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3rd Symposium of the Belgian Wildlife Disease Society

Wildlife disease surveillance




Friday 16 October, 2009

Queen Astrid Military Hospital, 1120 Brussels

Information: http://wildlife.var.fgov.be
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Organising Committee:
Paul Heyman, Paul Tavernier, Stefan Roels, Alexandre Dobly

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Program

08:30 - 09:00 Registration

09:15 Welcome	*Gen. D. Levillez (Ass. Chief Of Staff Well Being - Be)*
09:20 Opening	*S. Roels (VAR - Be)*

Wildlife Disease Surveillance in Belgium
Chairmen: P. Heyman & M. Govaerts

09:30 Wildsurv project	*P. Tavernier (VAR/UGhent - Be)*
09.50 Tick species in Belgium	*M. Madder (UGhent - Be)*
10:10 Mosquito species in Belgium	*W. Van Bortel (ITG - Be)*

10:30 – 10:55 Coffee Break

11:00 Bats as reservoir of RNA viruses	*S. Van Gucht (ISP - BE)*
11:20 Q-fever	*D. Fretin (VAR - Be)*
11:40 Rodent-borne Diseases	*K. Baert (INBO - Be)*

12:00 – 13:55 Lunch/Poster session (on site)

Wildlife Disease Surveillance:
The European Perspective
Chairmen: S. Roels & M. Dispas

14:00 Wildlife Disease Surveillance on the Iberian Peninsula	*Chr. Gortazar (IREC - ES)*
14:30 Wildlife Disease Surveillance in N-Europe	*A. S. Hammer (Nat Vet Inst - DK)*

15:00 – 15:25 Coffee Break

15:30 Wildlife Disease Surveillance in the Balkans Region	*M. Hukic (Univ. Sarajevo - BIH)*
16:00 EU-FP7 Wildlife Disease Surveillance in the EU (WildTech)	*C. Billinis (Univ Thessaly - GR)*
16:30 Closing remarks & poster awards	*P. Heyman (RLVBD - Be)*

16:50 End of the day & Coffee

Introduction : **Wildlife disease surveillance**

Disease outbreaks in man or animals are associated worldwide with pathogens emanating from wild animals. In the last decades socio-economic and environmental changes (climate) have contributed substantially to the increased risk of disease outbreaks caused by pathogens occurring in wild animals. Consequently the need of a well organised and continuous risk analysis in this area has increased as well.

For Belgium, an efficient network for active and passive surveillance of pathogens in wildlife, mainly game, exists in Wallonia since 2001 namely the “Réseau de Surveillance de la Faune sauvage en Région Wallone”. Furthermore, a number of organisations and institutes in the entire country are qualified to take part in networks for the surveillance of pathogens in wildlife. However, research initiatives in this field were limited or poorly coordinated until recently. An integrated organisation of sampling, diagnostics and reporting has become necessary at the national level, not only on behalf of the public health and animal health, but also in order to allow international communication including the annual reporting of notifiable diseases to the Office International des Epizooties (OIE). Therefore, last year we started up a project founded by the Federal Government (RT DG4) called WILDSURV with the goal to detect the gaps in the surveillance of wildlife diseases in Belgium, to improve this surveillance and to put forward a basic frame for an integrated surveillance system at the national level.

At the European level (on the other hand), several EU member states are currently developing projects to organise or to improve their surveillance of pathogens in wild living animals. Prior to this BWDS Symposium, two meetings were organized in Brussels namely yesterday and the day before. These meetings were organised in collaboration between the European Wildlife Disease Association, The Belgian Wildlife Disease Society, and the new EU project Wildtech, with he objective to bring together people from the different EU member states in order to discuss the various national projects and to try to develop common guidelines on which a supra-national EU surveillance system for wildlife diseases could be based.

Our third BWDS Symposium is somewhat complementary to this European expert meeting and will present different projects on wildlife diseases surveillance; starting in the morning with our own Belgian initiative Wildsurv. As we have realised that in The Netherlands a team of the National Institute for Public Health and the Environment (called RIVM) is working on a parallel project with strong links to Wildsurv, we are aiming to cooperate between the two countries at the Benelux level. Further on, the morning sessions include other “Belgian” wildlife disease surveillance themes. We will continue in the afternoon with reports about surveillance projects in Western, Northern and Eastern European countries (respectively presented by speakers from Spain, Denmark and Bosnia-Herzegovina) and finally we conclude with the presentation of the newly started European initiative called WILDTECH.

We hope you all will have an interesting meeting both on a scientific as on a social level.

Roels S.

Operational Direction Interactions and Surveillance, Veterinary and Agrochemical Research Centre (CODA/CERVA), Belgium

Vice president of the BWDS

Oral presentations

The WILDSURV project: towards an integrated wildlife disease surveillance in Belgium.

Tavernier P[1], Linden A[2], Dispas M[1], Pirot P[2], Roelandt S[1], Heyman P[3], Dewulf J[4], Roels S[1]

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WILDSURV is a Belgian Federal Government granted project funded from March 2008 to March 2010. The objective is to identify gaps and to determine priorities in the surveillance of wildlife-borne diseases in Belgium, taking into account the differences between the regions (mainly Flanders, Wallonia and the Brussels region). Although wildlife management is a regional matter, zoonotic diseases are dealt with at the federal (national) level, demanding therefore a coordination of wildlife disease surveillance by the Federal Government. Moreover the communication with the OIE (Office International des Epizooties) concerning infectious diseases in animals is a federal competence as well. Information obtained through the project will allow advising the implementation of an integrated wildlife diseases surveillance system, depending on political decisions.

In the first stage of the project a list of pathogens with their possible wildlife hosts in the different Belgian regions was generated. The pathogens list, corresponding to the “hazard identification” of a classical risk analysis, was based on the new (2008) OIE notification form for wildlife diseases but includes also other pathogens for which a possible release on the Belgian territory cannot be excluded. This list is however not restrictive and more pathogens can be included if necessary. Information about pathogens and their hosts was obtained from a broad literature study which is meant to be continued in the future in order to allow a continuous prioritisation (“dynamic ranking”) of pathogens for surveillance purposes, based on the most recent knowledge. A new database WILDTOOL was developed to store and process the collected data. These data are classified within four “criteria” including “host presence”, “impact”, “transmission characteristics”, and “occurrence” which correspond to the elements of a qualitative risk-evaluation as stated by the OIE Terrestrial Animal Health Code (risk characterisation, release- and exposure assessment). Some of the criteria can be scored directly with Yes / No (host presence, occurrence) but an optional “second level”

scoring will allow the use of more detailed numerical data that are available for these criteria, offering a refined end-result. For the criteria “impact” and “transmission”, scores assigned to “sub criteria” (compilations of data obtained from literature and stored in a checklist) will be resumed to “criteria scores”.

WILDTOOL will be put at the disposal of the end-users, being the competent authorities at various departments of the national and regional governments in the areas of public health, animal health, game management, pest control and conservation. The end-users will have the possibility to run queries based on the assignment of a chosen set of “weights” for the sub criteria (according to the specific purposes of the end-user) and on the choice of a specific region and a specific target group. Each query will produce a score for one chosen pathogen or a prioritisation list based on the ranking of scores for multiple pathogens.

On the other hand an inventory of the available resources for sampling and diagnosis was made through sending out an inquiry to institutes and organisations qualified for these purposes. By comparing the results of the inquiry with the prioritisation results, suggestions for basic surveillance networks can be made and lacunas in wildlife disease surveillance can be identified. Results will be submitted as far as feasible within the project possibilities to a quality assessment procedure including expert opinion (NUSAP method).

Taking into account the existing structures and the different competences at the federal and regional level, agreements will have to be made concerning the use of available resources in function of the different purposes of the stakeholders and the prioritisation results. The WILDSURV project fits in and converges with parallel projects in other EU countries such as The Netherlands and France which offers opportunities for supra-national cooperation and towards the future for an integrated European approach of wildlife disease surveillance.

This study was funded by the Federal Public Service of Health, Food Chain Safety and Environment (contract RT 07/5 WILDSURV)

Ticks in Belgium: the importance of sampling methods.

