disease - Classical Swine Fever - Aujeszky's Disease - *Trichinella* spp.

Worldwide, the population density of wild boar is increasing, leading a higher contact rate between hosts with regard to the transmission of infectious diseases. The knowledge of diseases circulating in wildlife populations is significant not only for conservation and livestock production but also for public health. Here we report the results of a 4 years monitoring program of wildlife disease implemented in Emilia-Romagna region, Italy during 2006-2009. Wild boar blood sera were analysed for the presence of antibodies against Swine Vesicular Disease Virus (SVDV), Classical Swine Fever Virus (CSFV) and Aujeszky's Disease Virus (ADV), while samples of muscular tissue were examined for the presence of *Trichinella* spp. larvae, according to Regulation (EC) 2075/2005. Samples from 28,035 wild boars were collected during 4 hunting seasons. No antibodies against CSFV (0/6,716) and SVDV (0/5,812) were detected, while 1,311/5,632 sera resulted positive for ADV. The prevalence rates were 31.9%, 35.2%, 21.6% and 31.3% in 2006, 2007, 2008 and 2009, respectively. *Trichinella* spp. larvae were detected in 1/28,035 wild boar. The parasite was detected in a young male wild boar hunter-killed at the end of December 2009 and was identified as *T. pseudospiralis* by multiplex PCR. Wild boar populations have been reported to be infected by ADV almost worldwide in a variable proportion. The seroprevalence in Emilia Romagna is high, so it may be important to consider the possible role of wild boar as reservoir for domestic pigs, in particular for outdoor pig herds. The continuous monitoring of SVDV and CSFV circulation in wild populations is pivotal for the demonstrated epidemiological connection with domestic swine outbreaks and the significant economic impact of such diseases. The monitoring program confirms the very low circulation of *Trichinella* spp. in the regional wildlife populations; moreover it has allowed to detect a species which had never been reported before in mammalian hosts in Italy.

## POSTER PRESENTATION 128

### Wildlife fauna monitoring plan in Emilia Romagna region (Italy): health status of roe deer (*Capreolus capreolus*) population

Spaggiari, Brunella<sup>1</sup>; Rugna, Gianluca<sup>2</sup>; Licata, Elio<sup>3</sup>; Frasnelli, Matteo<sup>2</sup>; Barigazzi, Giuseppe<sup>2</sup>; Gelmini, Luca<sup>2</sup>; Massi, Paola<sup>2</sup>; Renzi, Maria<sup>2</sup>; Ricchi, Matteo<sup>2</sup>; Merialdi, Giuseppe<sup>2</sup>

<sup>1</sup>Ausl Bologna; <sup>2</sup>Izslser; <sup>3</sup>Regione Emilia-Romagna

Key words: *Capreolus capreolus* – monitoring – diseases

Roe deer (RD) (*Capreolus capreolus*) was included in target species of Emilia Romagna monitoring plan of wildlife during 2008-2009 with the aim of gathering information on population health status, prevalence of zoonotic agents and relevant infectious diseases for interacting domestic livestock. Serological investigations were run for *M. avium* subsp. paratuberculosis (MAP), *Brucella* spp., *B. burgdorferi* and *T. gondii* antibodies on hunter-killed RD. Direct investigations for MAP, *Brucella* spp., VTEC, *Y. enterocolitica*, *Salmonella* spp. and gastrointestinal parasites were performed on either found-dead or sick RD. Overall 576 RD were examined: 464 hunter-killed and 112 either found-dead or sick. During post-mortem examination of carcasses or viscera, gross signs of enterocolitis and diarrhoea were found in 14% of cases. Significantly, 55% of found-dead RD exhibited diarrhoea while only 4% of hunted ones did. Serological investigations for *Brucella* spp. yielded negative outcomes, while *B. burgdorferi* infection was found in 56/273 individuals. Sixty-three sera out of 248 tested positive for *T. gondii*, a medium-to-high prevalence mainly involving adult RD. MAP antibodies were found in 4/353 healthy RD. On the other hand, MAP PCR-positive RD (7/35) were diarrhoeic individuals. EAE gene+ *E. coli* was detected 13/94 animals with statistically significant differences between shot and found-dead/sick RD. Moreover, the pathogen was prevalent ( $p < 0,05$ ) in diarrhoeic animals. *Salmonella* spp. was isolated from 2 non-diarrhoeic shot RD. Eight percent of RD tested positive for *Y. enterocolitica* Biogroup 1A, which includes non-pathogenic European strains. Gastrointestinal strongyles occurred at high prevalence (46/131) even though low parasite burdens prevailed. When present, low level coccidia parasitism (16/131) almost always co-occurred with worms and rarely associated with diarrhoea. On the base of the results of this survey the general health status of this RD population appears generally satisfactory and diarrhoea was found to be associated with pathogens commonly found wild ruminants.

## POSTER PRESENTATION 129

### Wildlife networks : useful tools for monitoring side effects of pesticides on wildlife. Example of SAGIR (France)

Mastain, Olivier<sup>1</sup>; Poulsen, Véronique<sup>2</sup>; Berny, Philippe<sup>3</sup>; Decors, Anouk<sup>1</sup>; Alix, Anne<sup>4</sup>

<sup>1</sup>National Hunting and Wildlife Agency; <sup>2</sup>French Food Safety Agency; <sup>3</sup>VetAgro Sup; <sup>4</sup>Ministry of Agriculture

Key words: Pesticides, wildlife, toxicovigilance, SAGIR, network

The existing wildlife networks such as SAGIR can provide important and useful information on effects of PPP on birds and mammals. Its main aim is to check, record, and report wild animal mortality incidents, and to alert authorities in case of abnormal mortality.

The information for each case is entered into a database for forthcoming investigations. Up to now, over 58,000 records including 119 different species, nearly 10,000 toxicological tests and a few more 2,000 confirmed poisoning events have been diagnosed and registered in the national database.

As a warning system, SAGIR showed for instance the side effects on wild birds and mammals through misuse, abuse or approved use of PPP. Some well-chosen examples of major mortality outbreaks associated with poisoning are shortly described to show that SAGIR is helpful in : i) showing that PPPs have an effective impact on wildlife health; ii) describing the major risk factors (climate, agricultural practice, illicit use); iii) describing preventive measures to be implemented (training, information, color of seed coating, ...). In that way SAGIR results can connect the outcome of the risk assessment performed in the regulatory context for PPP, bringing a feedback of field monitoring.

However, some limiting factors are still preventing the SAGIR network from describing the real acute impact of PPP on non-target species. The post-registration studies carried out in 2009 highlighted the difficulties in field to: i) observe the dead animals (vegetation, predation, decomposition, ...); ii) exhaustively collect the dead animals (observers' motivation); iii) detect some of the substances in animal tissues.

The analysis of SAGIR data is restricted to acute effects such as short-term mortality after application of the PPP. The analysis is unable to address other possible impacts such as delayed mortality from more subtle debilitation, reproductive effects or indirect effects mediated through pesticides-induced changes in cover or food supply.

In relation with the limiting existing factors such as detecting dead animals in the field and the potential sublethal effects of PPP, protocols must be improved to increase monitoring of the side effects of PPP in agricultural areas in France. That way, the knowledge collected from networks can feed the current risk assessment area on two aspects: i) a better interpretation of the conclusions of the risk assessment

performed for PPP, particularly for substances that have been used for years; ii) post-registration studies based on strong and operational protocols.

## POSTER PRESENTATION 130

### Results of an ongoing study on the role of wild deer as an infection source for domestic animals and humans

George Valiakos<sup>1,2</sup>, Lisa Yon<sup>3</sup>, Vassiliki Spyrou<sup>4</sup>, Mark Artois<sup>5</sup>, Antonia Touloudi<sup>1,2</sup>, Paul Barrow<sup>3</sup>, Periklis Birtsas<sup>6</sup>, Mike Hutchings<sup>7</sup>, Marina Sofia<sup>1</sup>, Dolores Gavier-Widen<sup>8</sup>, Christos Iacovakis<sup>1,2</sup>, Christos Sokos<sup>1,2,9</sup>, Alexis Giannakopoulos<sup>1</sup>, Charalambos Billinis<sup>1,2,10</sup>

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We report the first results from an ongoing survey in Greek Roe Deer (*Capreolus capreolus*) for multiple viral and bacteriological pathogens known to affect wild and domestic animals and/or humans. Blood and organ samples were collected from 50 wild deer shot during the 2008 - 2009 hunting seasons in controlled hunting areas. To date, sera have been tested by enzyme-linked immunosorbent assay for the presence of antibodies against *Toxoplasma gondii*, *Neospora caninum*, Infectious Bovine Rhinotracheitis virus (IBR), *Mycobacterium paratuberculosis*, *Trichinella spiralis* and *Chlamydophila abortus* and for Bovine Viral Diarrhoea Virus (BVDV) antigen. Further, tissue samples were examined by PCR for *Mycobacterium bovis*. Additional PCR tests will be done for BVDV, *Mycobacterium paratuberculosis*, *Toxoplasma gondii* and Malignant Catarrhal Fever.

