BELGIAN WILDLIFE
DISEASE SOCIETY
(BWDS)

Belgian Wildlife Disease Society

2nd Symposium - October, 13th 2007

"Wildlife Diseases Environment & Man"

Queen Astrid Military Hospital
Bruynstraat
Brussels, Belgium

http://wildlife.var.fgov.be/

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08:30 - 09:00 Registration

09:15 Welcome ***(Div Well-Being/Health - Be***)

09:20 Opening & short presentation
P. Tavernier (UGent- Be)

SESSION 1 : Vector-borne diseases
Chairmen : P. Heyman & P. Butaye

09:30 Bluetongue
K. Declerq (CODA/CERVA - Be)

09.55 Bluetongue: results
A. Linden (ULG - Be)

10:10 West Nile virus & Chikungunya
H. Zeller (Institut Pasteur - Fr)

10:35 Coffee Break

SESSION 2 : Aquatic environment related problems
Chairmen : V. Luyasu & J. Van Wichelen

11:00 Sea mammals
T. Jauniaux (ULG - Be)

11:25 Decline of the European eel
C. Belpaire (INBO - Be)

11:50 Toxic Bluegreen algae
J. Van Wichelen (UGent - Be)

12:15 Lunch (on site)

SESSION 3 : Population dynamics

Chairmen : S. Roels & A. Dobly.

13:35 Population dynamics in Geese in Flanders
K. Devos (INBO - Be)

14:00 Rodent Ecology
A. Dobly (CERVA/CODA - Be)

14:25 Honey bee & Wild bee ecology
F. Jacobs (UGent- Be)

14:50 Coffee Break

SESSION 4 : Epidemiology & Control

Chairmen : K. Walravens & A. Linden

15:15 Control of TBC in France
J. Hars (ONCFS - Fr)

15:40 Besnoitiosis
A. Leitao (IICT - Pt)

16:05 Tick Borne Encephalitis
M. Kunze (Univ Vienna - Aus)

16:30 Closing remarks & acknowledgements
P. Heyman (MoD BE - Be)

16:55 End of the day & Coffee

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The Organizing Committee and the Scientific Committee thank the sponsors for their support to the Second Symposium of the Belgian Wildlife Diseases Society.

Introduction : Wildlife Diseases, Environment and Man

Evolution is determined by the capacity of living organisms to adapt to continuous changes in their environment. Adaptive mechanisms include altering food resources, behaviour, and population dynamics (i.e. the size and the distribution of populations) but also immunological, hormonal and metabolic readjustments. Natural selection is the result of the genetic background of these adaptations. Infectious agents, in ecological terms called micro- and macroparasites, form an inherent part of ecosystems. They contribute to maintain ecosystem balances through population control and they can determine evolutionary responses (Goater and Holmes, 1997). Environmental changes and the adaptations they induce in living organisms will cause adaptations of infectious agents as well. Ultimately, environmental conditions appear to be a key determinant of the outcome of many host-parasite interactions (Moore and Clayton, 1997).

More drastic environmental disturbances have been induced by contingent events. Under such conditions potential pathogenic agents may emerge and effects on biodiversity (whether at the ecosystem, species or genetic level) may occur. The Hantavirus Sin Nombre outbreak in New Mexico (USA) in 1993 was related to increased rodent densities due to climatologic conditions induced by an El Niño phenomenon (Martineau, 2001). El Niño events were also responsible for 50% population decreases in Galapagos penguin (***Spheniscus mendiculus***) numbers over a 20 years period. The periodicity and magnitude of El Niño events has increased in the last three decades (Root et al., 2007). Arbovirus infections in animals and humans show an increasing emergence pattern which is linked to global climate changes combined with anthropogenic factors (Toussaint et al, 2006)

More and more anthropogenic induced environmental changes constitute very important challenges to ecosystems. They create new interfaces and offer opportunities for infectious agents to conquer new niches through rapid adaptations and by crossing species boundaries (Osterhaus, 2005). Habitat loss through land use is thought to be the main reason for the emergence of several Australian bat viruses having a zoonotic potential such as Hendra virus, Nippah virus, Menangle virus and ABLV (Breed et al., 2005). Trade of exotic species is a major concern as proven by the detection of highly pathogen H5N1 avian influenza virus in smuggled eagles in Belgium (Van Borm et al., 2005). The mushrooming of “raptor shows” in Europe is responsible for increased trapping and export of birds of prey in their native countries, resulting in a rapid decline of species that occupy the upper levels of the food chains (Virani, pers. comm.). The introduction of grey squirrels (***Sciurus carolinensis***) carrying parapoxvirus into the UK led to a dramatic decline in native red squirrel ***(Sciurus vulgaris***) numbers (Sainsbury and Ward, 1995).