Madder M[1], Claerebout E[2], Losson B[3], Lempereur L[3], De Cat A[2], Heylen D[4], Tack W[5], Yackers Y[6], Demeersseman M-A[6] and Verheyen K[5]

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Ticks are one of the most efficient vectors of a variety of protozoa, rickettsiae, bacteria and viruses, some of which causing important diseases to domestic and wild animals as well to humans. The distribution of some of these ticks has been studied in detail, especially for those ticks that transmit economically important diseases or zoonoses.

For the last 10 years, several studies have been initiated in Belgium to monitor the presence of various ticks or their parasites and to determine the influence of changing habitat on the population density of ticks. These studies mainly focus on the sheep tick *Ixodes ricinus* as this is the most common and important tick species in Belgium. The tick sampling methods used in these studies to collect free-living ticks are not recommended to monitor all possible tick species in a certain region as the success of for instance dragging is entirely dependent on the questing behaviour and type of life-cycle of the tick species. Many of the local tick species exhibit an endophilic behaviour and are therefore not picked-up by drags. Furthermore, the phenology of the many ticks species encountered in Belgium seems significantly different and largely adapted to host activity. Both for endophilic and exophilic ticks sampling of parasitic stages also requires accurate information on host preference, apart from the phenology, as ticks can display a monotropic, ditropic and telotropic behaviour. It is obvious that sampling protocols for ticks often need to be adapted in relation to the target species.

An overview is given of the advantages and drawbacks of the most universal tick sampling techniques in relation to the observed species in Belgium.

MODIRISK: Mosquito species in Belgium

Van Bortel W

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Knowledge of the taxonomic and functional biodiversity of both endemic and invading mosquito species as well as the factors driving change is missing in Belgium. Acquiring this knowledge is an essential step towards understanding current risk on (re)emergence of mosquito-borne diseases in Europe. Therefore the project MODIRISK aims at (1) making an inventory of endemic and invading mosquito species in Belgium, (2) assessing the population dynamics of endemic and invasive mosquito species and their interrelationship and (3) developing spatial mosquito distribution models.

The inventory of mosquitoes was achieved by a large scale mosquito survey based on a spatial sample design. Major biotopes such as urban, rural and natural areas were selected. In total, more than 900 sites throughout Belgium were included in the study. Additional sampling took place in potential “import risk areas” such as harbors, airports and tire companies. Mosquitoes were morphologically identified.

A total of 26508 individuals, belonging to 23 species and 6 genera, were collected and morphologically identified. The number of species will certainly increase because some species complexes, such as *Anopheles maculipennis* complex, still need to be identified by molecular means. The most species rich genus in Belgium is *Ochlerotatus* whereas *Coquillettidia* is only represented by 1 species. The most abundant species was *Culex pipiens* which is found in a large variety of breeding sites. *Coquillettidia richiardii* was the second most prevalent mosquito. This is however due to one study site, a nature reserve in the harbour of Antwerp, where more than 3700 specimens of this species were collected. Interestingly is the general occurrence of *Anopheles* species, mainly *An. claviger and An. plumbeus.*.

Data on the distribution of endemic and invasive mosquitoes in Belgium will be further used for the development of predictive spatial models on the presence/absence of mosquitoes and will contribute

to understand the impact of eco-climatic changes on their distribution. Furthermore selected species will be studied in longitudinal studies. Data will be linked up and compared with similar ongoing projects in The Netherlands. An improved understanding of the biodiversity of mosquito vectors is an essential step towards a better understanding of the ecology of the diseases they potentially transmit.

MODIRISK (Mosquito vectors of disease: spatial biodiversity, drivers of change, and risk) partners:

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Dangerous zoonoses from bats: is it coincidence or is there more?

Van Gucht S

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Seventy-five percent of newly emerging infectious diseases in humans are zoonotic. More and more, it appears that bats are important reservoir species of zoonotic viruses, which sometimes cause serious disease in humans [1-4]. Famous examples are the SARS coronavirus [5], Ebola virus [6], Marburg virus [7], rabies virus [8], Nipah [9] and Hendra viruses [10]. But also other zoonotic viruses have been detected in bats, such as astroviruses [11], West Nile virus [12], tick-borne encephalitis virus [13], Rift Valley fever virus [14] and Toscana virus [15]. A review published by Calisher *et al.* in 2009 [4] mentions 66 different viruses that have been isolated from bats worldwide. Among these viruses, a striking number causes serious and lethal illness in humans or domestic animals, such as dogs, pigs and horses. For some virus genera and families, the number of different viral species that have been isolated is remarkable. For example, bats seem to be potential hosts for ten different types of rabies viruses (genus *Lyssavirus)*. In fact, epidemiological phylogeny studies more and more point towards the concept that bats are the original reservoir of lyssaviruses from which spill-over infections have occurred to other species [16]. Such spill-over events can yield a single dead-infection in an individual specimen, or, if the virus can properly adapt to its new host and all the socio-environmental conditions are right, initiate a temporary or long-lasting infection chain in the new host species. Eighteen different types of flaviviruses have been detected in bats [4]. Since the discovery that bats are the reservoir of the SARS coronavirus, numerous new coronaviruses have been discovered in bats and it is presumed now that bats are the reservoir gene pool for all mammalian coronoviruses [17].

The more spill-over events occur, the higher the possibility that a virus can adapt to its new host and meet all the conditions to spread in the new species and cause an epidemic. It is presumed that due to the growth of the human population, deforestation, increased land-use for agriculture, animal husbandry and urbanisation, the number of spill-over events is increasing. In certain areas of the world, such as south-east Asia or the Amazon forest, the habitats of humans and bats are more and more closing in on each other. For example, in the Amazon region vampire bats are attracted by live

stock of local villages and thrive on their blood. The increase in bat numbers facilitates rabies virus circulation. When live stock numbers suddenly decrease, the bats feed on humans and transmit rabies [18]. This phenomenon has already led to dramatic rabies epidemics in certain Amazons settlings.

From these examples, it is tempting to conclude there is a strange alliance between bats and viruses. But is this really so? Is there really a predisposition in bats to be carriers or reservoirs of viruses or are the recent findings merely a statistical coincidence? The truth is that not enough is known about virus epidemiology in bats and bat physiology or immunology to properly answer this question.

For sure, it is evident that certain characteristics render bats into suitable hosts for long term circulation and propagation of viruses. For one, bats often live in large colonies at very high densities allowing stable circulation of pathogens in an endemic equilibrium. Roosting and grooming behaviour favours transmission by direct contacts. Bats can also be migratory, allowing fast spread of viruses over large distances. Some bats have life spans of 20 to 30 years, allowing long term persistence and excretion of viruses by individual specimens. Interspecies transmission is facilitated in mixed colonies of different bat species. Although not proven, some researchers think that the immune response of bats may be different from other mammals and rather favours persistence and subclinical carriage of viruses, rather than full clearance of the virus or lethal disease [4]. The latter obviously reducing the chance of spread in the population. For example, the antiviral interferon response can be diminished or delayed during and after hibernation [19]. Some scientists hypothesize that there is a relative close evolutionary relationship between megachiropterian bats and primates, based on some striking anatomical and physiological similarities, which may facilitate pathogen transmission between both orders (see “The flying primate theory”: [20]). Bats belong to an ancient order and were among the first mammals to evolve [4]. Relative to other mammalian orders, bats have evolved little overtime. Certain families of viruses, such as henipaviruses and lyssaviruses, seem to have maintained themselves in bats since ancient times. So viruses which have co-evolved with bats may use cellular receptors and enzymes for their replication that are well conserved in mammals in general [4]. On the other hand, the occurrence of these viruses is very much dependent on the specific bat species and the geographical location. For example, Ebola is mainly found in certain fruit bats in Central Africa [6], whereas the SARS coronavirus is mainly present in horseshoe bats in southeast China [5].

Perhaps the most obvious explanation is the shear fact that bats are one the largest orders of mammals and represent a huge biomass all over the world [4]. The biodiversity of bat species is impressive: in fact twenty percent (925/4600) of all mammal species are bats. It is thus no wonder that a significant proportion of zoonotic viruses originate from bats. Nevertheless, it would be interesting if more studies would come out on the physiological and immunological nature of virus-bat interactions.

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Q Fever.

Fretin D

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Q fever is a zoonosis which is caused by an obligatory intracellular bacterium *Coxiella burnetii*. This disease, described for the first time among workers in Australia, is now recognized as being endemic worldwide except in New-Zealand (1). Multiple hosts can serve as reservoir of infection, including many wild and domestic mammals, birds and ticks. Domestic ruminants (Sheep, goats and cattle) are recognized as the main sources of human infection. Inhalation of contaminated aerosols is the primary route of human infection. Parturient products and feces of infected animals are the principal sources of contamination.