Antibodies against *Toxoplasma gondii*, *Neospora caninum*, Infectious Bovine Rhinotracheitis virus (IBR), *Trichinella spiralis* and *Chlamydophila abortus* were detected in 15%, 5%, 38%, 15% and 5% of the tested sera, respectively. All sera were negative for the presence of antibodies against *Mycobacterium paratuberculosis*. BVDV antigen was detected in the sera of 78% of wild deer examined. Tissue samples tested by PCR for *Mycobacterium bovis* were negative.

These results indicate that wild deer may be carriers of several pathogens such as IBR, *Toxoplasma gondii*, *Neospora caninum*, *Chlamydophila abortus* and *Trichinella spiralis* which can be transmitted to domestic ruminants in regions where domestic ruminant farms are adjacent to land inhabited by wild deer. More significantly, these findings

suggest that wild ruminants, such as deer, may act as a reservoir for significant livestock diseases such as BVDV and Bovine Herpesvirus-1.

The research leading to these results received funding from the European Union Seventh Framework Programme (2007-2013) under grant agreement n° 222633 (WildTech).





# Workshops

## Workshop: Bovine tuberculosis in wildlife: what's new?

Organizers: Pauline Nol and Dolores Gavier-Widén

9.00-9.15 Welcome and Introduction: Dolores Gavier-Widén. National Veterinary Institute (SVA), Uppsala, Sweden

### Plenary lectures

9.15-10.00 Bovine tuberculosis in wildlife in Europe: Christian Gortázar, IREC, Ciudad Real, Spain

10.00-10.45 Bovine tuberculosis in wildlife in North-America: Pauline Nol, Colorado, USA

10.45-11.15 COFFEE BREAK

### Session I (contributed papers). Ecology, epidemiology and strains of bovine tuberculosis in wildlife

11.15- 11.30 Bovine Tuberculosis in wildlife in France: an update. Jean Hars. Office National de la Chasse et de la Faune Sauvage (ONCFS), Unité Sanitaire de la Faune, Gières, France

11.30.11.45 Preliminary results of the prevalence and distribution of mycobacterial infections in Eurasian badgers (*Meles meles*) in Spain. Ana Balseiro. Spain

11.45-12.00 Field epidemiology of wild boar livestock interactions in South Central Spain. Joaquín Vicente. Spain

12.00-12.30 Open discussion: the challenges of bovine tuberculosis in wildlife.

Panel: C. Gortazar, Pauline Nol, D. Gavier-Widén

12.30-14.00 LUNCH BREAK

### Session II (contributed papers): vaccines, control and management of bovine tuberculosis in wildlife

14.00-14.15 Vaccination of Eurasian wild boar with inactivated *Mycobacterium bovis* results in immune responses and protection after challenge similar to BCG: Preliminary results. Bea Beltrán. Spain

14.15-14.30 Immunological responses following oral BCG vaccination against bovine tuberculosis in the Eurasian badger (*Meles meles*). Roland Ashford. Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey, UK

14.30-14.45 The development of a bait for the delivery of an oral BCG vaccine formulation against bovine tuberculosis (*Mycobacterium bovis*) to wild badgers (*Meles meles*). Sonya Gowtage. Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey, UK

14.45-15.00 Impact of group size and external sources of infection on the efficacy of vaccination for reducing bovine tuberculosis in badgers. Joanne Hardstaff. UK

15.00-15.15 BadgerBCG: the journey to the first licensed tuberculosis vaccine for a wildlife species. Mark Chambers. Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey, UK

15.15-15.30 General discussion on vaccines for bovine tuberculosis

### Session III: Oral summaries of poster presentations

15.30-15.40 *Mycobacterium bovis*, *Mycobacterium tuberculosis* and *Mycobacterium avium* infections in wildlife animals in the Bieszczady region (Poland). Katarzyna Bartoszek and Blanka Orłowska

15.40-15.50 Tuberculosis and other mycobacterial infections of wildlife in Hungary. Szilard Janosi

16.00 Conclusions-closure of the workshop. Pauline Nol and Dolores Gavier-Widén

## ORAL PRESENTATION

### Bovine tuberculosis in wildlife in Europe, a review

Christian, Gortázar<sup>1</sup>

<sup>1</sup>Instituto de Investigacion en Recursos Cinegeticos

**Key words:** Bovine tuberculosis

Bovine tuberculosis (bTB) is a chronic zoonotic infection of wild and domestic mammals caused by *Mycobacterium bovis* and closely related members of the *Mycobacterium tuberculosis* complex (MTBC). As bTB prevalence has dropped in livestock, the relative importance of a potential wildlife reservoir may increase. Thus, wildlife aspects need to be considered in the strategy to control bTB. Herein, we review the current knowledge on bTB in European wildlife, identifying areas for future research and management. Bovine TB has a complex epidemiology, often with climate and habitat-mediated peculiarities. Thus, the role of wild and domestic hosts in bTB epidemiology varies among regions. Three wildlife taxa are regionally defined as true *M. bovis* maintenance hosts in Europe: the Eurasian badger (*Meles meles*) in Great Britain and Ireland, the Eurasian wild boar (*Sus scrofa*) in the Iberian Peninsula, and cervids belonging to the subfamily Cervinae in different European regions. However, these species are regarded as spillover hosts elsewhere, and their status can change in time depending on their local abundance and management. Moreover, the real contribution of wildlife to slowing down the progress of bTB control in cattle is still a matter of discussion. As our knowledge on wildlife diseases grows, disease control becomes more often an option. Monitoring is needed to identify changes in disease occurrence and to measure the impact of interventions. Setting up stable, comprehensive and accurate schemes at different spatial scales should become a priority for health authorities and wildlife managers.

## ORAL PRESENTATION

### Bovine tuberculosis in wildlife: A low down and an update on the United States and Canada

Pauline Nol<sup>1</sup>

<sup>1</sup>DVM, MS, PhD

Bovine tuberculosis (BTB) has been known to exist in North American livestock since the mid to late nineteenth century. Bovine tuberculosis has been demonstrated in a variety of wild species over the last 100 years as well; however, there are a few foci of disease in North American wildlife that have had and continue to have significant impact on the region. These foci include white-tailed deer (*Odocoileus virginianus*) in Michigan, USA and Minnesota, USA; feral swine (*Sus scrofa*) in Molokai, Hawaii, USA; elk (*Cervus elaphus manitobensis*) in Riding Mountain National Park, Manitoba, Canada; and bison (*Bison bison* spp.) in Wood Buffalo National Park, Alberta, Canada. There are both unique qualities and shared aspects among these wild examples of BTB infection, and these will be examined from historical, managerial, and epidemiological perspectives, emphasizing the valuable lessons and new innovations scientists, managers, policy makers, and the public can gain on both local and international levels.

## ORAL PRESENTATION

### Bovine tuberculosis in wildlife in France: an update

Hars J.<sup>1</sup>, Zanella G.<sup>2</sup>, Richomme C.<sup>3</sup>, Garin-Bastuji B.<sup>4</sup>, Boschiroli M.L.<sup>4</sup>

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<sup>2</sup>Agence Française de Sécurité Sanitaire des Aliments (AFSSA), Lerpaz; <sup>3</sup>Lerrpas, Unité Pathologie des Animaux Sauvages, Nancy, France; <sup>4</sup>Unité Epidémiologie and Unité Zoonoses Bactériennes, Maisons-Alfort, France

**Key words:** Tuberculosis; *Sus scrofa*; *Cervus elaphus*; *Meles meles*; France

In 2000, France was declared officially bovine tuberculosis (TB) free and, until then, *Mycobacterium bovis* had never been found in free-living wildlife in mainland France. However, since 2001, TB has been revealed in wild animals in several areas: initially in 2001, in the Brotonne Forest (Normandy, north-western France), where *M. bovis* infections concerned red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*), with apparent prevalence of 14 % and 28 % respectively. Roe deer (*Capreolus capreolus*) and badgers (*Meles meles*) did not seem affected. Despite the implementation of adapted control measures, infection prevalence and clinical features still increased, which led to the exceptional decision of totally depopulate the forest of red deer, considered as TB-maintenance hosts. This measure seems to be effective, as the prevalence in wild boars has decreased ever since (< 5% in 2009), suggesting a role of spillover host in this forest.