It is clear that threats to human and animal health and to biodiversity should be considered and studied simultaneously. The most intriguing question is whether evolutive mechanisms are able to cope with the highly increased (in frequency and intensity) anthropogenic challenges to the environment, and if so, what will be the cost at the level of biodiversity and human and animal health. Are we really facing

global pandemics as well as a global loss of biodiversity ? In illustration, current extinction rates are estimated 50-500 times background and are believed to be increasing with serious consequences for the future of life (Woodruff D.S., 2001). In the meantime and for the first time in the history of mankind, the world population had doubled in 1999 within a forty years period (Martineau, 2001).
Insight into these questions requires to study each disease or biodiversity problem through a case by case approach, taking into account the extremely complex interactions within and between ecosystems. Data input from monitoring programs is needed for ecological and epidemiological studies which are closely linked. This Symposium presents a number of important disease and biodiversity problems stressing the need of a full ecological approach for studying and managing wildlife related diseases.

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ORAL PRESENTATIONS

Bluetongue in the North of Europe.

Frank Vandenbussche, Tine Vanbinst, Elise Vandemeulebrouck, Nesya Goris, Koen Mintiens, **Kris De Clercq**

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Bluetongue (BT) is a non-contiguous disease caused by an orbivirus of the family Reoviridae. The bluetongue virus (BTV) serogroup consists of 24 serotypes. BTV is transmitted by arthropods of the genus Culicoides and its distribution worldwide is restricted to regions that contain competent vectors. Its reliance upon an arthropod vector that is normally exclusively found in tropical and temperate areas restricted BT for a long time to America, Australia, Africa and some regions of Asia. However, due to climatically changes BTV recently spread northwards into the Mediterranean Basin, where 5 serotypes of bluetongue (1, 2, 4, 9 and 16) have been identified in more than 12 countries. In the summer of 2006, BTV emerged for the first time in North-Western Europe and quickly disseminated over large parts of the Netherlands, Belgium, Germany and the North of France. Virus isolation and subsequent characterization demonstrated that the epidemic was caused by a BTV serotype 8 strain that had previously only been found in the Republic of South Africa, Nigeria, Kenya, Central America, the Caribbean and probably in India and Malaysia. Apart from the classical clinical signs in sheep, clear clinical signs in cattle especially characterized the BTV8 epidemic. In July 2007 BT re-occurred almost simultaneously in Germany, Belgium and the Netherlands. The virus has now emerged further to the East of Germany and more to the South into Luxemburg and France.

Bluetongue in wild cervids in Southern Belgium : data 2006

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Bluetongue (BT) was reported for the first time in Belgium in August 2006. The original cluster was first located in the border area common to Belgium, Germany and the Netherlands. The disease then spread over a large area along an east-west axis with a new cluster in the west of Belgium and a second one in Germany (EFSA, 2007). From August 2006 until February 2007, more than 2000 cases (cattle and small ruminants, especially) have been reported in Belgium, France, Germany, the Netherlands and Luxembourg (EFSA, 2007). BT is not a threat to human health. In veterinary medicine, consequences of a BT outbreak may be serious in some breed of sheep. On the other hand, recovery is the usual outcome in infected cattle. In the face of this emerging infectious challenge in Belgium, federal authorities imposed drastic measures to control the spread of a disease with such huge negative economic consequences for the farmers due to animals movement bans. Controlling a vector-borne disease such as BT is intrinsically difficult. The involvement of wild ruminants is one of the crucial questions to be addressed for circumscribing the epidemiology of the disease. In this context, the preliminary study reported here was carried out to determine whether wild ruminants could be considered as reservoirs for the BT virus or, at least, as indicators of its circulation.

In active surveillance, 684 wild hunter-killed ruminants (***Cervus elaphus*** and ***Capreolus capreolus***) were sampled (serum and spleen) during the autumn 2006. A second series of 262 sera from wild cervids samples in 2005 was joined. Geographic distribution of the samples covered 25 of the 37 areas (« cantonnements ») known to shelter wild cervids in Wallonia. The sera were screened for antibodies to BTV using a commercial competitive ELISA (ID.VET) and the spleen fragments were sought for the virus itself by RT-PCR. In passive surveillance, all cervids found dead since August 2006 were necropsied at the Veterinary Faculty with special emphasis on lesions suggestive of BT.