The diagnostics of Q fever is difficult and epidemiological studies are often based on serological investigations. *C.burnetii* does not grow on standard laboratory bacteriological media and isolation is long, difficult and hazardous to perform. In addition, it requires biosafety level 3 condition. For humans, the diagnostic is mainly based on serological analysis and the immunofluroescence assay (IFA) remains the most common method used to detect antibodies against *C.burnetii*. The complementation fixation test and ELISA are the routine tests for serodiagnosis in animals.

In Belgium, Q fever is present in domestic ruminants. The situation in wildlife is unknown. The objective of the study was to evaluate by serology the prevalence of C. burnetii in wild ungulates.

Sampling of rodents and surveillance of rodent-borne diseases in Belgium.

Baert K

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Worldwide rodents are the largest order of mammals with some 1500 species divided into 30 families. Some rodent species are of immense importance for the human society because of their depredation on crops , the structural damage they may cause and as carriers of disease. This is especially true for synanthropic or commensal species like the brown rat (*Rattus norvegicus*), the black rat (*Rattus rattus*) and the house mouse (*Mus musculus*) which are known as carriers of a number of pathogenic agents. This means that they can spread and transmit diseases easily to men and domestic animals. However, rodents living in the wild or open field can also be infectious, especially in densely populated countries.

Rodent-borne diseases (RBD) are spread to humans, livestock and companion animals directly through bite wounds or indirectly through contact with urine, faeces or contaminated food and water or by means of vectors such as ticks, mites and fleas. In this last case both rodents and parasites can act as disease reservoirs.

In Belgium there are 16 different rodent species. Most studies performed in Belgium on RBD are once-only and limited in sample size. These limited data being insufficient to obtain a realistic picture, a systematic surveillance is necessary in order to evaluate the true risks of RBD. As the ecology of each rodent species will influence the transmission and the epidemiology of the diseases they carry, ecological studies should be implemented simultaneously.

Rodent sampling is the essential “limiting factor” to obtain the necessary materials for RBD research. Samples can be obtained through cooperation with professional rodent controllers, in which case only pest species can be sampled in a non-systematic way. Moreover, the frequent use of rodenticides for pest control often renders impossible the sampling procedures. In most cases rodent-trapping is the method of choice. Depending on the species and the tissues needed, the rodents can be caught dead or alive. The use of snap traps is only appropriate when working on pest species or when there is a

need for tissue samples. Working on protected species or collecting blood samples demands the use of live traps. For serological research blood can be taken through retro-orbital, cardiac or saphenous vein puncture.

It is obvious that rodent sampling on a scientific basis is time consuming and represents often the bottleneck of reliable research. This is probably one of the reasons that so far, most of the RBD in Belgium are not well documented.

This presentation will focus on some rodent-sampling techniques in the field and on rodent species and their ecology in relation to RBD. Based on studies performed in Belgium and the neighbouring countries, an overview of the most relevant RBD, including hantaviroses, leptospirosis, EMCV and echinococcosis will be presented.

Wildlife Disease Surveillance in Spain

Gortazar C

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Wildlife diseases are increasingly relevant to human and animal health, and conservation. In consequence, wildlife disease surveillance and control is receiving increased attention (Gortázar et al. 2007). Wildlife disease control begins with surveillance (Wobeser 2002), knowing which diseases are present, their past and current distribution and the trends in their prevalence. Proper implementation of a complete surveillance effort must be a priority of the veterinary authorities, as it is accepted that those countries that conduct disease surveillance of their wild animal populations are more likely to detect the presence of infectious and zoonotic diseases and to swiftly adopt countermeasures (Morner et al. 2002). This presentation introduces the Spanish wildlife disease surveillance scheme, and the interactions with regional schemes and with wildlife disease research in Spain.

The Environment and Agriculture Ministry (MARM), in coordination with the 17 Autonomous Communities, is responsible for animal health. The draft of the Spanish wildlife disease control strategy, prepared in 2008 by IREC for MARM, includes two documents, (1) a wildlife disease surveillance plan, and (2) a wildlife disease contingency plan. For surveillance purposes, Spain is divided into six “bio-regions” depending on wildlife disease risk factors, habitat, and host distribution (Figure 1).

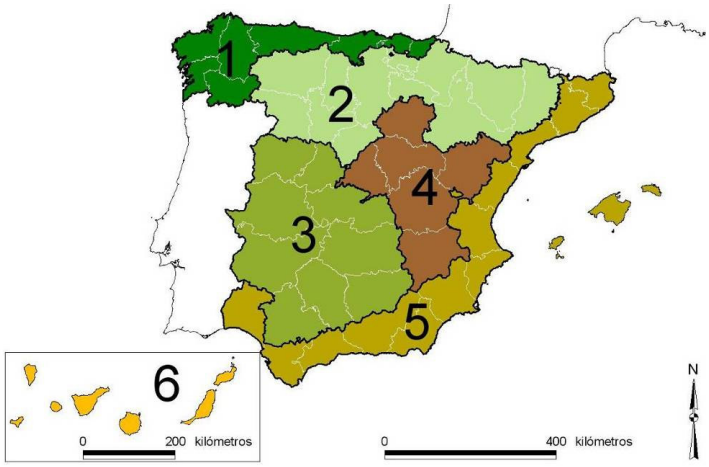

Figure1.- Bio-Regions defined in the (draft) wildlife disease surveillance plan.

Within each of these bio-regions, the surveillance plan proposes a minimum of animals of the most relevant species that should be sampled by active surveillance, mainly from random hunter-harvested wildlife. Animal species sampled include mainly wild boar, red deer, and waterfowl. Other game birds, lagomorphs and other ungulates are also considered, but with a reduced sampling effort. Carnivores and protected species are only considered in passive surveillance. In its current version, the total annual sampling goal is 3741 birds, 345 lagomorphs, 1980 wild boar, and 1385 wild ruminants. This active surveillance sample size is divided by regions and provinces to facilitate the work division among administrations.

Diseases of particular concern include TSE, avian influenza, West Nile, bluetongue, viral diseases of swine and wild boar, bovine tuberculosis, brucellosis and trichinellosis, among others. Current knowledge on their situation in Spain will be briefly presented, highlighting the opportunities for improved disease surveillance.

In addition to surveillance, action is being taken regarding disease control. Since July 2009, a new Royal Decree regulates the control of relevant diseases in any translocated wildlife, including the release of farmed animals for hunting purposes. Research is providing new diagnostic tools to

contribute to this improved disease control effort. Research is also focusing on disease control tools that may contribute to the wildlife disease contingency document, for instance by identifying risk factors and better management practices, or by developing specific tools such as vaccination.

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Wildlife disease surveillance in Denmark

Hammer AS

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The national Danish wildlife disease surveillance were founded in the early 30´ies, at a governmental institution belonging to the Agricultural Ministry and mainly dedicated to production of serum and vaccines for veterinary applications and disease surveillance - Statens Veterinære Serumaboratorium (SVS). Initially the main purpose of the wildlife section was to support eradication programs for severe contagious diseases like brucellosis and tuberculosis, by identifying possible reservoirs in wildlife. For more than 70 years this institution has conducted passive as well as active surveillance of diseases in Danish wildlife, making it one of he oldest – very likely he oldest - continuous national wildlife disease surveillance program in Europe. Many things have changed through the years: the institute changed ministry and name 5 times, it merged with other institutes and separated from others and the source and level of funding alternated. Finally funding for wildlife disease surveillance reached such dangerously low levels that survival of the section was threatened. Active surveillance was cut back to a minimum involving surveillance of only a couple of zoonotic agents (trichinella and avian influenza) and passive surveillance was maintained by minimal resources.

Recently funding from the Danish Forestry Agency has made it possible to take new initiatives. A National Center for Wildlife Health has been founded and for the first time the main focus of research and surveillance are changing from zoonotic agents to agents with relevance for wildlife health and management. The Center for Wildlife Health is cross-disciplinary and cross-institutional and managed from the National Veterinary Institute, were an epidemiologist has been added to a working group additionally including three pathologists, a virologist and a PhD-student. The Center also enabled establishment of a coordination group including representatives from the Danish research institutions engaging in wildlife research. In addition to representatives from the Danish Technical University, the group also includes representatives from the Danish Environmental Research, the Danish Forestry Agency and the Danish Food Safety Authorities. The purpose of he group is to coordinate and facilitate research activities and act as a counsel in case of outbreaks or other wildlife health emergencies.