In Burgundy (east-central France), TB in cattle has re-emerged in the last 7 years and, since 2007, grouped cases have also been identified in wild boars. Furthermore, in highly bovine-TB prevalent zones, 16 cases were confirmed in 2009 in badgers (n = 223) which shared the same territory with infected herds, highlighting the gravity of the situation. A strong reduction of these wild species' populations was decided to avoid spillback of TB to cattle.

In the Mediterranean island of Corsica and in the Pyrenees (south-western France), sporadic detection of TB in wild boars, recognized as good epidemiological sentinels, seems to reveal the persistence of the infection either in cattle and/or in the environment.

Independently of the concerned region, the same genotypes of *M. bovis* strains, defined by spoligotyping and MIRU-VNTR analysis, were isolated from wildlife and neighbouring cattle, showing that TB evolves in a multi-host complex system that may hamper the sanitary management of this notifiable disease.

## ORAL PRESENTATION

### Immunological responses following oral BCG vaccination against bovine tuberculosis in the Eurasian badger (*Meles meles*)

R. Ashford<sup>1</sup>; S. Lesellier<sup>1</sup>; S. Palmer<sup>1</sup>; S. Gowtage<sup>1</sup>; D. Dave<sup>1</sup>; L. Chamberlain<sup>1</sup>; B. Grundy<sup>1</sup>; D. Dalley<sup>1</sup>; R.G. Hewinson<sup>1</sup>; B. Catchpole<sup>2</sup>; F. E. Aldwell<sup>3</sup>; M. Chambers<sup>1</sup>

<sup>1</sup>Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey, KT15 3NB, UK; <sup>2</sup>Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Hertfordshire, AL9 7TA, UK; <sup>3</sup>University of Otago, Centre for Innovation, Dunedin, New Zealand

**Background:** BCG vaccination of wildlife reservoir species against bovine tuberculosis provides one potential approach to control of the disease where livestock are at risk of infection. In such situations oral vaccination, via a bait, will often be preferable, to enable the largest possible proportion of the wildlife population to be targeted. Data regarding fundamental immunological responses to vaccination and disease progression are beneficial to the development of vaccination strategies.

**Methods:** Here we report the results of an oral BCG vaccine efficacy study in the Eurasian badger (*Meles meles*). Captive badgers were administered oral BCG vaccine and subsequently challenged with virulent *Mycobacterium bovis*. A group of control animals received no vaccine. Peripheral blood mononuclear cells (PBMCs) were collected regularly throughout the course of the study. Vaccine efficacy was assessed on the basis of pathology post mortem. Changes in the expression of mRNA for key cytokines (interferon-gamma, tumour necrosis factor-alpha, interleukins 2, 4, 6 and 10) were measured using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) assays.

**Results:** Oral vaccination with BCG provided protection against *M. bovis* challenge, as demonstrated by reduced pathology post mortem. Measurement of key cytokines associated with the adaptive immune response to tuberculosis revealed responses to both vaccination and disease challenge.

**Conclusions:** We have demonstrated that oral vaccination with BCG provides a degree of protection against virulent *M. bovis* challenge in an experimental setting, and is associated with characteristic changes in the expression of a number of key cytokines.

## ORAL PRESENTATION

### The development of a bait for the delivery of an oral BCG vaccine formulation against bovine tuberculosis (*Mycobacterium bovis*) to wild badgers (*Meles meles*)

S. Gowtage, K. Palphramand, A. Nadian, G. A. Williams, S. Lesellier, S. Palmer, M. Chambers

**Key words:** Badger (*Meles meles*); Bait; BCG; Bovine tuberculosis; Oral vaccination

**Background:** The vaccination of wildlife is one of the tools being considered for the control of bovine tuberculosis in the UK. Successful oral vaccination of wild badgers with BCG will require a targeted, attractive and highly palatable bait which is compatible with the vaccine delivery system and is therefore capable of delivering the vaccine formulation, be it to the oral cavity or the gastro-intestinal tract.

**Methods:** Bait palatability and preference studies were undertaken on captive and wild badgers in England. Bait candidates were screened in preference tests with captive badgers to gauge attractiveness, uptake and handling. Selected baits were then tested in field preference studies using a well-studied wild badger population. Measures of bait preference included daily bait disappearance rates and General Linear Modelling was used to assess factors affecting bait disappearance (e.g. bait type) by both badgers and non-target species. Different ways of delivering the vaccine were evaluated using a biomarker presented to captive badgers in a range of palatable baits.

**Results:** A total of seven different baits were field tested between August 2009 and May 2010. In captive animals and in field trials the uptake ranged between 92-98% and 58-98%, respectively. Uptake by non-target species ranged between 0-27%. The performance of the different vaccine delivery methods varied as the number of animals with detectable biomarker after consumption of the bait ranged between 50% and 100%.

**Conclusions:** Field trials have successfully identified a number of highly palatable baits with low non-target uptake. Further work is required to refine the vaccine delivery system. Many different factors must be addressed before a final product could be considered for licensing, such as practicalities of large scale manufacture, vaccine stability, bait environmental stability, cost, and efficacy of the best combination of vaccine formulation, delivery system and bait. Such work is currently underway.

## ORAL PRESENTATION

### BadgerBCG: the journey to the first licensed tuberculosis vaccine for a wildlife species

Mark A. Chambers<sup>1,2</sup>; Sandrine Lesellier<sup>1</sup>; Deanna Dalley<sup>1</sup>; Sonya Gowtage<sup>1</sup>; Roland Ashford<sup>1</sup>; Si Palmer<sup>1</sup>; Dipesh Davé<sup>1</sup>; Laura Chamberlain<sup>1</sup>; Ben Grundy<sup>1</sup>; Fiona Rogers<sup>1</sup>; R. Glyn Hewinson<sup>1</sup>

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**Background:** Increased incidence of bovine tuberculosis (TB) in the United Kingdom caused by infection with *Mycobacterium bovis* is a cause of considerable economic loss to farmers and Government. The Eurasian badger (*Meles meles*) represents a wildlife source of recurrent *M. bovis* infection to cattle in the UK and their vaccination against TB with *M. bovis* Bacille Calmette-Guérin (BCG) is one of the disease control options being pursued by British government. In March 2010, a licence was granted for an injectable form of the BCG vaccine for use in Eurasian badgers (*Meles meles*) in the UK. The vaccine, called BadgerBCG, will be used to vaccinate badgers from July 2010 as part of a deployment project funded by the government.

**Methods:** A series of defined studies were conducted in both captive and wild badgers in order to demonstrate vaccine safety and efficacy.

**Results:** Highlights from the programme of work will be presented to demonstrate the safety and efficacy of BadgerBCG to both captive and wild badgers. Data will be presented on the immune response of badgers to vaccination and the efficacy of the vaccine based on assessment of experimental infection *post mortem*.

**Conclusions:** BCG administered intramuscularly was demonstrated to be safe to captive and wild badgers. Efficacy was demonstrated as reduction in the severity of disease in vaccinated badgers following experimental infection of captive animals with *M. bovis*. Evaluation of the vaccine in wild badgers provided evidence for a notable biological effect of vaccination. Pointers will be given to those interested in licensing vaccines for other wildlife species in Europe.

## ORAL PRESENTATION

### **Bovine tuberculosis in wildlife, a European problem with global perspective**

Dolores Gavier-Widén, National Veterinary Institute (SVA).  
Uppsala, SE-75189, Sweden

*Mycobacterium bovis*, the cause of bovine tuberculosis (bTB), has a wide host-range, including members of the orders Marsupialia, Carnivora, Primates, Rodentia, Lagomorpha, Artiodactyla, and others. *M. bovis* has multiple reservoirs, and often persists asymptotically in carrier stages. In Europe, the overall prevalence of bTB is slightly increasing. Officially tuberculosis free (OTF) countries, for example, France, Germany and Austria, as well as not-OTF countries reported BT in wildlife in recent years.

In many parts of the world, eradication of bovine tuberculosis has been hindered because of wildlife reservoirs, for example Eurasian badgers (*Meles meles*) in Britain. Wild boar (*Sus scrofa*) and red deer (*Cervus elaphus*) are increasingly recognized problems as hosts of bTB in Europe. *M. bovis* shed by wild animals may persist in soil and water causing infection of domestic animals. Gathering of animals, such as in pens or at watering points, facilitates spread of bTb, it is an important disease of farmed cervids, llamas and alpacas. Pets, dogs and cats, are also susceptible to *M. bovis* infection. The impact of bTB on conservation is well exemplified by the cases reported in Iberian lynx (*Lynx pardinus*) and European bison (*Bison bonasus*) in Poland.