All sera sampled in 2005 were negative. In 2006, the apparent seroprevalence was 0.58 % (95 IC = 0,16 - 1,49). All the spleens analysed (327 from seronegative and 2 from seropositive animals) were RT-PCR-negative. These data (false/true and positif/negative results) will be discussed orally as well as the impact of possible biases in the data. In passive surveillance, no massive mortality case of cervids has been reported by the field collaborators in autumn 2006. At the Faculty, from the 102 wild cervids found dead (or shot for sanitary reason) and necropsied (between 8/2006 and 8/2007), only one roe deer (***Capreolus capreolus***) presented suggestive BT lesions, but the case was not confirmed by RT-PCR.

On the basis of the 2005-2006 data collected in southern Belgium, it is concluded that (1) there is no indication of massive BT infection in wild cervids in the areas studied and (2) these wild ruminants do not seem to play an important role as reservoirs for the BT virus. The wildlife BT surveillance program is currently working to follow the situation in 2007.

West Nile and Chikungunya: Emerging diseases?

Hervé Zeller

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West Nile Virus

West Nile (WN) is an arboviral disease present in Africa, Southern Europe and Russia, the Middle East and more recently in the Americas. The virus is a ***Flavivirus*** transmitted to birds by mosquitoes (mostly from ***Culex*** species) usually without detectable pathogenicity. Ornithophilic mosquitoes can accidentally bite sensitive hosts such as humans or horses. Non apparent infections are recorded in more than 70% of the cases, influenza-like illness in 20%, and a severe neuroinvasive illness in 1% of the cases with fatality rate up to 10% in elderly patients. Since 1994, frequent outbreaks in humans and/or horses had been reported in the Mediterranean basin. In 1997-1998, WN virus was isolated from dying wild birds (storks, raptors…) in Israel and several flocks of domestic geese were infected. In 1999, WN virus emerged in downtown New York causing a massive mortality in a large variety of birds species (crows and blue jays were particularly sensitive) and fatal neuro-invasive illness in humans and horses. The virus had more than 99% homology to the Israel 98 strain. Rapidly all the US states were infected as well as the southern part of Canada and the Caribbean region. A few equine cases were reported in Argentina in 2006.

The WN virus can infect a large variety of hosts including fox squirrels, alpacas, dogs, alligators or amphibians. In Europe, WN disease which is known since decades seems to have a limited impact on wildlife compared to the situation in United States. Meanwhile new pathogens can be introduced in any place. In 2001, a high mortality was noticed in blackbirds in Wien, Austria and Usutu virus was isolated from dying birds. No disease in mammals was noticed. The mode of introduction of this virus which is present in Subsaharian Africa remained completely unclear.

Chikungunya virus

Chikungunya (CHIK) virus (in makonde: "which bends up") is an ***Alphavirus*** (Togaviridae family) transmitted by ***Aedes*** sp. Mosquitoes, and widely distributed in Africa (Esat/West/South) and Asia. Clinical symptoms in humans are fever with strong arthralgia (sometimes with recurrency for months) and possibly exanthema. In 2005-2006 a large outbreak occurred in the Indian Ocean region and the virus was upgraded as an emerging threath. Longitudinal studies in the 1950's had shown that monkeys were involved in the cycle in East Africa but real impact of CHIK on wildlife is unknown. In Asia, no evidence of a wildlife cycle had been reported. Antibodies to CHIK had been reported in various vertebrates but none of them can be considered as a reservoir. Transovarial transmission in ***Aedes*** mosquitoes allows the maintenance of the virus. The disease is considered as a non fatal illness. However in La Reunion, severe cases and several fatalities possibly related to the viral infection had been observed particularly in elderly fragile patients. Simultaneously foeto-maternal transmission cases with neurological illness in newborns were reported in women who were infected during the last week of pregnancy. An adaptation of the virus to ***Aedes albopictus***, the main mosquito vector in the Indian Ocean, had been detected in the early phase of the outbreak. This mosquito species had been recently introduced in Europe and its area of distribution is extending. A consequence of this outbreak was the upsurge of studies on CHIK virus and pathogeny, on mosquito vector competence and control, and on transmission cycles.

These two examples reveal the importance of a multidisciplinary approach for a better understanding of the ecology of arthropod-borne viruses and their impact on wildlife and emphasize the need of coordinated surveillance programmes.

Cyanobacterial blooms in Flanders: nature, occurrence, toxicity and potential threat to wildlife.