Wildlife Disease Surveillance in the Balkan Region

Hukic M

Clinical Microbiology, Clinical Center University of Sarajevo, Bosnia and Herzegovina

The countries of Balkan Peninsula, in the last decade of 20th century and the first decade of 21st century, became the place of outbreaks of emerging and re-emerging diseases. The majority of them are wildlife zoonotic diseases and vector-borne diseases. Among the etiological agents of emerging and re-emerging infectious diseases are bacterial, viral and parasitic organisms that naturally reside in animal and arthropod hosts.

The most prevalent zoonotic and vector-borne diseases on Balkan Peninsula are: brucellosis; Leptospirosis, Listeriosis, Tularemia, Q-fever, Lyme disease, Anthrax, Rabies, viral hemorrhagic fevers, Sandfly fever, Tick borne encephalitis, Leishmainiasis, Echinococcosis and Trichinelosis.

During the acute stage of illness, the clinical signs and symptoms and available laboratory tests frequently do not point to a particular diagnosis. Epidemiological factors determined by the ecology of the bacteria are often the most useful diagnostic clues. The recognition of these evolving problems emphasizes the need for development of better laboratory diagnosis methods, for surveillance for and tracking of disease, and for continued research into factors contributing to transmission of the organisms. The continual appearance of previously unidentified infections requires prospective national strategies for timely recognition of the syndrome, identification of the agent, establishment of criteria and methods for diagnosis, optimization of the treatment regiment, and determination of successful approaches to prevention and control. There is urgent need for a communication channel among Balkan Peninsula countries.

Since 1992, the WHO is coordinating wildlife diseases surveillance in most of Balkan Peninsula countries. Although new technology and communication has extremely improved in the last decade, still there is a need for better exploitation of communication technologies like the Internet and other media in the field of emerging diseases.

EU-FP7 Wildlife Disease Surveillance in the European Union (WildTech)

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Surveillance networks and rapid detection of emerging and re-emerging pathogens that threaten human health and domestic livestock has become a priority due to their increasing incidence. Of the emerging pathogens 60% are zoonotic and 72% of these have wildlife reservoirs; examples are avian influenza, severe acute respiratory syndrome, rabies virus, West Nile virus, Ebolah and Nipah virus.

To address this problem the Wildtech consortium aims to construct a comprehensive surveillance system of wild animal diseases associated with public and animal health. We will develop cutting edge molecular and serological technologies for the detection of existing and new emerging pathogens in wild animal populations, which will enable for the first time, simultaneous screening of individual samples for a wide range of pathogens. Eventually, to construct a pan-European surveillance framework for wildlife diseases, we will develop a fully accessible data base for wild animal infections arising from this project and from other world-wide networks.

To serve this scope the WildTech consortium is broad-based involving extensive and essential collaboration between partners in UK (coordinator), France, Germany, Netherlands, Greece, Croatia, Sweden and Canada with links to the USA and, most importantly, its Associate Partners (APs) from Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Italy, Poland, Portugal, Romania, Spain, Slovenia, Switzerland, the Netherlands, Albania, Andorra, Botswana, Croatia, Russia, United Arab Emirates, Turkey and UK.

Poster abstracts

Transmission of Macaviruses in different species of ruminants

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Malignant catarrhal fever (MCF) is a fatal disease of different species of Artiodactyla caused by a group of closely related Gammaherpesviruses (GHV) recently classified into the genus Macavirus. Until now there are 6 distinct Macaviruses known to cause MCF: alcelaphine herpesvirus 1 (AlHV-1), ovine herpesvirus 2 (OvHV-2), caprine herpesvirus 2 (CpHV-2), white tailed deer-MCF-virus (MCF-WTD), an unclassified Macavirus from Ibex and a virus resembling alcelaphine herpesvirus 2 (AlHV-2). Other closely related viruses are the hippotragine herpesvirus 1 (HiHV-1 in roan antelope) and GHV in springbok, impala, oryx, muskox and aoudad. Until now AlHV is the only Macavirus that can be propagated in cell culture. Reservoir hosts which do not develop clinical disease are sheep, goat and wildebeest. Several species of Artiodactyla are susceptible to MCF, for example bison, cattle, deer and moose. In addition pigs, goats and experimentally infected rabbits show typical clinical symptoms like fever, central nervous disorders, erosions of mucosa, severe nasal and lacrimal discharge, corneal opacity and skin lesions. Diagnostic methods concerning MCF are currently based on OvHV-2-specific-PCR, histopathology and a competitive-inhibition-antibody-ELISA.

In a survey on samples on 199 wild and 176 small domestic ruminants we employed different PCR and Realtime PCR methods. Amplified fragments were cloned, sequenced and used for phylogenetic analyses. From 18 wild ruminants, 4 members of the Macavirus-group were detected: OvHV-2, CpHV-2, unclassified (Ibex), unclassified (Waterbuck). Interestingly single- and also double-infection with OvHV-2 and CpHV-2 occurred in goats whereas sheep samples contained only OvHV-2-specific DNA. We could show that more than 50% of tested goats were infected with a GHV, 70% of these with CpHV-2. Additional investigations along this line are required to confirm our preliminary data.

Studies on MCF have concentrated on the transmission of OvHV-2 from sheep to cattle. Our results show that additional host-species as well as additional viruses should be considered. According to our work there is a risk of transmission of OvHV-2 and other Macaviruses to endangered species in zoos and mixed species environment, the latter has become more common in recent zoo management.

Can Surveillance of Mortality in Wildlife help to detect Emerging Diseases?

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Since 1986 France, as a country, is covered by a wildlife mortality monitoring system, called SAGIR. A network of technicians from the federations of hunting and the National Hunting and Wildlife Agency (ONCFS), collect carcasses found by hunters and the general public, and submit them for investigation by veterinary laboratories, in each "département". The collected data are analyzed and results reported annually. More than 50.000 cases of mortality in wildlife were recorded between 1986 and 2007, representing 244 species of terrestrial mammals and birds, and were attributed to 220 different causes of mortality. Our aim is to use the data collected by the network to study the feasibility of early detection of unusual health events we call "abnormalities".

We first analyzed the functioning of the network, in order to evaluate its ability for the early detection of health events affecting wildlife. Syndromes of interest were then defined by a statistical typology of the lesions observed on the carcasses, using factorial analysis in order to identify the information which best explains the observed variability, followed by clustering techniques. We then carried out trend analyses on selected historical data, to establish the "background noise" of occurrence of these syndromes, characterized by multiple features (time, space, nature (species, habitat,…). Existing abnormality detection methods will then be adapted. Variation of occurrence of a syndrome should be detected by the methods as an abnormality and will lead to an alarm signal. The poster will describe the mortality monitoring system and the data collected; it will then illustrate some of its characteristics regarding detection of unusual health events. Then, it will detail the syndrome definition and trends, and finally present the abnormality detection methods chosen.

The study poses questions about the relevance of the use of mortality surveillance data for a broader purpose and gives arguments in favor of the use of this type of data for the detection of emerging diseases.

Monitoring of rabies in bats in Albania

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In the North of Albania, different cases of rabies have been reported. These cases include rabies in pets. Other cases of rabies in pets were also reported previously, in 1992-2006. Unfortunately a lot of people were also infected by rabies especially by bites of infected foxes or wolves, in few cases by infected dogs, and sometimes the source is not known. This has prompted the Ministry of Agriculture and Food to undertake a study to monitor rabies not only in wild carnivores, but also in bats. The monitoring study in wild bats has started in 2006 and is carried out by the Institute of Veterinary and Food Safety in collaboration with experts of the Faculty of Veterinary Medicine and the Veterinary Directorate in the Ministry of Food and agriculture.

We have received bats from different counties of Albania, especially from those problematic areas where rabies was seen in domestic animals. Methods used are immunofluorescence and biological diagnosis by inoculating cerebral extract in mice and kavire. Until now, our work has not revealed any positive case. However, we will apply soon new and mores sensitive diagnostic methods. Fully analyzed sample results will be available as a result of the involvement of the veterinary medicine faculty in the WildTech project. This will illuminate the rabies situation in bats in Albania.