The true extent of *M. bovis* infection humans is not really known but it is estimated to be of up to 10% of the cases of human tuberculosis in some countries. There is evidence that i.) *M. bovis* may persist in the human population even where it has been eradicated from cattle, ii) HIV predispose to *M. bovis* infection and augments the possibility of transmission between humans, iii) results from occupational exposure to aerosols, iv) Reactivation may occur with age, v) *M. bovis* is resistant to pyrazinamide, a frequently used drug for treatment of tuberculosis.

The persistence of bTb today, in Europe and globally, is compounded by limitations of diagnostic tests and vaccines, poor surveillance and insufficient knowledge of epidemiological patterns. In wildlife, new detections of *M. bovis* have widened its ecological niche in nature.

## Workshop: Bat Diseases and Zoonoses

Programme September 13<sup>th</sup> 2010, 13:30- 18:00

**Convenors/ Moderators** **Wim H. M. van der Poel, Central Veterinary Institute, Wageningen University and Research Centre**  
**Peter H.C. Lina, Naturalis, Leiden**

13:30 – 13:45	Wim H. van der Poel	Welcome and Introduction
13:45 – 14:15	Stephen Rossiter	Bat genetics and climate change
14:15 – 15:00	Alex Barlow, Gudrun Wibbelt	Bat “white nose syndrome”
15:00 – 15:15		BREAK
15:15 – 15:45	Wim H.M. van der Poel	Bat related zoonoses
15:45 – 16:15	Juan E. Echevarria	Rabies surveillance in bats in Europe
16:15 – 16:30		BREAK
16:30 – 17:30		Free communications (15 min per abstract)
17:30 – 18:00		Plenary discussion

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## ORAL PRESENTATION

### Bat White-Nose Syndrome

Barlow, Alex<sup>1</sup>

<sup>1</sup>VLA Langford

Key words: Bat, White-Nose Syndrome, Geomyces

White-Nose Syndrome (WNS) is a newly emerging disease associated with mass mortality in insectivorous bats. It was first reported in hibernating bats in, New York State, in February 2006. 13 states in North Eastern and Mid Atlantic USA and two Provinces in Canada are now affected. Bat population falls ranging from 80-97% have been recorded. The total losses are estimated to be over one million bats. This is the most catastrophic decline of Northern American wildlife caused by an infectious disease ever recorded. Severely affected bats can have visible fungal growth around the muzzle, ears and wing membrane. A specific fungus, *Geomyces destructans* has consistently been isolated from affected bats. It may only be an opportunist infection but no other infectious agents have been implicated. The dying bats often have little or no fat reserves. It is suggested that “WNS transmission” occurs at roost and may occur during autumn bat swarms, as well as by spread from people visiting the caves.

The criteria for WNS are;

- Mass mortality of bats
- Aberrant behaviour
  - Day time flight, near cave/hibernacula entrances
  - Roosting near entrances, where there are light and temperature fluctuations
- *Geomyces destructans* confirmed by culture and/or PCR
- Histopathology; Affected areas of skin with cutaneous fungal infection with *G. destructans* fungal hyphae replacing hair follicles, associated sebaceous and sweat glands.

The depletion of this number of insectivorous bats may lead to a significant increase in insect population. Some bat species eat approximately 3,000 insects per night feed. These insects may be vectors for zoonotic diseases, diseases of farmed and wild animals and may also be crop pests. This level of mortality could lead to the possible extinction of rare bat species in North America.

## ORAL PRESENTATION

### Bat population genetics and climate change

Rossiter, Steve<sup>1</sup>

<sup>1</sup>Queen Mary University of London

Key words: bats genetics disease

\* please note this is a review paper by invitation and the format of Methods and Results does not apply.

The potential role of bats in hosting and spreading zoonotic pathogens means that understanding patterns and levels of connectivity among bat populations is of growing concern. Indeed, this issue is likely become increasingly important in the face of habitat and climate change, with the responses of host populations having knock-on effects for disease transmission dynamics. Population genetics approaches offer powerful tools for evaluating and quantifying levels of gene flow among bat populations. In this paper I will review the current state of our knowledge of population genetic structure and gene flow in undisturbed bat populations. I will consider some of the trends emerging from recent studies, which have collectively highlighted the importance of ecological, morphological and behavioural factors in shaping gene flow and genetic subdivision in bats, both in undisturbed and modified landscapes. Finally, I will discuss how environmental change caused by human activity – both now and in the future - might alter natural patterns of gene flow and connectivity in bats, and how these changes could have consequences for our own health.



## ORAL PRESENTATION

### Rabies surveillance in bats in Europe

Juan E. Echevarría<sup>1</sup>

<sup>1</sup>Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain, email: jeecheva@isciii.es

A total of 946 lyssavirus-infected bats have been recorded in Europe from passive surveillance (1977-2009), mostly based on bats collected by the public or involved in human contact. Two European bat lyssaviruses (EBLV1 and EBLV2) has been found in Europe associated with either serotine bats (*Eptesicus serotinus* and *Eptesicus isabellinus*) or two species of *Myotis* (*Myotis daubentonii* and *Myotis dasycneme*) respectively, whereas rabies cases due to EBLVs in other bat species have only occasionally been reported.

More than 95% of the infected bats found in Europe were serotines with EBLV1. Most cases come from the northern part of the distribution areal of *Eptesicus serotinus* (The Netherlands, Germany, Denmark, Poland), with a progressive decrease to the south, which is interrupted by a cluster of cases from Southern Spain associated to *Eptesicus isabellinus*. Studies on EBLV1 phylogeography show most strains in a quite homogeneous cluster called EBLV1a extending from Northern France to Russia in a west-east axis, with most of the genetic variability to the south (France, The Netherlands, and Spain) in several clusters named as EBLV1b. EBLV2 has been found associated to *Myotis daubentonii* in the UK and Germany, but to *Myotis dasycneme* in The Netherlands. A few infections have been found in the exotic bat species *Rousettus aegyptiacus* in a zoo in The Netherlands (EBLV1), and as a single case illegally imported in France (Lagos Bat Virus).

Active surveillance is mostly based on the sampling of healthy bats captured in the field. Data from *Eptesicus isabellinus* colonies from the south of Spain show bats both with EBLV1 antibodies or EBLV1 RNA in oral cavity with no alteration in the physical condition. Only EBLV2-antibody positive bats have been found by the same approach in the United Kingdom despite intensive search of viral RNA in oropharyngeal swabs. Other published works from Spain show both EBLV1 antibody and viral RNA in the blood of several bat species different from *Eptesicus* sp. A third lyssavirus, the West Caucasian bat virus (WCBV) has been found associated to the cave bat (*Miniopterus schreibersii*) in the European face of the Caucasus.

Despite both approaches provide complementary information, passive surveillance should be given priority, as it is focused in the most immediate concern for the Public Health. Active surveillance should be considered as a complement for analysing the prevalence, dynamics and epidemiology of lyssavirus infections in bat host reservoirs, as well as to search for previously undetected lyssaviruses on bat species underrepresented on passive surveillance.

## ORAL PRESENTATION

### Bats as a source of emerging zoonoses

Wim. H. M. van der Poel<sup>1</sup>

<sup>1</sup>Central Veterinary Institute, Wageningen University Research, Netherlands; National Centre for Zoonoses Research, University of Liverpool, United Kingdom. Email: wim.vanderpoel@wur.nl

An increasing number of bat species is identified as reservoir host of zoonotic pathogens, mainly viruses but also bacteria, implicating potential public health hazards. Bats classified in the order Chiroptera, are the most abundant and widely distributed non-human mammalian species in the world. The order Chiroptera includes over 930 species, which is over 20% of all mammalian species. There are several theories why an increasing number of emerging zoonoses is observed in bats, but it may be mainly a number case. Lyssaviruses have emerged from bats in America (Genotype 1 rabies virus, RABV), Europe (European bat lyssavirus, EBLV) and Australia (Australian bat lyssavirus, ABLV), whereas Nipah virus is the most important recent zoonosis of bat origin in Asia. Bat species in Africa have been identified as the reservoir hosts of filoviruses. Ebola virus has been detected in some megachiropteran fruit bats, and natural Marburg virus infection was detected in the common African fruit bat, *Rousettus aegyptiacus*. Horseshoe bat species (genus *Rhinolophus*) have been identified as the reservoir host of coronaviruses closely related to SARS-coronavirus. Bacterial zoonoses detected in bats include the genera *Borrelia* and *Bartonella* and more are reported. New zoonotic pathogens may emerge from bat reservoirs and known ones may spread to a wider geographical range. To assess the threats posed by zoonoses of bats, there is a need for accurate knowledge of the factors underlying disease emergence and for effective surveillance programmes. Primary efforts should be focussed on the implementation of effective passive and active surveillance systems for known zoonotic pathogens including RABV in sanguivorous bat species (family Phyllostomidae), Henipaviruses in pteroid bat species, Filoviruses in African fruit bats and EBLVs in insectivorous bats. Secondly detection methods for zoonotic pathogens that may emerge from bats should be implemented. Analyses of data from surveillance studies can shed more light on the dynamics of bat zoonoses. Subsequently studies will have to be performed to assess the public health hazards of such pathogens, i.e. infectivity and risk of infection for people. In future efforts could be made to develop a rapid response system.