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Although algal blooms are a natural phenomenon in water bodies, their occurrence and geographic distribution have increased worldwide, mainly as a result of anthropogenically induced eutrophication. These fast and strong algal proliferations have far-reaching esthetical, ecological and economical consequences. Many bloom-forming species belong to the Cyanobacteria (also known as bluegreen 'algae'), which are known for their potential production of toxic substances, such as hepatotoxins, neurotoxins and dermatotoxins, which are a direct threat for waterfowl, fish, cattle and pets. Probably the best known recent example of their toxicity for wildlife is the mass mortality among the Lesser Flamingo in some Kenian lakes (Krienitz et al. 2003, Codd et al. 2003). Also humans can become affected after contact with cyanobacterial toxins but in most cases symptoms are restricted to skin irritation and gastro-intestinal problems. Therefore, a maximal accepted microcystin concentration in drinking water was set on 1 µg L^{-1} (Falconer et al. 1994) and based on this a maximal accepted daily intake of 0.4 µg microcystin kg^{-1} bodyweight was launched by the World Health Organization (Chorus & Bartram 1999). Why Cyanobacteria produce toxins remains largely a mystery but one of the main reasons is believed to be protection against zooplankton grazing.

In the frame of the national BELSPO research projects B-Blooms and B-Blooms2 (http://www.bblooms.ulg.ac.be), a corporation between the Universities of Namur, Ghent and Liege, efforts were made to get a better picture of the magnitude of these problematic blooms in Belgian surface waters. Monitoring was started in summer 2003 and since than 104 different algal blooms, mainly in ponds and small lakes, were sampled in Flanders. Dominant bloom-forming taxa were isolated and cultivated. The species composition was assessed with microscopical and molecular tools, which was complemented by the analysis of the photosynthetic pigment composition, the concentration of cyanotoxins, and the genotypic diversity.

Cyanobacteria dominated about 82 % of all blooms, with ***Microcystis*** (46 %) and ***Planktothrix*** (18 %) being the most dominant genera. Blooms of ***Anabaena (13 %), Aphanizomenon (11%)***, ***Woronichinia, Pseudoanabaena, Anabaenopsis*** and ***Limnothrix*** (all less than 5 %) were less frequently observed. Toxin analysis for the presence of microcyctins, the most common cyanotoxin, was carried out for 56 blooms. In 38 % (21 blooms), high microcystin concentrations could be detected. ***Microcystis*** acted as the toxin producing agent in 19 cases while ***Planktothrix*** was responsible for microcystin production in 2 cases. However, molecular analysis revealed that two microcystin synthetase genes (***mcyB*** and ***mcyE***) were present in almost every sample, which is indicative of the widespread potential for toxin production in Flemish waters. Two toxic cyanobacterial blooms coincided with a mass mortality among waterfowl, with paralysis of wings, legs and neck ('limber neck') as the most frequently observed external symptoms. In Pond Leeuwenhof (Drongen, August 2004) and Pond Tiens Broek (Tienen, August 2005), liver samples of several victims were tested positive respectively negative for botulism toxin, indicating that cyanotoxins might have directly affected the waterfowl in the latter. We hypothesize that cyanobacterial blooms can lead to mortality among waterfowl, either directly by the production of cyanotoxins, which in turn provides dead bodies where ***Clostridium botulinum*** can proliferate, or indirectly by causing anoxia, which favors the production of botulism toxins. This is further supported by a 15 year spanning time series, in which a strong and positive correlation was found between avian botulism outbreaks in Belgium (as percentage of treated birds in wildlife rehabilitation centers) and hot summers with low precipitation, conditions known to favor cyanobacterial blooms. Improvement of the water quality is thus expected to be the only long-lasting remedy against mass mortality amongst waterfowl in the future.

In addition to the monitoring study of cyanobacterial blooms, laboratory experiments were carried out to learn more about the toxin production, growth rate and colony formation of different ***Microcystis*** strains and to unravel the potential importance of grazing by the Water flea ***Daphnia magna*** on these characteristics. Four genetically different ***Microcystis*** strains were isolated from Pond Leeuwenhof (two toxic and two non-toxic) and four from Pond Tiens Broek (also two toxic and two non-toxic). All ***Microcystis*** strains were grown with and without kairomones (pheromone-like chemicals that can induce protective responses in prey organisms) from ***Daphnia magna*** and in addition, the four strains from Pond Leeuwenhof were also grown together with grazing ***Daphnia magna*** individuals. The results from these experiments showed a high diversity in ***Microcystis*** strains considering toxin-production, growth rate and colony formation. Moreover, no correlations could be found between these characteristics. The differences in these characteristics were also larger between strains than between treatments for each strain and different strains also differed in reaction to treatments. Toxin production, growth rate and colony formation were influenced by ***Daphnia*** grazing and/or kairomones from ***Daphnia*** in some of the strains only. Also, ***Daphnia magna*** could control the growth of non-toxic strains, but failed to do so when toxic strains were present. In conclusion, zooplankton grazing seemed to play a role in the toxicity of ***Microcystis*** blooms, however, more studies are needed to completely understand its role.