Observations on foot and mouth disease in vaccinated and unvaccinated wildlife in the United Arab Emirates

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Foot-and-mouth disease (FMDV) is a contagious but usually nonlethal disease of ruminants. The virus is endemic in domestic livestock populations in the Middle East, but is rarely reported in wildlife. We present observations on two outbreaks of FMD in one unvaccinated and a second vaccinated collection of wild ungulates in the UAE.

Site 1 (unvaccinated) was a collection of dorcas gazelles (100) and sika deer (25). Site 2 (vaccinated) was a collection comprising blackbuck (~80), sand gazelle (~20), Arabian oryx (~45), mountain gazelle (~8), impala (~48) and spekes gazelle (~36) where most animals had been vaccinated annually against FMD since 2006. Affected animals were euthanased and submitted to the Central Veterinary Research Laboratory (CVRL, Dubai) for investigation. Necropsy and virology was conducted at the CVRL. Viral isolates were sent to the World Reference Laboratory at the Institute for Animal Health (UK) for characterization. The majority of gazelles and deer were severely lame and approximately 50-60% of the gazelles died at site 1 over a 6 wk period. Most free-ranging blackbuck were lame and FMD was confirmed in one euthanased blackbuck female and 4 dead juvenile animals at site 2. Oral and cardiac lesions were observed and FMD virus type O was isolated. The virus was closely related to FMD strains from India (Ind-2001) and Iran (Irn-2001).

The source of infection was not confirmed for either outbreak, but was suspected to be from dairy cows imported from Iran (site 1) and an adjacent infected sheep farm (site 2)

Except for a study by Kilgalon et al[1] the immunological response to any FMD vaccine has not been established in exotic ungulates. The authors concluded that a single dose of FMD vaccine may not elicit a sufficient antibody response in Arabian oryx to confer lasting protection. Our observations indicate that, although the animals at site 2 were only vaccinated annually, they were afforded good protection when exposed to the same FMD strain that caused high mortality in unvaccinated gazelle at site 1.

Investigations regarding *Rickettsia* spp. in ixodid ticks collected from European hedgehogs (*Erinaceus europaeus*).

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The European hedgehog (*Erinaceus europaeus*) is a common natural host of *Ixodes (I.) hexagonus* and *I. ricinus*, both vectors of zoonotic pathogens including rickettsial agents. Several recent studies describe the detection of *Rickettsiae* in both ixodid ticks and Giroud et al. suggested the reproductive host of these ectoparasites, *Erinaceus europaeus*, as reservoir of *Rickettsia* or *Neo-Rickettsia* species. In a study regarding the presence of *Rickettsiae* in hedgehog ticks, we investigated a total of 1,400 ticks collected from 33 hedgehogs in Germany. In addition, 150 ixodid ticks from eight hedgehogs originating from North Yorkshire were examined accordingly. German ticks were stored in 96% ethanol until further investigation whereas the ticks from North Yorkshire were retained at –80°C immediately after collection. Ticks collected included all life cycle stages. So far, 120 adult ticks were screened individually by a Pan-*Rickettsia* Real-time PCR targeting the citrate synthase (*gltA*) gene. This analysis revealed a prevalence of 4% *Rickettsia*-PCR-positive ticks. Species identification is currently performed by sequence analysis of amplicons of the *ompA* and *ompB* genes. Results regarding rickettsial species distribution and prevalence will be compared to data obtained from wild rodents and flagged ticks from Germany.

Failure of emergence of *echinococcus multilocularis* in foxes in the north of belgium anno 2007-2008

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Echinococcus multilocularis is a small cestode that lives in the mucosa of the small intestines of foxes. Eggs are shed in the faeces and can be ingested by rodents, which serve as intermediate hosts. Foxes are infected by ingestion of contaminated rodents. Humans can be infected by ingestion of eggs, which develop into multiple cysts in the liver. Aggressive proliferation of cysts leads to a lethal liver condition, called alveolar echinococcosis.

E. multilocularis is known to be endemic in a wide area of central Europe. This area extends as far as the south of Belgium (Wallonia), the southeast of the Netherlands, the east of France and Switzerland (Eckert and Deplazes, 2004). In Wallonia, the cestode seems to maintain itself stably in the fox population. Between 2003 and 2004, 25% of 990 examined foxes tested positive (Hanosset *et al*., 2008). The highest prevalences (41 to 62%) are found in the Ardennes and Fagne-Famenne. These areas are rather densely forested and located at relatively high altitudes between 400 to 700 meters above sea level.

The north of Belgium, including the regions of Flanders and Brussels, is more urbanized and has been colonized entirely by red foxes since the nineteen eighties. A temperospatial analysis of compiled epidemiological data from 1996-2003 predicted a northwest spread of the cestode from Wallonia and the Netherlands towards Flanders and Brussels (Vervaeke *et al*., 2006). In 2007-2008, we have tested 187 foxes, which were shot or found dead in the regions of Brussels and Flanders, with the intestinal scraping technique. None of the examined foxes carried *E. multilocularis* cestodes.

We found no proof of emergence of *E. multilocularis* in foxes in Flanders and Brussels, despite the nearby highly endemic region. The possible reasons are not known, but may include differences in altitude, climate, fox diet or low abundance of suitable intermediate hosts. We conclude that the current risk for the public is low, but further surveillance is warranted.

Tick-borne encephalitis (TBE): screening of the Belgian dog population as sentinels

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Tick-borne encephalitis (TBE) is a pathogenic flavivirus infection in humans and is the most important tick-borne virus in Europe. The Western subtype TBE-virus causes meningo-encephalitis with high morbidity and long term sequelae, but with relatively low mortality (0-3.9%). Though in 50% of canine cases, no clinical signs are detected, TBE can cause a febrile illness with lethargy, anorexia and multifocal neurological signs. The condition can follow a peracute lethal, a sub-acute or a chronic course. The most applied diagnostic test is IgG detection by ELISA. Positive results should be confirmed, especially in areas of low prevalence or where TBE is diagnosed for the first time. In endemic areas, canine seroprevalence is often higher than in humans. Symptomatic treatment (fluids, non-steroidal analgesics/antipyretics, anticonvulsants) is recommended in affected animals but a vaccine is not yet available for dogs.

Human TBE is emerging in several Northern and Western European countries and several canine TBE cases were diagnosed in dogs living in or visiting endemic areas. Given the dramatic increase in human cases in recent years, TBE is likely to be more frequently diagnosed in dogs in the future as awareness in the veterinary community rises. Though Belgian dogs travel to endemic areas and though dogs tend to have more frequent contact with the tick vectors of TBE (*Ixodes ricinus*), clinicians do not routinely test for TBE in canine meningoencephalitis cases and veterinary surveillance is currently non-existent in Belgium.

Until now, no autochthonous human or canine cases were reported in Belgium, but if TBE were to emerge, it could pose an important threat to canine and public health. Targeted serological screening of sentinel animals such as dogs and wildlife would contribute in a cost-effective way to answer the question whether or not TBE is present in Belgium. A commercial all-species ELISA test (Progen Biotechnik, Heidelberg, Germany) was adapted and validated for canine sera. A set of sera from northern (n=521) and southern (n=192) Belgium (n=713) was screened for TBE antibodies (IgG). Preliminary results are presented and discussed.

Sero-epidemiological study of four pathogens in cervids from Belgium and Norway

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Wild mammals may act as natural reservoirs for many diseases. Yet serological studies carried out in wildlife are limited. In the context of global changes, it is however essential to obtain a better knowledge of the pathogens circulating in wild populations from different geographical regions. In this study we tested for serological indicators of the presence of four pathogens in cervid species found in two regions with different climatic conditions (Belgium and Norway). We looked for the agents of leptospirosis (*Leptospira interrogans*), paratuberculosis (*Mycobacterium avium subsp. paratuberculosis*), Q fever (*Coxiella burnetii*) and bluetongue (bluetongue virus). In Belgium, we tested sera from 39 wild roe deer (*Capreolus capreolus*) and 15 domestic red deer (*Cervus elaphus*) for the four pathogens. In Norway all tested cervids were wild with the following sample sizes: leptospirosis in 20 reindeer (*Rangifer tarandus*), 15 elks (*Alces alces*) and 20 red deer, paratuberculosis in 100 reindeer and 50 elks, and finally Q fever and bluetongue in 100 reindeer, 50 elks and 97 red deer. The seroassays used included the microagglutination test for *Leptospira*, and ELISA tests for the other pathogens. In Belgium, roe deer presented a seroprevalence of 5.1% for leptospirosis (n=39), 5.6% for paratuberculosis (n=36), 2.7% for Q fever (n=37) but appeared free of bluetongue (n=34). The domestic red deer appeared free of leptospirosis but showed a seroprevalence of 6.7% for paratuberculosis and Q fever, and of 66.7% for bluetongue (n=15). All tested cervids from Norway appeared free from the four targeted pathogens, despite a sample size four times bigger than in Belgium. We suggest that this is partially due to the climatic differences between the two studied regions. This observation is particularly relevant for the insect-borne bluetongue virus, which was only found in Belgian domestic red deer. The presence of all four studied pathogens in wild or domestic cervids in Belgium can have implications for control measures and public health.