## ORAL PRESENTATION

### **Geomyces destructans colonisation of bats occurs in several European countries without mass mortality**

Gudrun Wibbelt

**Background:** Alerted by early press postings on White-Nose Syndrome European bat researcher discussed the implications of the findings in the US and a possible occurrence of WNS in Europe. Discussions were controversial as many had never noted fungal growth on hibernating bats, while others remained who had occasionally seen hibernating bats exhibiting white patches on nose and wing membranes. But as arouse uneventfully from hibernation, identification of the fungus was not attempted.

**Methods:** During 2008/2009 winter's hibernacula census bat researchers from four different European countries (Germany, Hungary, Switzerland and United Kingdom) conjointly searched for hibernating bats colonized by fungus similar to WNS in the US. Touch imprints with adhesive tape as well as clipped hairs were retrieved and submitted to the Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany, for further investigation. Single hairs were examined by for the presence of characteristic *G. destructans* conidia, touch imprints were examined by light microscopy. All samples were subject to isolation attempts and molecular investigations.

**Results:** Twenty-one out of 23 samples revealed *G. destructans*. Similar to a case report of a single hibernating bat in France (Puechmaille et al. 2010) genetic comparison of selected fungal genes from European and US strains showed 100% identity. Still, there mass mortality associated to fungal growth has never been reported in Europe.

**Conclusion:** Besides homology in distinct parts of the genom virulence factors could differ between the US and the European strains, explaining the marked differences in regard to mortality events at either side of the atlantic ocean. Alternatively, the immune system of European bats could have adapted towards the fungus resulting in superficial fungal colonization without deteriorating effects.

## Workshop: Novel tools

### Chaired by Eva Warns-Petit and Marc Artois

The epidemiological information retrieved from general wildlife disease surveillance is difficult to evaluate. Indeed, specimen sampled are not representative of a population (sampling is mostly opportunistic, frequently obtained from hunted animals). In addition, the targeted population distribution is hardly known. Therefore, neither true disease prevalence or incidence can be calculated, nor proper risk analysis of disease occurrence be done. But this surveillance can provide useful information on diseases of concern to animal or human health.

The purpose of this one-day workshop is to provide interested people with the opportunity to discover modern tools used at various steps of disease surveillance, presented by scientists that have been implicated in the design and use of them. It will be concluded by a round table discussion aimed at brainstorming on how these tools could be applied to improve wildlife health surveillance systems.

The workshop is organized by members of the veterinary public health department of the veterinary campus of Lyon, France (VetAgro Sup). It is linked to a research project called « WildTech », on novel technologies for surveillance of emerging and re-emerging infections of wildlife, which is funded since 2009 by the European Union FP7.

Programme Monday September 13<sup>th</sup> 2010

<b>Introduction</b>	
11.00-11.15	Introductory talk on wildlife disease surveillance (Marc Artois, VetAgro Sup Lyon, FR, m.artois@vetagro-sup.fr)
11.15-11.45	WILDTOOL, a flexible tool for first line prioritization of wildlife-borne diseases (Paul Tavernier & J. Dewulf, Ghent University, BE, paul.tavernier@ugent.be)
<b>Data capture in the field</b>	
11.45- 12.15	Using mobile phones to capture and transmit case data in real-time, example of West Nile virus surveillance in equine population (Camargue - southern France) (Jocelyn de Goër, INRA Clermont, FR, jgoer@clermont.inra.fr)
12.15-12.45	A syndromic surveillance for the early detection of outbreaks among military personnel (Nina Faure, Institut de Médecine tropicale du Service de Santé des Armées, Marseille, FR nina698@yahoo.fr)
BREAK	
<b>Sample analysis</b>	
14.00- 14.30	Pathogen detection microarrays and their application in wildlife disease surveillance (Abu-Bakr Abu-Median, University of Nottingham, UK, Abu-bakr.Abu-median@nottingham.ac.uk)
<b>Databases</b>	
14.30- 15.00	The CCWHC database and experiences from the DWHC (A. Gröne <sup>1</sup> and K. Brown <sup>2</sup> ; <sup>1</sup> The Dutch Wildlife Health Centre (DWHC), Utrecht, NL; <sup>2</sup> The Canadian Cooperative Wildlife Health Centre (CCWHC), Saskatoon, CA)
14.45-15.00	Impact of group size and external sources of infection on the efficacy of vaccination for reducing bovine tuberculosis in badgers. Joanne Hardstaff. UK
15.00-15.30	The wildlife health monitoring network system (Joshua Dein, USGS Wisconsin, USA, jdein@wisc.edu)
BREAK	
<b>Data analysis</b>	
16.00-16.30	Use of laboratory data for detection of unusual health events (Lucy Snow, VLA Weybridge, UK, l.snow@vla.defra.gsi.gov.uk)
16.30-17.00	Monitoring wildlife diseases: syndromic surveillance applied to post mortem findings (Eva Warns-Petit, VetAgro Sup Lyon, FR e.petit@vetagro-sup.fr)
17.00- 17.30	Support of wildlife disease epidemiology and surveillance by GIS (Christoph Staubach, FLI Wusterhausen, D, christoph.staubach@fli.bund.de)
BREAK	
<b>Final round table</b>	
Discussion on the use of these tools to improve wildlife disease surveillance with a special focus on the design of a pan-European system	

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## ORAL PRESENTATION

### **WILDTOOL, a flexible tool for first line prioritization of wildlife-borne diseases**

Tavernier P.<sup>1,2</sup>; Roelandt S.<sup>1</sup>; Dewulf J.<sup>2</sup>; Roels S.<sup>1</sup>

<sup>1</sup>Wildsurv project, Operational Direction Interactions and Surveillance, Veterinary and Agrochemical Research Centre, Groeselenberg 99, B 1180 Brussels; <sup>2</sup>Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B 9820 Merelbeke

“WILDTOOL” is an electronic tool to rank pathogens from wildlife in Belgium in function of their risk and their need for surveillance in wildlife. The ranking is based on a semi-quantitative risk-evaluation. The system is a first line approach in which the “hazard identification” includes a broad range of pathogens. It offers a maximal flexibility to the users who can select their own parameters for prioritisation of pathogens, according to their responsibilities (public health, domestic animal health, economy of livestock breeding, biodiversity, wildlife management).

These choices include a target group susceptible to the pathogens (humans, companion animals, production animals, or wildlife itself, including game, protected and pest species), a Belgian region (Flanders, Wallonia, Brussels region, or Belgium as a whole), a set of “weights” for the different criteria determining the prioritisation (i.e. the relative importance that the end users attach to the criteria), and a first or second level of ranking (respectively emphasizing comprehensiveness and refinement of the prioritisation).

Starting from a broad literature search, an algorithm is used (Yes/No choices for host presences and pathogen occurrences) to select pathogens from the hazard identification list for the first and the second level ranking. Qualitative scores (4 options) are assigned to a number of pathogen-specific criteria. These scores are reviewed by experts specialised in the different pathogens. Each score is converted to one of 5 numerical values which are subsequently multiplied with the given weights. These products are added up. The final score for each pathogen is expressed as a percentage of the highest possible score per pathogen. In the second level ranking additional detailed data about host-presence and pathogen occurrence are used to refine the first level ranking scores.

A criterium for which no information is available for a particular pathogen, obtains the median numerical score through which the criterium is maintained (score differs from zero) but with a minimal effect of this score on the final pathogen score. The prioritisation result is presented as a list of pathogens, ranked according to their total scores. The uncertainty is expressed as the relative amount of “unknown” information for each pathogen.

Prioritisation results can be compared with identified networks for sampling and diagnosis, allowing to recognise surveillance gaps and to suggest improvements.