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The decline of the eel.

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The European eel, ***Anguilla anguilla***, is a long distance migrator, living in continental waters but spawning in the Sargasso Sea. Larvae grow up during a sedentary phase in fresh water or estuarine habitats. Mature silver eels migrate downstream the river for a transatlantic migration towards the spawning grounds. Since the 1980s the eel stock has decreased dramatically: glass eel recruitment collapsed to ca 1% of the level of the 1970s. The stock is considered outside safe biological limits and current fisheries are not sustainable. The European eel has recently been listed on the UN CITES Appendix II list and the European Union agreed on the development of an international eel recovery plan including strict measures for restoring eel stocks. Anthropogenic factors like exploitation, habitat loss, pollution and transfer of pathogens have been suggested as possible reasons for the decline. However also natural processes may be involved (e.g. climate and oceanic currents change, predation). We discuss here the actual status of the stocks in Flanders through the glass eel recruitment series available for River IJzer and through the results of the fish stock assessment network. We will focus on two factors potentially responsible for the decline of the species: pollution and pathogens. It has been reported that high bioaccumulation of persistent organic pollutants and pathogens may be restrictive factors, disabling a long distance migration and a successful reproduction. Eels in Flanders are heavily polluted by a high number of persistent organic contaminants (1). Since the early 1980s a swimbladder nematode (***Anguillicola crassus***) has infected eels in Flanders (2). The colonization has been initiated by man and the parasite seems highly infective to new eel populations. Intercontinental trade of live eels (and maybe also paratenic hosts) seems to be the first infection route, subsequently, spreading has been enhanced additionally by trade between eel farms, transport procedures and restocking practises.

1. Maes J et al. in press. Spatial and annual variation of polychlorinated biphenyls, organochlorine pesticides and heavy metals in European yellow eel (***Anguilla anguilla*** L.) (Flanders, Belgium). Environmental Pollution doi. 10.1016/j.envpol.2007.07.21.
2. Audenaert V et al. Spatio-temporal Dynamics of the Nematode ***Anguillicola crassus*** in Flanders, Belgium. Diseases of Aquatic Organisms 2003, 56 : 223-233.

Causes of death of marine mammals: potential relationships with human activities

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Relationships between marine mammals strandings and anthropogenic activities have frequently been suggested, particularly involving pollutants in heavily polluted areas such as the Southern North Sea. To understand the cause of death of cetaceans and pinnipeds, and the potential links with human activities, marine mammals stranding networks appeared in Europe at the end of eighties. For the coastline of Belgium and northern France, the multidisciplinary research network MARIN (Marine Animals Research & Intervention Network) investigates the cause of death of stranded mammals and performs pathological and toxicological analyses on collected samples. The aim of the present paper is to summarize the most frequent causes of death of marine mammals in European waters, highlighting those linked with human activities. Such a link was initially suspected during the first epizootic of morbilliviral disease occurring in seals. Completely unknown before, morbillivirus infection in marine mammals was described in 1988, and proved responsible for the death of 18.000 harbor seals (***Phoca vitulina***) in the North Sea (population estimate 25.000). Since then, morbillivirus infections have been described worldwide, both sporadic cases and large epizootics, among various species of seals and cetaceans, including endangered species such as whales. It was frequently suggested that chemical pollution was a contributing factor but epizootics in the North Sea emerged after a southward shift of arctic seals, a species where the disease is endemic. A cause of death directly linked with human activities is the incidental capture of marine mammals in fishing nets. During the nineties, it was estimated that for the North Sea, every year ca. 7.000 harbor porpoises (***Phocoena phocoena***) were caught in the nets of the sole Danish fishingfleet, from a total population of 350.000. Considering the low reproductive rate, incidental captures represent a major threat for that species. Along the coast of Belgium, it was considered at the end of the nineties that 20% of the porpoises found dead or stranded had been by-caught. Nowadays, figures rank between 45 and 50%, with similar numbers for the UK, the Netherlands and Germany. Noise pollution can also affect marine mammals, more particularly the Low Frequency Active Sonars (LFAS) used to detect submarines during naval exercises. The first evidence was reported in 1996 in Greece when a mass stranding of very rare beaked whales occurred just after a military exercise using LFAS. Other similar mass strandings of beaked whales occurred in relations with LFAS in the Bahamas (2000, 17 whales), Madeira (2000, 3 whales), and the Canary Islands (2002, 18 whales). For the latter, whales were presenting evidence of acoustic traumas.