Zoonotic diseases from wild rodents

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Many infectious diseases have emerged from wildlife over the past decades. The largest number of them are zoonotic diseases which means that they are naturally transmitted from animals to men. In this context it is interesting to evaluate possible zoonotic risks from wild rodents that live often in close proximity to humans. This poster based on a literature study presents a schematic overview of the most important rodent-borne zoonoses and their importance in Europe. Bacterial diseases that can be transmitted from rodents to humans include leptospirosis, rat-bite fever, Lyme disease, tularemia, rickettsial diseases and pseudotuberculosis. The plague is mentioned because of its historical importance in Europe. Other diseases are caused by viruses such as hantavirosis, lymphocytic choriomeningitis, tick-borne encephalitis, encephalomyocarditis and cowpox. Though rodents can be a reservoir for zoonotic parasites such as toxoplasmosis (transmitted to humans through oocysts excreted by cats) or multilocular echinococcosis (transmitted to humans through eggs excreted by foxes), there are apparently only very few parasites known that are transmitted directly from rodents to humans. An example is *Giardia lamblia* for which rats and beavers have been reported as a possible infection source for humans.

For each disease we indicate the etiologic agent, its wildlife hosts, the most important way of transmission and the impact on humans. The gross occurrence of the disease and its evolution in Europe are mentioned.

The extensive literature study including treatment advices was presented as a Masters thesis at the Ghent University and is available on request.

Naturally Acquired Anthrax Antibodies in a Cheetah (*Acinonyx jubatus*) in Botswana

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An outbreak of anthrax in the Jwana Game Reserve in Jwaneng, Botswana, was first observed when three cheetahs (*Acinonyx jubatus*) died of the disease in November 2004. In the aftermath of this event, banked serum samples collected from 23 wild-caught cheetahs were examined, by the inhibition enzyme-linked immunoassay (ELISA), for antibodies to the protective antigen (PA) of *Bacillus anthracis*. Of the 23 cheetahs, 16 regularly accessed the reserve. Antibodies to PA were detected in one cheetah collected in May 2004, indicating the disease was occurring well before it was first noticed. This appears to be the first demonstration of naturally acquired anthrax antibodies in cheetahs. The finding of one antibody-positive animal amongst at least 16 potentially exposed individuals is consistent with existing reports that it is uncommon for cheetahs to develop natural immunity to anthrax.

Presence of Methicilline-Resistant *Staphylococcus aureus* ST398 in rats on Belgian pig farms.

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In the past, rodents have been associated with transmission of different diseases. However, they have not yet been associated with Methicilline-Resistant *Staphylococcus aureus* (MRSA). The objective of this study is to investigate the prevalence of MRSA sequence type (ST) 398 in rats on pig farms.

Fifteen rats have been sampled. From each rat one swab was taken from the anterior nares, another swab was taken from the skin behind the ear, the fur on the abdomen and back, and the tail. The rats originated from 3 different pig farms. The first 10 rats originated from farm A and were pooled in 2 groups (5 rats in each). One rat came from farm B, and 4 rats came from farm C. Swabs were enriched in nutrient broth (Oxoid) supplemented with 6.5% NaCl for 24 hours and was then sub-cultured on a selective chromogenic media for MRSA. Characteristic colonies were confirmed MRSA by using a multiplex-PCR for 16S *r*RNA, *mec*A and *nuc.* Further characterization was done by staphylococcal protein A (*spa*) typing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing and Multilocus sequence typing (MLST).

Farm A and C were found positive for MRSA. In farm A, two different *spa* types were identified, type t011, a common type in pigs and t4872. In farm C, all isolates belonged to *spa* type t011. SCC*mec* types IVa and V were found in farm A, and type V in farm C. MLST showed ST398.

Our results indicate that MRSA in rats is highly prevalent and can be isolated both from pooled and single samples. This indicates that rats may play an important role in the dissemination of the animal associated MRSA ST398. Striking is the first detection of a new *spa* type t4872 that has not been detected before in Belgium. This may be indicative for a constant evolution of the strain, perhaps under the influence of colonizing different animal species.

The major threats to tiger conservation in india

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The tiger (*Panthera tigris tigris*) is the spirit of the Indian jungle. Census of 1972 revealed tiger population to be approximately 1827 in the country. As a result, a tiger project, was commissioned in 1973 with the objective of its perspective in-situ conservation. Species population evolved as follows 3024, 4005, 4334, 3750, 3565 and 3163 respectively in the year1979, 1984, 1989, 1993, 1997 and 2001-02. The declining trend after 1989 census is 1347%, 5.0%, and 11.27% (may be little less, as census data of 3 tiger reserves of the 3 states yet to be documented officially). The threats to tiger conservation are related to poaching, infighting, electrocution and diseases. Annual loss due to poaching is reckoned by Government depart as 14, 38, 39, 46, 41, and 79 respectively in year 1998,1999,2000,2001, 2002 and 2003. The WPS reported poaching losses as 121 in 1995, 152 in 1996, 88 in 1997, 44 in 1998, and 81 in 1999. Country had lost nearly 200 tigers alleged to poaching alone between 2000-2002 (3 years). In many cases deaths are not specific cause related. Recent investigation of skeletal abnormalities revealed many cases of chronic ante-mortem fractures, related to traumatic insults caused due to poaching as well as intra-specific conflicts. Important diseases recorded are rabies, pasturellosis, paragonimiasis, gnathostomiasis. There is need to conduct a comprehensive study to assess the losses on account of dispersal phenomenon vis-à-vis territory profiles. This alludes to need to improve in the diagnosis and data recording system at the national level.

Bacterial & viral diseases of captive and free ranging Indian wild mammals

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One of the most important bacterial diseases encountered in Indian is tuberculosis (Tb), detected in deer, antelopes, primates (*several species*), chitals (*Axis axis*) and including several carnivores of which the susceptibility to the disease may differ.. *Mycobacterium bovis* has been detected in deer and antelopes and bears. Paratubercuosis has been diagnosed in wild ruminants.

Clostridium perfringens type D and C and C have caused deaths is bear and chitals (*A.axis*) chousinghas (*Tetraceros quadrcornis*) respectively. The latter have also been affected by Braxy (*Clostridium septicum*). Death of an elephant (*E. maximus*) calf is registered due to Blackquarter. Brucellosis has been detected in chitals (*A. axis*) with orchitis and blackbucks (*A. cervicapra*).

Pasteurellosis has been diagnosed in deer (*Axis axis*), antelope (*Antelope cervicapra, Boselaphus tragocamelus*), tiger (*P. tigris*), snow leopards (*Uncia uncia*).In wild as well captive artiodactylid species and elephants outbreaks of anthrax were recorded. Salmonellosis (*Salmonella* Enteritidis) cause mortality in pigmy hog (*P. salvania*); *S.* Choleraesuis in rhesus monkeys (M. mulatto) and *S.* Typhimurium in rhinoceros (*R. unicornis*) and cheetah (*Acinonyx jubatus*).

Of the viral diseases, foot lesions due to foot and mouth disease (FMD) have been detected in gaur, deer, antelopes and wild boar, wild hog-deer (*A. porcinus*). Rinderpest (RP) has been known since long in wild gaurs and in deer, however, presently the disease is contained. Peste Des Petitis Ruminants (PPR) and blue tongue (BT) are emerging viral diseases. Classical swine fever was detected in wild pigs (*S. scrofa cristatus*).