## ORAL PRESENTATION

### **Using mobile phones to capture and transmit case data in real-time, example of West Nile virus surveillance in equine population (Camargue - southern France)**

Jocelyn de Goër<sup>1</sup>

<sup>1</sup>INRA Clermont, jgoer@clermont.inra.fr

Syndromic surveillance is an investigative approach in which health officials, assisted by an automated data acquisition system, are able to monitor disease indicators in real time in order to detect an emergence early, even before laboratory case confirmation, in order to rapidly implement preventive measures. Interest in such systems developed following the events of September 11, 2001, primarily for the detection of bioterrorist attacks. Their use later was expanded to the surveillance of infectious diseases. In this project, we are developing these technologies for the surveillance of infectious equine diseases.

“S2IAP (Surveillance des Syndromes Infectieux et Alerte Précoce – Surveillance of infectious syndromes and early alert)” is a software platform that allows a veterinary surveillance network to capture clinical descriptions of observed cases via two options:

- A system that can be used directly in the field, at “horse’s bedside”, allowing input and then transfer (via a Smartphone) to a central server observed clinical case. The transmission of data is made via the mobile telephone’s Internet connection.
- A secure Internet website permits clinical descriptions to be entered and consulted and provides access to feedback on all declared cases.

## ORAL PRESENTATION

### A syndromic surveillance for the early detection of outbreaks among military personnel

Nina Faure<sup>1</sup>; Hervé Chaudet<sup>1</sup>; Gaëtan Texier<sup>1</sup>; Jean-Baptiste Meynard<sup>1</sup>; Xavier Deparis

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**Background:** Several thousand French soldiers are deployed throughout the world every year, exposing them to natural and aggressive risks. Epidemiological surveillance is one of the Military Health Services' missions needed to maintain the operational capacity of these forces. Developing an early warning capacity is one of the priorities of surveillance, in order to detect as soon as possible an unusual event, evaluate its potential impact and provide information to assist medical responses.

For this purpose, a prototype of a real-time epidemiological surveillance was set up in French Guyana in October 2004: the "système de surveillance spatiale des épidémies au sein des forces armées en Guyane" (2SE FAG).

**Methods:** The system was designed to allow, in near real time, geo-location and epidemiological analysis of cases of fever occurring in members of the armed forces anywhere in this South America French overseas territory where tropical diseases responsible of outbreaks exist. It was initially designed for fever surveillance in an equatorial area affected by many febrile tropical diseases, including dengue fever and malaria; the surveillance domain has been extended to all symptoms and deaths since February 2008.

The system's architecture is composed of 2 kinds of independent networks working together: a recording network and an analysis network; it permits to provide in real time some dashboards directly operational for the commanders.

**Results:** The operational use of the system allowed an early warning for a dengue fever outbreak in French Guiana in 2006 and a quicker public health response by the armed forces than by the civilian authorities.

In 2006, it has been decided to extend the concept by deploying its second generation within the French armed forces in Djibouti. Since then, it has shown its usefulness for early warning during different real and simulated situations, even an interallied NATO exercise.

In its last and current architecture the whole surveillance system, named "Alerte et surveillance en temps réel" (ASTER), encompasses several declaration and surveillance networks located in duty areas and a global epidemiologic data analysis network located in France.

**Conclusions:** A real time surveillance system is an essential alarm disposal; however it is only an information tool within the complex activity of piloting the sanitary situation. It must

be integrated within the whole situation expertise supports, represented also by medical intelligence, epidemiological investigations and prediction of the epidemiological phenomenon evolution.

Medical, technological, human and organisational aspects have to be taken into account to develop real time surveillance within the armed forces, and also specific military constraints.

## ORAL PRESENTATION

### Pathogen detection microarrays and their application in wildlife disease surveillance

Abu-Bakr Abu-Median<sup>1</sup> BVSc MSc PhD

<sup>1</sup>The University of Nottingham, School of Veterinary Medicine and Science, Sutton Bonington Campus, Nr Loughborough, Leics LE12 5RD, UK

**Background:** Early and accurate detection of the aetiological agent(s) is a crucial factor in disease control. With the current threat imposed by emerging and re-emerging diseases, it is essential to have a rapid and specific assay for pathogen detection and characterisation. Many detection methods are aimed at identifying single or a few known targets often relying on knowledge gained from disease symptoms. Existing techniques to screen for a wider spectrum of pathogens, or for the detection of novel or emerging pathogens, suffer from severe limitations. Pan-microbial detection microarrays were developed within the last eight years predominantly targeting human pathogens. Recently, several arrays were developed for specific human and plant pathogens. A great advantage of a diagnostic microarray is that a single field or clinical sample can be analysed for the presence of multiple pathogens in a single operation without prior knowledge of their identity. Detection microarrays targeting viruses of veterinary importance were developed and validated for some key viruses very recently. WildTech will expand the detection of pathogens (viral, bacterial and parasitic) to wildlife using novel technologies such as microarrays (both oligonucleotide- and protein-based).

**Methods:** Methods described here are for oligonucleotide-based microarrays. Oligonucleotides (50-70 mer) are designed from fully or partially sequenced genomes of pathogens of interest using publicly available software. These oligonucleotides (probes) could be designed to cover each pathogen from family to species or even genotype/serotype levels. The designed probes will be synthesised commercially and printed onto a solid platform. These arrays are stored at room temperature and ready to use.

The sample to be tested (target) will be processed prior to testing on the array. The sample could be in the form of cultured material or tissue. The nucleic acid will be extracted, randomly-amplified and labelled. The labelled target will then be hybridised to the probes on the array. Fully automated hybridisation is now available. Following hybridisation, arrays are scanned, images captured and raw data generated. Different array fabrication, amplification, hybridisation and analysis methods have been developed.

**Results:** Raw data will be processed using dedicated analysis software. Numerical figures will be plotted as charts or heatmaps, accompanied by statistical analysis, identifying the detected pathogen(s). Examples of detection of key veterinary

viruses in farm animals will be presented.

From our experience, it takes around 15 hours from sample to final result. With further optimisation, time taken will be potentially reduced.

**Conclusion:** Microarrays offer wide-spectrum detection with the capability of discovery of new pathogens.

Therefore, the use of microarrays during outbreaks and/or disease surveillance would save time and help with early decisions to control spread of disease. While they have the potential, detection microarrays are yet to be used as routine surveillance tools for veterinary use. Currently, microarrays require high sophistication, and cost per test is still high compared to other diagnostic methods. Several conditions must be taken into account for use of microarrays in wildlife disease surveillance including, but not limited to; time of sample collection, suitability and handling of the sample collected. Recently, new portable formats of microarrays have been developed with less sophistication without compromising the output. These platforms could be used in smaller non-specialised laboratories close to the field, and with minimum training. It should be noted that microarrays in some cases only provide the first evidence on the nature of a pathogen – therefore, the technology should be considered complementary to existing diagnostic tools

## ORAL PRESENTATION

### The CCWHC database and experiences from the DWHC

A.Gröne<sup>1</sup>; K.Brown<sup>2</sup>

<sup>1</sup>The Dutch Wildlife Health Centre (DWHC), Utrecht, The Netherlands; <sup>2</sup>The Canadian Cooperative Wildlife Health Centre (CCWHC), Saskatoon, Canada

The CCWHC database was created as a data repository used by the five different CCWHC sites. This data is then used to generate reports which are sent to submitters and finders as well as for summary reports. The database is focused on providing a data storage medium for all types of wildlife disease related lab data. There is currently no significant epidemiological component incorporated, with data analysis being facilitated through exporting of data. Once data is exported from the database, tools such as SPS, ArcGIS and Excel are used to perform analysis and further reporting. The database does feature end-user reporting.

The main advantage of using the CCWHC database is that it is a fully supported, custom built application dedicated solely to wildlife disease-oriented data. The data model was designed around the generally common work flow points of sample receipt, testing and diagnosis, with analysis, again, being considered outside the functionality of the system. Large lists of scientific reference data (such as taxonomy and anatomy) were generated which are used to speed up data entry and ensure a high rate of data consistency and validity. The modularity of the database allows, that other projects or other centres are added easily. The DWHC was added in 2008 and all final data from the post-mortem investigations in wildlife have been stored on the CCWHC database in a separate section since that time. The existing reference data were adjusted to more specific European needs. The format of the reports follows in largely that of the CCWHC, but with the DWHC logo. Heading of the reports are in English, the text is in Dutch. At the moment, data is copy-pasted from the lab systems, an import option is being investigated. Easily available support from the IT unit in Saskatoon is via mail or phone.