Finally, collisions with ship also represent a threat for marine mammals, more specifically in areas of dense shipping such as the southern North Sea.

Population dynamics in geese (Flanders, Belgium)

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There are many wildlife diseases that are known to effect birds. During the last years, especially avian influenza or 'bird flu' - caused by influenza A viruses - received much attention on an international and national level.

We can assume that viruses are relativily widespread among wild birds, although there is still little knowledge about the mechanisms of the spread of viruses in bird populations. It is however clear that the conditions that favour the spread of viruses are related to the behaviour and ecology of bird species. The most important factors are:

- the migration pattern
- habitat use during migration and winter
- gregariousness during migration and winter
- degree of mixing with other species during migration and winter

Based on these factors, high risk species can be defined. The threat posed by bird species with respect of introducing and spreading viruses in a particular country or region can also be expected to be related to the number of indivuals of that species entering the country (and the length of stay in that country). It is therefore very important to have good information on the ecology and the status of bird species. For that information, we relay on bird monitoring, census and ringing projects.

In Flanders (Belgium), the numbers of waterbirds in winter can be as high as 600.000 to 800.000 individuals (including waders and gulls). Considering the turnover of birds, the actual number visiting our country must be considerably higher. Looking at their behaviour and ecology, most goose species can be regarded as high risk species in relation to the spread of viruses. The number of wild geese wintering in Flanders has more than doubled during the last 10 tot 15 years. The most numerous species are White-fronted Goose, Pink-footed Goose and Greylag Goose, which all have different migration routes. Traditional wintering areas are quite localised in the polders, but several new areas have recently been colonised.

A totally different story is that of introduced and feral goose species which were very succesfull in colonising a large part of Flanders, such as Canada Goose, Barnacle Goose and Egyptian Goose. Their numbers have increased to more than 15.000 birds in recent years. Most of them show no long distance migration and occur year round in the same region. Their distribution and habitat choice is often very different from wild goose species, with a preference for more urbanised areas, park areas etc… In some areas however, mixing between feral and wild goose populations exists. This means that infected migrating birds could pass viruses to feral, non migrating populations.

As it is not preferable to prevent migrating birds to visit traditional wintering or stopover places (e.g. by culling or flushing birds), the current policy in most countries is to take measures which concentrate on preventing contact between wild and domesticated birds. This is also the case in Belgium where areas with large numbers of high risk species have been defined on a map and where preventing measures were taken (mainly keeping poultry indoors).

Although there are indications that H5N1 viruses can spread through infected wild birds (over short distances and with very localized outbreaks), it is more and more recognized that trade in poultry and poultry products plays a major role in recent outbreaks of avian influenza H5N1 and is probably even the main mechanism of global dispersal of the virus.

Rodent-borne hantaviruses: The ecology of an emerging zoonosis

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Rodent-borne hantaviruses constitute important emerging health risks. These RNA viruses were described in the Old World in 1951 and only in 1993 for the New World more deadly serotypes. They cause potentially deadly human hemorrhagic fevers worldwide while reservoir species apparently remain symptom-free. They are carried by various adult Muridae. In Belgium, bank voles (***Myodes*** [earlier, ***Clethrionomys***] ***glareolus***) is known to carry Puumala hantavirus and to transmit a mild form of haemorrhagic fever with renal syndrome to humans (Nephropathia epidemica). The contagion mainly happens through infected dust inhalation and the disease can drive to hospitalisation. Norway rats (***Rattus norvegicus***) carry Seoul serotype, which rarely transmits to humans in our regions. A new thread could come from yellow-necked mice (***Apodemus flavicollis***) living in south Belgium and susceptible to carry Dobrava serotype. Based on the results of 3-year wildlife surveillance in Brussels, we present the current state of knowledge of this public health concern.

For the voles we use the non-destructive capture-mark-recapture method while we destructively sampled the rats. We used ELISA rapid test and RT-PCR on lungs and kidneys of positive rats (with Paul Heyman from the Research Laboratory for Vector-Borne Diseases, Ministry of Defence, Division Well-Being/Health).