Feline panleucopenia (FPL) outbreaks occurred in large cats neonates, red panda (*Ailurus fulgens*). Canine infectious hepatitis (ICH) caused death of wolves (*Canis lupus*) and a black bear *(Ursus americanus*).Calcivirus infection was related to stomatitis in a hybrid lion (*P. leo*). Inclusion body hepatitis (IBH) has been diagnosed in leopards (*P. pardus*). Rabies claimed several deaths in tigers (*P. tigris*), hyaena (*H. hyaena*), wolves (*C. lupus*), bears (*M. ursinus*) and rhinoceroses (*R. unicornis*).

Prevalence of Toxoplasma gondii in Belgian Foxes

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Toxoplasma gondii is caused by an obligate intracellular parasite. It is a zoonotic parasite, member of the phylum Apicomlexa. *T. gondii* has a worldwide high prevalence as it infects most warm blooded animals, including humans. Few studies are available on the occurrence of this parasite in wild animals. In this study we investigate the prevalence of this parasite in Belgian foxes.

Foxes (*Vulpes vulpes*) are carnivores; they hunt live prey (especially rodents). They are also opportunistic feeders and eat a wide variety of other foods ranging from insects to fruits like berries. Once infected through ingestion of contaminated prey, the disease evolves in an asymptomatic chronic fase resulting in the formation of life long persistent tissue cyst (as in all hosts). The highest concentration of those cysts can be found in brain and heart tissue.

Brain samples from foxes were obtained from the Belgian Reference Laboratory for Rabies. In total, 278 samples were tested by Real Time PCR for the presence of *T. gondii* brain cysts. The amplified DNA target was the 529 bp *T.gondii* "repeat element" (AF146527). To check for inhibition, the cellular r18S gene was used.

The prevalence was found to be: 12.9% in Flanders, 19.17% in Wallonia and 13.82% in Brussels. In total 16.19% tested positive.

The presence of *T.gondii* seems to be rather common in foxes. As they are at the top of the food chain in Belgian wildlife, they are a good indicator to reflect the spread of this parasite in the environment. Further investigation of their prey could give some interesting clues on transmission of toxoplasmosis in wildlife.

Canine distemper virus outbreak in a beech marten population in Flanders

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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 morbillivirusses 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 intracytoplasmatic 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.

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 characterized 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. The concerning CDV-strain will be identified in the nearby future in order to compare the virus with already described strains. It will help us to understand this CDV outbreak and his influence on the beech marten population, and the possible virus transmission amongst different carnivore species.

A three-year survey on *Anaplasma phagocytophilum* in wild cervids populations in Southern Belgium.

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Through an active surveillance program, 1071 wild hunter-killed ruminants [749 red deer (*Cervus elaphus*), 279 roe deer (*Capreolus capreolus*) and 43 mouflons (*Ovis aries*)] were sampled (serum and/or spleen) during autumns of 2004 to 2006. The objective was to determine the prevalence of *Anaplasma phagocytophilum* in these wild populations present in Southern Belgium (24 forest districts studied in Wallonia). Serum samples were screened for anti-*A phagocytophilum* antibodies using an Indirect Immunofluorescent Assay (IFA IgG). Spleen fragments for 45 roe deer, seroreactive by IFA, were sought for bacterial DNA by PCR.

Overall, apparent seroprevalence were higher in roe deer [91 % (IC 95 % : 87,64-94,36)] than in red deer [44,3 % (IC 95% : 40,74 - 47,86)] and mouflon [58,1 (IC 95% : 43,4 - 72,8)]. In red deer, apparent seroprevalence decreased significantly with time (from 54,9 % in 2004 to 37,5 % en 2006) and results were significantly higher for cervids sampled in south forest districts (51,1 %) than for those sampled in center forest districts (39,8 %). In roe deer, apparent seroprevalences were maintained with time (88,2 % in 2004 to 94,7 % in 2006) but no significant difference in seroprevalence was seen for year or region of sampling. Of 45 roe deer tested, 21 spleen fragments (46,7 %) were *msp*-2 PCR positive.

In conclusion, this study gives the first insights of presence of *A. phagocytophilum* in wild cervids populations in Southern Belgium. These cervids, and specially roe deer, could be used as sentinels in epidemiologic studies related to this pathology. However, further studies are needed to determine if variant strains of *A. phagocytophilum* present in wildlife are human-infective strains.

Causes of mortality and diseases in hare (*Lepus europaeus*) in Southern Belgium.

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Since a few years, a decline of European brown hares (*Lepus europaeus*) populations has been reported in some regions of Southern Belgium. Different reasons have been suggested such as intensive agricultural practices, increasing pressure of predators, climatic conditions and/or infectious diseases.

From November 2006 to April 2009, we collected 50 hares (46 adults and 4 juveniles less than 2 months old; 29 males and 21 females). Animals were found dead or euthanized by hunters for ethic reason; most of the carcasses were frozen before examination. After necropsy, livers were tested for European Brown Hare Syndrome virus (EBHSV) by RT-PCR. Targeted bacteriologic and parasitologic analyses were performed according to suggestive gross lesions.

The body condition was reported as emaciated for 26 hares, poor for 10, and good for 14. RT-PCR results for EBHS were positive in 5. The cases concerned 4 adults and one juvenile. Gross lesions consisted in mild to severe pulmonary congestion (n = 5), hemorrhagic content in the small intestine (n = 4), discoloration and loss of consistency of the liver (n = 2), hepatic congestion (n = 1) and mild enlargement of the spleen (n = 2). Seven other hares presenting lesions suggestive of EBHS were negative by RT-PCR, probably due to the poor quality of the samples. *Yersinia pseudotuberculosis* was isolated from 4 adult male hares showing alteration of the body condition and mild to severe splenomegaly, as well as various lesions in internal organs. Pseudotuberculosis was suspected in eleven but could not be confirmed by isolation. Other pathologies were intestinal parasitism (8 cases), pasteurellosis (3 cases) and traumatic injuries (5 cases), One case of stress and one case of starvation in leverets, one case of purulent pericarditis due to *Staphylococcus aureus* and one case of cardiomyopathy.

These results show a wide diversity in causes of death, with a predominance of infectious diseases and chronic pattern. The presence of EBHS has been confirmed in Southern Belgium, without evident epizootic mortalities. Additional data are needed to strengthen the epidemiologic picture of the diseases in hare populations in our country.

Sero-epidemiological study of the presence of hantaviruses in domestic dogs and cats from Belgium

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Hantaviruses are worldwide rodent-borne pathogens that infect humans but also other animals. Infection occurs via rodent bites or inhalation of aerosols contaminated with rodent excreta. Domestic dogs and cats are probably more exposed to rodent contamination than humans. However few data are available on dog and cat hantavirus seroprevalence. We therefore screened serum samples from pet dogs and cats in Belgium. As rodent populations and human cases are larger in forested southern than in northern Belgium, we expected higher pet seroprevalence there than in the north of Belgium. We tested 534 sera (410 dogs and 124 cats) using IgG ELISA and IFA tests. We analyzed the effect of the owner's address as well as pet sex, age and blood test results on hantavirus status. We detected hantavirus antibodies in both pet species in northern and in southern Belgium with a higher seroprevalence in cats than in dogs (16.9% vs. 4.9%). Additionally more dogs were infected in southern than in northern Belgium (10.5% vs. 3.0%). This was not the case in cats. However, in the south but not in the north, positive cats were found in more densely forested localities than negatives ones. Finally, the blood analysis showed that more positive cats than negative ones had erythropenia. The high seroprevalence in cats could be linked to their specialisation in catching small rodents. Our study clearly indicates that, in Belgium, hantavirus infections are present in dogs and cats. These companion animals represent potential candidates for bio-indicators for this zoonosis.

Control of efficiency of oral anti-rabies vaccination in wild animals in the Kaliningradskaya Oblast of the Russian Federation

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The animals in the Russian Federation have been affected by rabies for many years. The epidemic situation of this disease has noticeably deteriorated for the last few years. Rabies is reported in wild, domestic and farm animals. In addition several human rabies cases are reported annually. Oral anti-rabies vaccination seems to be the only valid measure to control and eradicate rabies. The estimation of bait consumption is needed to evaluate the efficiency of oral anti-rabies vaccination in wild carnivores. To address the issue we used a widely applicable method with tetracycline used as a marker.