## ORAL PRESENTATION

### The Wildlife Health Monitoring Network System

F. Joshua Dein<sup>1,2</sup>; Megan K. Hines<sup>1,3</sup>; Christine M. Marsh<sup>1,3</sup>

<sup>1</sup>Wildlife Disease Information Node - National Biological Information Infrastructure; <sup>2</sup>US Geological Survey - National Wildlife Health Center; <sup>3</sup>University of Wisconsin - Nelson Institute for Environmental Studies

Integrating different types of wildlife disease surveillance data together provides critical information for understanding wildlife disease patterns and their potential impact on wildlife, human, and domestic animal health. To help address this need, The Wildlife Disease Information Node (WDIN), part of the US National Biological Information Infrastructure (NBII), is building the Wildlife Health Monitoring Network (WHMN), a Web-based open source system with interchangeable modules that support data entry, storage, reporting, analysis, and exchange in collaboration with many partners. The goal of this system is to create tools for institutions and individuals to track and store wildlife health data in a way that is useful and meaningful to them, as well as allowing them to share their data, as they deem appropriate, with the rest of the Network.

The WHMN system consists of the following components:

- An **Information Model** to provide the framework for the representation of the semantic and lexical connections that exist between the information carried in data fields.
- An extensible Data Architecture (**Wildlife Health Integrator: WHI**) based on semantic web concepts that can accommodate the potential broad ranges of observational data that may be collected or imported from monitoring activities.
- A web data collection tool, the **Wildlife Health Event Reporter, WHER**
- The **Administrative Application**, that handles such tasks as maintaining various vocabulary lists, source database connection information, processing ETL (Extract Transform Load) functions, and basic data editing.
- The **Wildlife Health Analysis and Visualization (WHAV)** tools. Web Services that will make the information contained within the WHMN data architecture available in a variety of formats to external users and applications.

## ORAL PRESENTATION

### Use of veterinary laboratory data for the detection of unusual health events

Lucy Snow; Paul Duff

**Background:** Routine scanning surveillance data from submissions to diagnostic laboratories can provide important information about the health of animal populations. A key objective for any disease scanning surveillance system is to increase the likelihood of early detection of important changes in the health of animal populations, including the incursion of exotic diseases, the emergence of a new disease, or changes in known diseases.

**Methods & Results:** In the UK, the Veterinary Laboratories Agency (VLA) provides a diagnostic service to veterinary practitioners and other stakeholders across the UK. While the vast majority of submissions to VLA are from livestock, wildlife also makes up an important component of the scanning surveillance data under the VLA Diseases of Wildlife Scheme (VLADoWS) and over 400 species of UK vertebrate wildlife have now been added to the submission data input process. All submissions received are allocated a diagnostic code based upon defined criteria. Where it is not possible to make a diagnosis, submissions are categorised into broad system related groups based on bodily systems and allocated a “diagnosis not reached” code for each of these groups. These latter codes are particularly important for detection of new and emerging diseases. Automated statistical analyses and built-in alerts notify epidemiologists and veterinarians of potentially significant changes in diseases diagnosis and trends both for known pathogens (e.g. Salmonella), but also of submissions where a diagnosis cannot be reached.

**Conclusion:** The development of a single, standardised data collection system; consistent diagnostic criteria and harmonised recording, enables the collation of the disease surveillance data for both livestock and wildlife. Although the greater challenges posed by wildlife surveillance can impose limitations on any analyses, it still provides a useful tool that should enable action to be taken and resources to be appropriately targeted.

## ORAL PRESENTATION

### Support of wildlife disease epidemiology and surveillance by GIS

Christoph Staubach<sup>1</sup>

<sup>1</sup>Friedrich-Loeffler-Institut (FLI), Institute of Epidemiology, Wusterhausen, Germany

**Key words:**

**Background:** Geographic information systems (GIS) and spatial epidemiology are playing more and more an important role in animal disease control. For quite some time GIS is also used in applied wildlife disease epidemiology and surveillance. With the help of GIS and spatial statistical methods the spatial and temporal spread of diseases can be analysed and its risk described. Furthermore, GIS support countries to target and report surveillance of notifiable wildlife diseases more effectively.

**Methods:** Georeferenced and aggregated data on administrative level of hunted foxes were used to better describe the association of environment and occurrence of *Echinococcus multilocularis*. Vectorized data of lakes and rivers, villages, streets and forests as well as a digital terrain model of the area were used to describe the topography of the landscape. Furthermore, Landsat Thematic Mapper satellite images were processed to identify different land-use classes describing the homerange of the foxes with a higher resolution. The area-specific prevalence of the disease, time-trend and influence of environmental co-variables were estimated using spatial logistic regression within Frequentist and Bayesian statistical frameworks. Using the example of Classical Swine Fever (CSF) in wild boar different surveillance strategies were developed on the basis of spatio-temporal analysis of the CSF situation in wild boar and domestic pigs of new accession or member state and neighbouring countries, respectively. Especially hunting bag, different risk factors and spatial configuration were included in the determination of the sample size for each spatial unit and time period.

**Results:** An association between the proximity of humid areas and the occurrence of *E. multilocularis* could be demonstrated for the first time by this field study. Possible consequences for the explanation of heterogenous spatial distribution patterns and prospective risk estimates of the occurrence of *E. multilocularis* are discussed. The increased prevalence and spread of *E. multilocularis* in the Federal State Thuringia are described as well as methodological issues. The spatially targeted surveillance and control programmes of the Republics of Slovenia and Croatia are introduced as an example how decision makers may be supported by GIS technology.

**Conclusion:** These studies mentioned above stress the importance and usefulness of GIS within the framework of wildlife disease epidemiology and surveillance programmes. Nevertheless, availability of data, limits of the approach and statistical assumptions have to be carefully considered.



## Workshop: “Wildlife forensics”

### Programme

9.00 Arrival of participants & welcome

#### Part 1: LECTURES

30 minutes	<b>Introduction</b>	Definition of a forensic case, appropriate investigation procedures. Dr. Frank van de Goot, The Centre for Forensic Pathology, The Netherlands; email: bathory@live.nl
30 minutes	<b>Taphonomy</b>	Determining the time of death (in days, weeks, or more): criteria to be considered, influencing factors, limits. Dr. Frank van de Goot.
45 minutes	<b>Wildlife poisoning</b>	Acute vs. chronic poisoning, clinical symptoms, macro- and microscopic lesions caused by common poisons, adequate sampling and preservation of samples, applications and limits of applied techniques. Dr. Philippe Berny, Laboratoire de Toxicologie, Campus vétérinaire de Lyon, Université de Lyon, France ; email: p.berny@vetagro-sup.fr
30 minutes	<b>Wounds I: Soft tissue injuries</b>	Dr. Frank van de Goot
30 minutes	<b>Wounds II: Bone injuries</b>	Identifying intra-vitam and post-mortem injuries caused by different tools; assessment of shot wounds. Bone injuries 30 min. Dr. Reza Gerretsen, The Netherlands Forensic Institute, The Netherlands.
45 minutes	<b>Diagnostic of predation</b>	Investigation procedure and criteria to be considered when attempting to identify predators based on prey carcasses; use of genetic tools. Dr. Marie-Pierre Ryser, Centre for Fish and Wildlife Health, University of Bern, Switzerland; marie-pierre.ryser@itpa.unibe.ch

#### Part 2: CASE STUDIES (30-45 min each)

<b>Intoxications</b>	Ph. Berny
<b>Ballistic &amp; cut wounds</b>	F. van de Goot
<b>Diagnostic of predation</b>	M.-P. Ryser

#### Discussion and closing remarks

18.00 End of the workshop

## ORAL PRESENTATION

### Wildlife forensics: introduction

Frank van de Goot<sup>1</sup>

<sup>1</sup>The Centre for Forensic Pathology, The Netherlands; email: forensischpatholoog@live.nl

Animal cruelty is a widely spread phenomenon concerning mostly animals in domestic situations or animals as part of the human food system. Often in such cases of animal cruelty are generally “easier” to investigate than wildlife forensic cases. However, in any situation, the approach should be the same and aims at observing, collecting and securing as much evidence as possible. This is particularly important because a public prosecutor could be willing to take the case to court. Also from the defense point of view, following specific standards will guarantee an optimal chain of evidence. This standard forensic approach can be of tremendous use if wildlife animal cruelty must be taken to court in the future. In the Netherlands, since 2010 these acts of crime are no longer seen as vandalism but as an actual criminal act with sufficient punishment if proven.