We firstly showed that 31% of adult rats carried Seoul hantavirus in Brussels. Despite local extinctions we found hantavirus at least once in all areas where voles were captured. The seroprevalence reached 57% at one time in one high-density area. Moreover, wood mice (***Apodemus sylvaticus***, sympatric to bank voles) can be infected with a similar seroprevalence. At low density, positive voles had interconnected home ranges forming clusters of very high seroprevalence. The vole seroprevalence was density-dependent both at low and high density but with two different dynamics. Finally the large multi-annual vole population variations were positively correlated with human case epidemics.

These last results open the possibility of human infection risk prediction. This could be done by the direct estimation of rodent populations or maybe by the examination of other more accessible bio-indicators (such as wild boars for example).

Honeybee and Wild Bee Ecology in relation to Pollination, Bee Pathology and the use of bees as Bio-Indicators.

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The honeybee is the second most important cultivated animal species at world level. As honeybees can be easily integrated into agricultural and other ecological habitats, problems in relation to competition between domesticated and wild species are described and discussed. An overview of several bee diseases is given, with special attention to those parasites and pathogens, which are recognised today as “very dangerous” and in a fast evolution by the interrelationship between different species as vectors and/or hosts: ***Varroa jacobsoni, Nosema spec***. and ***Aethinia tumida.***
The collection and residue-analysis of biological samples from bees, nest materials, the honey and pollen, makes it possible to study the “natural value” of particular environments, taking into account the specific background of each species. In conclusion, the value of bees as bio-indicators will be discussed.

Referenties:
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www.beekeepers.pbwiki.com

Monitoring and management of bovine tuberculosis in free-living red deer (*Cervus elaphus*) and wild boars (*Sus scrofa)* in the Brotonne forest (Normandy. France)

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In 2000, France was officially declared bovine TB free, thanks to an eradication plan in domestic cattle based on a test and slaughter program started in 1954. Before 2001, ***Mycobacterium bovis*** had never been reported in free-living wildlife. However, since 2001, ***M. bovis*** infections have been identified accidentally in wild ungulates of four geographically distinct regions: in red deer and wild boars in Normandy (2001) and Burgundy (2003), and in wild boars in Corsica (2003) and Pyrenees (2005). In these areas, like throughout the French territory, wild ungulates densities have increased greatly over the last 25 years. Epidemiological surveys were carried out in each region in order to establish whether TB reservoirs exist in wildlife.

In 2001-2002, in the "Brotonne forest" located in Normandy, the apparent prevalence of ***M.bovis*** infection was of 28 % in wild boars and 14 % in red deer. Control measures, such as the reduction of red deer and wild boar population levels, the ban on artificial game feeding and the destruction of hunted animal viscera were implemented to stop TB spread. Despite these measures, TB seemed to worsen in the affected animal species as in 2005-2006, the apparent prevalence of infection was of 23 % in red deer and around 30 % in wild boar, with an increased proportion of lesions, preferentially in mesenteric lymph nodes in red deer and in retropharyngeal nodes in wild boars. Red deer disclosed abscessed lesions with caseation in organs and lymph nodes while in wild boar, lesions were predominantly caseo-calcareous and occurred mainly in the lymph nodes. Only one infected roe deer and one red fox were found infected. ***M. bovis*** was not isolated from badgers.

Spoligotyping and MIRU-VNTR analysis allowed to determine that all wildlife ***M. bovis*** strains and strains isolated from nearby cattle had the same genotype, demonstrating a real epidemiological link between wild and domestic infections. Facing this situation, the veterinary services decided in 2006 (***i***) to wipe out the red deer population, considered as the primary reservoir of the disease and (***ii***) to dramatically reduce the wild boar population, considered as a spillover host. The first data collected during the 2006-2007 hunting season seem to disclose a decrease of the prevalence in both species.

On the contrary, in other French regions where TB has been identified in wildlife, only few cases have been recorded, without any apparent reservoir. In this latter situation, TB cases disclosed in wildlife could be the consequence of occasional previous contaminations from cattle breakdowns.

These results raise the question of the emergence and the management of TB in wildlife, in a country where this disease is almost eradicated in cattle.

Besnoitiosis: a re-emerging disease in Europe?