The only oral vaccine available on the market on the Russian Federation is ORALRABIVAC (PZB, Pokrov, Russia). This vaccine is based on the live attenuated rabies virus strain RV-97 and is now in use for all vaccination campaigns conducted in Russia. The vaccine contains tetracycline.

This important EU funded project started in 2007, with the attempt to eradicate rabies in the Kaliningradskaya Oblast. Two vaccination campaigns have been conducted. The tests carried out after bait distribution showed tetracycline presence in teeth of 15 out of 51 animals (or 29.41%). Tests carried out 1-2 months after the last vaccination showed tetracycline in teeth of 27 out of 51 animals (or 52.94%).

In conclusion, the correlation between the percentage of bait consumption and the number of seropositive animals (foxes, raccoon dogs, martens) was obtained. The vaccination of wild carnivores in this region of the Russian Federation will be continued for several years.

***Chlamydophila psittaci* in Belgian wild birds and feral pigeons**

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Chlamydophila (C.) psittaci is a bacterium causing psittacosis, ornithosis or generally called chlamydiosis. *C. psittaci* has been found in 469 different bird species. There are 7 avian *C. psittaci* outer membrane protein A (*ompA*) genotypes. The different genotypes have a certain host specificity and a different virulence. *C. psittaci* causes respiratory disease in birds leading to a systemic infection. The transmission is mainly aerogenic. *C. psittaci* is a zoonotic agent, thus can infect humans. Human infections vary from unapparent to severe systemic disease with interstitial pneumonia and encephalitis. When it comes to *C. psittaci* infections in wildlife, Belgium is a "blind spot". The aim of this study was firstly, to evaluate several diagnostics for Belgian wildlife and secondly, to collect preliminary data on the prevalence of *C. psittaci* in Belgium wild birds and feral pigeons.

Two pharyngeal swabs were taken from each bird: one swab with DNA stabilising reagent (*C. psittaci*-specific nested PCR) and one swab with chlamydial transport medium (culture and immunofluorescence staining). The *ompA* genotype was determined by use of the genotype-specific real-time PCR and/or the genotype-specific micro array.

Twenty-one birds housed in the Birds Rescue Centre of Merelbeke and 61 feral pigeons caught Ghent were examined. 14 on 21 (67%) birds were negative by nested PCR. 5 on 21 (24%) samples were possible false negatives. One of them was found positive by culture. 2 on 21 (10%) samples were positive by both nested PCR and culture. 3 on 21 (14%) wild birds were positive. Positive birds were: a Carion Crow (*Corvus corone corone)*, a Long-Eared Owl (*Asio otus*) and a European Magpie (*Pica pica*). Samples of the Carion Crow and the European Magpie were *ompA* genotype A. The sample of the Long-Eared Owl still needs to be genotyped. One on 61 pigeons (*Columba livia*) (1,6%) was positive.

According to the results, *C. psittaci* is present in Belgian wild birds and feral pigeons, consistent with other countries. It is important to collect additional samples from a broad range of wild birds.

Johne's disease in a herd of Sika deer in Belgium

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Johne's disease or paratuberculosis is a chronic infection caused by *Mycobacterium avium subsp. paratuberculosis*. The epidemiology and pathogenesis are best understood in cattle, sheep and goats, although the disease occurs commonly in many free-ranging and captive ruminants. Numerous species of wild mammals and birds are naturally infected, though not necessarily diseased. A debate exists as to the role of this bacterium in the pathogenesis of Crohn's disease in humans.

In april 2009, a male 2 year old Sika deer (*Cervus nippon*) was submitted for necropsy with a history of chronic weight loss and depression, evolving to chronic diarrhea and finally death. The animal was cachectic and dehydrated. Enteritis without thickening of the intestinal wall and moderate swelling of the mesenteric lymph nodes were observed. Histopathology showed a severe granulomatous enteritis with foamy macrophages in the propria mucosae near the villous tips. In the mesenteric lymph nodes, there was multifocal depletion and distortion of lymphoid follicles with diffuse infiltration of foamy macrophages and giant cells. Numerous acid-fast intracytoplasmic rod-shaped bacteria were seen in the macrophages on Ziehl-Neelsen stain. The presence of *M.avium subsp. paratuberculosis* was confirmed by bacteriological culture of intestinal contents.

Paratuberculosis in wild deer is widespread geographically. In countries with large scale deer farming, including New Zealand, Canada, the USA and some European countries, paratuberculosis can be a significant cause of economic loss. In cattle, young animals are more susceptible to infection, which may evolve into clinical symptoms around 2 to 5 years of age. In deer, Johne's disease can occur in all age groups. Many infected animals, as well in cattle as in deer, become asymptomatic carriers. The main problem in control programs for paratuberculosis is the lack of an efficient clinical test to recognize these asymptomatic carriers. Several studies conclude that serological testing by Elisa should be used as a screening test in deer, despite it's low sensitivity in early infections. Fecal culture is recommended as the definitive diagnostic test but also has a low sensitivity. Post-mortem, histopathology is less reliable in early or subclinical infections. PCR assay for DNA detection has recently been documented as a promising ante-mortem and post-mortem test.

Contamination patterns of feather-associated microorganisms of barn swallows (*Hirundo rustica*) from the radioactively contaminated Chernobyl area.

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Research on the ecological and evolutionary role of avian microflora has recently increased, as resistance to parasites is considered to play a central role in sexual selection and in population dynamics. Little is currently know about the environmental and biotic factors that shape the establishment, variation and dynamics of the bird's bacterial communities.

On the surface of the Earth, natural levels of radiation vary among sites, thus exposing living organisms to different radiation levels. Several studies described how the radiation from Chernobyl reduces the reproductive success, the survival and diversity of animals. Aberrant phenotypes have been found to be more common in populations living in radioactive areas. Currently, practically nothing is known about the role of radiation on avian microorganisms. In this context the 1986 nuclear disaster from Chernobyl, Ukraine, provides a unique opportunity to study the ecological consequences of high radiation levels.

In this report we describe a study where we investigated the cultivable microbial load of swallow feathers from the Chernobyl area. We found a negative correlation between total cultivable bacterial loads and environmental radioactivity, whereas fungal loads and abundance of feather degrading bacteria were not significantly correlated with radiation. Furthermore, abundance of both total and feather degrading bacteria increased with barn swallow colony size, showing a potential cost of sociality.

Here we will discuss the potential behavioural and ecological mechanisms underlying these relationships.

Salmonella in free-living and captive native _Vipera_ snakes in Romania

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Reptile-associated salmonellosis in humans is an increasing public health issue. Apparently, bacteria from the genus *Salmonella* are component of the normal intestinal microbial community for most of the snakes, emerging this reptile species as a significant source of human *Salmonella* infections. The aim of this study was to determine the prevalence of *Salmonella* spp. in Romanian free-living and captive autochthon *Vipera* species.

We screened 16 free-living and 10 captive native *Vipera* snakes for presence of *Salmonella*. The studied snake species where the following: common European adder (*Vipera berus*) (captive n=5; free-living n=3), nose horned viper (*Vipera ammodytes ammodytes*) (captive n=5; free-living n=4) and the critically endangered *Vipera ursini rakosiensis* (free-living n=9). Fresh cloacal samples were collected from the individuals with a sterile cotton swab and immediately processed according to a standard protocol for the detection of *Salmonella* (according the OIE). Identification of the isolates was realized by using API20E identification systems (BioMerieux, Lyon, France).

Salmonella was isolated from 5 captive and from 10 free-living snakes (50% and 62.5%, respectively), obtaining a total number of 19 isolates. Concurrent shedding of multiple strains of the bacteria was detected in four free-living snakes. We observed significant differences between the species, the European adder having higher prevalence than the other two species (87.5, 44.44% and 44.44% respectively). 15 isolates out of 19 belonged to *Salmonella* Arizonae, while the other 4 strains were identified as *Salmonella* spp. with our methodology. *Salmonella enterica* serovar Arizonae (*S. enterica* subspecies IIIa) is common *Salmonella* isolate from reptiles and can cause serious systemic disease in humans and in snakes under different stressors such captivity (Köbölkuti & Czirják unpublished data).

Our results confirm the risk of transmission of *Salmonella* from free-living and captive *Vipera* snakes to humans and the high prevalence should be considered as a concern in captive breeding programs (e.g. fatal *Salmonella* infections in *Vipera* species under stress of captivity).

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