## ORAL PRESENTATION

### Post-mortem changes and time of death

Frank van de Goot<sup>1</sup>

<sup>1</sup>The Centre for Forensic Pathology, The Netherlands; email: forensischpatholoog@live.nl

A recurring problem in forensic medicine is the need to fix the time of death within the limits of probability. Evidence for estimating the time of death may come from three sources: (1) Corporal evidence, i.e. that present in the body; (2) Environmental and associated evidence, i.e. that present in the vicinity of the body; (3) Anamnestic evidence, i.e. that based on the deceased's ordinary habits, movements, and day to day activities. Many physico-chemical changes begin to take place in the body immediately or shortly after death and progress in a fairly orderly fashion until the body disintegrates. Each change has its own time factor or rate. Unfortunately, these rates of development of post mortem changes are strongly influenced by unpredictable endogenous and environmental factors. The most useful single indicator of the time of death during the first 24 hours post mortem is Algor mortis (body cooling). Further useful criteria are Rigor mortis (postmortal muscular stiffening) and Livor mortis (hypostasis, post-mortem lividity/suggillations). Later, the stage of decomposition (putrefaction) needs to be considered, including saponification or adipocere formation (modification of putrefaction characterised by the transformation of fatty tissues into a yellowish-white, greasy, wax-like substance with a sweetish rancid odour) and mummification (modification of putrefaction characterised by the dehydration or dessication of tissues). Maceration can also occur (aseptic autolysis). A further method that has been studied by several workers is the determination of the relationship between the rise of potassium concentration in the vitreous humour and the time since death.

## ORAL PRESENTATION

### Wildlife poisoning

Philippe Berny<sup>1</sup>

<sup>1</sup>Laboratoire de Toxicologie, Vetagro Sup, Campus vétérinaire de Lyon, Université de Lyon, 1 av Bourgelat, 69280 Marcy l'étoile France; email: p.berny@vetagro-sup.fr ou toxlab@vetagro-sup.fr

Wildlife toxicology is a relatively limited field of investigations, even though poisoning events may result in severe die-offs of wildlife species. It is generally acceptable to distinguish between acute poisoning, the tip of the iceberg, and chronic poisoning, probably often neglected, although there are not many reports and studies available to support this assumption.

Acute exposure to pesticide is a fairly common environmental problem and many toxicants are well-known for their effects on wildlife. A European survey clearly indicates that pesticides are among the most common toxicants involved in wildlife acute poisoning events. Chronic poisoning is better described by its implications on wildlife health and survival in a given environment. As a consequence, many scientists have developed programs and tools to monitor exposure to some contaminants (primarily heavy metals and persistent organic chemicals) but we still lack information on individual as well as population or ecological effects of many of these contaminants alone or in combination.

In this presentation, we will discuss some of the common toxicants involved in acute wildlife poisoning and present their clinical as well as pathological features (pesticides, heavy metals). Some chronic poisoning cases will be presented similarly (organochlorine, heavy metals).

In order to obtain usable results from toxicology screening, adequate sampling and preservation procedures need to be fulfilled. Together with detailed (as possible) case history, these practical issues will be detailed.

When toxicological data are obtained from a laboratory, interpretation is a delicate phase and we will focus on common rules as well as traps to avoid misunderstanding will be presented and discussed.

## ORAL PRESENTATION

### Soft tissue injuries and injury dating

Frank van de Goot<sup>1</sup>

<sup>1</sup>The Centre for Forensic Pathology, The Netherlands; email: forensischpatholoog@live.nl

In the forensic approach the differences between blunt injury and sharp injury can be very difficult. The only right way to approach an injury is in a descriptive manner. During this lecture different types of injury will be shown and the exceptions: Simple haemorrhage and bruising, including the histological and immunohistochemical approach of injury dating; Lacerations and tears, and the patterns often visible on the edges; Sharp injury, cuts and slashes; Gunshot injury (In and out); Heat induced injury, burns.

## ORAL PRESENTATION

### Forensic Anthropology, Trauma, skeletal remains

R.R.R. Gerretsen MD<sup>1</sup>, Forensic Anthropologist

<sup>1</sup>Netherlands Forensic Institute, Leiden University Medical Center, Barge's Anthropologica

After attending this presentation, attendees will have been introduced to the forensic anthropological technologies and methods available at the Netherlands Forensic Institute to the wildlife community in a forensic and medical context.

The osteologist is faced with three critical questions: Is the material human? How many individuals are represented? Of what antiquity is the material?

Proper evaluation of any skeletal remains usually requires collection and subsequent laboratory analysis of the bones. The recovery has to be executed under certain legal constraints. The spatial distribution of the skeletal parts has to be documented with written and photographic records. In case of cremated osteological remains certain procedures are followed preceding analysis to preserve the bone ashes.

It is likely that the forensic anthropologist will be asked to assist in interpreting skeletal defects that may have been caused by forces such as heat exposure and/or trauma to bones. The forensic anthropologist is familiar with the various effects thermal damage has on human remains, as well as knowledge of fracture patterns and cut mark morphology indicative of trauma. Cut mark analysis is possible in cases involving dismemberment in homicide and wildlife investigations. A saw blade, for example, is characterized by class features: the number of teeth per inch (TPI), push versus pull stroke cutting, tooth offset, and tooth width (blade thickness); valuable information for law-enforcement personnel. Chop marks can be identified on fresh and cremated remains. Finally, a scanning electron microscope is used to analyze microscopic parts left in the wound bed that might give an indication of which class of tool was used to damage the bone (Locard's principle).

The role of the forensic anthropologist has become vital to forensic investigations that involve skeletal remains.

## ORAL PRESENTATION

### Diagnostic of predation

Marie-Pierre Ryser-Degiorgis<sup>1</sup>

<sup>1</sup>Centre for Fish and Wildlife Health, Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, Postfach 8466, 3001 Bern, Switzerland; email: marie-pierre.ryser@itpa.unibe.ch

Diagnostic of predation (DP) consists in the identification of a predator based on the way a prey animal has been killed and eaten. DP is usually performed for three possible reasons: (1) in the frame of a police investigation, e.g. when domestic animals have been killed, wounded or mutilated; (2) when a domestic dog is suspected to have attacked wild animals; (3) to document the presence of wild carnivores (monitoring of protected wild species).

DP requires a rigorous procedure starting with records of evidence in the fields (e.g., species and number of dead and wounded animals, location, meteorological conditions, animal tracks) and followed by a post-mortem examination concentrating on lesions of skin, musculature and skeleton. DP is a challenging task, in particular because retrieved carcasses often are in an advanced stage of decay, or have been consumed by scavengers. Therefore, DP needs to include three major steps: (a) deciding whether the carcass can be appreciated or not (due to advanced decomposition or complete consumption); (b) determining whether the animal has been preyed upon by a carnivore or has died of any other cause and subsequently consumed by scavengers; (c) attempting to identify the predator.

In order to properly record evidence and interpret data, knowledge on the different hunting techniques of endemic predators and the way they consume their prey is a prerequisite. Genetic tools allowing the detection of DNA in saliva collected from bite wounds and from scats found around the prey may also complete the data set with precious information and contribute to the final diagnosis.

## Workshop: Rodent-borne pathogens

### Description

Rodent-borne pathogens threaten the health status of wildlife as well as that of domesticated animals and humans. The workshop aims to extend the conference theme, i.e., the interface between wildlife and public health, by focusing on rodent-borne pathogens. We will start with a selection of key speakers dealing with rodent-borne pathogens, the rodent reservoir, and resulting disease in humans. In the second part of the workshop we will have an open discussion on new developments in rodent-borne diseases in Europe. This workshop should be of interest to people from different disciplines, such as public health professionals, wildlife diseases specialists, ecologists, biologists and epidemiologists.

### Programme

#### Speakers

Eric van Gorp, Slotervaart Hospital, Amsterdam, The Netherlands, and Erasmus Medical Centre, Rotterdam, The Netherlands (Coagulation disorders in human patients with leptospirosis)

Åke Lundkvist, Swedish Institute for Infectious Disease Control, Solna, Sweden (Hantavirus infection in Europe)

Byron Martina, Erasmus Medical Centre, Rotterdam, The Netherlands (Recent advances in laboratory techniques for virus discovery)

#### Round-table discussion

Does prevalence of rodent-borne virus infections in humans relate to rodent population numbers?

Are newly discovered viruses in rodents a risk to public health?

Will climate change in Europe increase the prevalence of rodent-borne diseases in humans?

How do we monitor rodent populations for emerging infections?

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### Target audience

Public health professionals, wildlife diseases specialists, ecologists, biologists and epidemiologists

### Time and place

Monday 13 September, 2010, from 12.00 to 19.00 (Lunch, coffee, and tea included)

Strandhotel Seeduyn, Vlieland, The Netherlands

### Organizers

Ab Osterhaus and Thijs Kuiken, Department of Virology, Erasmus MC, Rotterdam, The Netherlands

Contact: [t.kuiken@erasmusmc.nl](mailto:t.kuiken@erasmusmc.nl)

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## Notes









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