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Species of the genus ***Besnoitia***, which are classified in the subfamily ***Toxoplasmatinae*** of the phylum Apicomplexa, are widely distributed intracellular protozoan parasites of vertebrates that have recently received increasing attention due to their, real or apparent, increased prevalence. In this scenario, besnoitiosis in domestic animals should act as an alert and draw our attention to the wild life. Although the first descriptions of bovine besnoitiosis, caused by ***B. besnoiti***, are from France and Portugal at the end of the XIX century and beginning of the XX century, its occurrence in Europe received little or no attention until the last decade of the XX century. Since then, numerous cases have been reported in Portugal, Spain and France. The life cycle of ***B. besnoiti*** is poorly understood and the definitive host, although its existence has been postulated, has never been identified. The capacity of bloodsucking insects for transmitting mechanically the parasite from infected to uninfected bovine has been demonstrated, and the existence of other intermediate hosts can’t be ruled out. For developing our studies there was a clear need for the isolation of naturally occurring ***B. besnoiti***. We succeeded to isolate the parasites directly by inoculation of bradizoites, obtained by mechanical disruption of bovine skin cysts, into cell cultures. By this process we obtained isolates from south of Portugal, north of Spain and southeast of Spain. In order to address the diversity among ***B besnoiti*** we determined the 18S, ITS1, 5.8S, ITS2 and 28S rDNA nucleotide sequence from these isolates, as well as from an isolate from Israel (kindly provided by Varda Shkap, Kimron Veterinary Institute). These sequences, together with rDNA sequences from other species of ***Besnoitia*** and related genus available in the GenBank database, were aligned according to their primary structure similarity using ClustalW. Phylogenetic analysis was inferred by genetic distance using Kimura two parameter, maximum parsimony and maximum likelihood. The extremely high level of identity found among our ***B. besnoiti*** sequences (99-100%) argues in favor of a clonal population, and for all rDNA regions it was possible to infer an independent cluster for the ***Besnoitia*** members which is formed by two distinct groups: ***B. akodoni***, ***B. jelissoni***, ***B. darlingi*** and ***B. oryctofellisi***, in one group, and ***B. besnoiti***, ***B. bennetti*** and ***B.***

tarandi in another, more homogeneous, group. This latter group would include ***B. caprae*** that has been claimed to be indistinguishable from ***B. besnoiti***. However, host species barriers for parasites within this group have to be further investigated. Presently, we are validating in house developed diagnostic methods to test samples from different animal species.

TICK BORNE ENCEPHALITIS

o. Univ. Prof. Dr.med. Michael Kunze

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Tick borne encephalitis (TBE) is a vector-borne disease with very specific epidemiological features. The endemic regions in Europe are quite well known, there is no specific treatment for the disease, but prevention is possible through vaccination. Other means of prevention are much less effective.

The Austrian example shows that by means of intensive social marketing an extremely high acceptance of TBE vaccination can be achieved, bringing the diseases more or less under control. It is now established, that TBE is more severe in middle and higher age groups, which does not mean that children are not affected.

While TBE is quite well controlled in countries like Austria, the disease becomes a travellers issue. The increasing mobility of people, the intensive summer-tourism leads to an “export” of TBE from countries where the disease is known, to countries where the disease is extremely rare or not known at all.

Therefore the disease most probably is underdiagnosed in countries and regions which where TBE is not endemic.

POSTERS

Developing a degree-day model to predict the spring emergence of Bluetongue *Culicoides sp.* vectors in Southern Europe, Italy.

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In August 2000, an epidemic of Bluetongue (BT) was noted in the south of Sardinia, Italy, and the disease persisted several years in the region (1). Several species belonging to the genus ***Culicoides***, and in particular ***Culicoides imicola***, are the main vector of the virus (2). Consequently, a trapping network was set up throughout affected areas in Italy to survey the vector population dynamics. In 2006, a new collaborative called EPI-STIS (Remote Sensing tools to study the EPIdemiology and Space/TIme dynamicS of diseases) project was initiated between Belgian and Italian partners to analyse the population dynamics of ***Culicoides*** in relation to landscape and environmental variables. One component of this project is the analysis of the trapping data to explore the predictability of the vector date of emergences as a function of climate. This would allow, for example, predicting when and where the vector are the most likely to emerge each spring. Because of technical difficulties in breeding ***Culicoides*** species, a degree-day model of development can not be derived from laboratory experiments. In this study, we explore how the data from the surveillance network of traps in Italy can be used to build a degree-day model of emergence of ***Culicoides***, and in particular ***C. imicola*** in Italy. Using data temperature of high temporal resolution resulting from 134 weather stations dispersed in the study area, combined with LST/MODIS data of higher spatial resolution, we interpolated daily temperatures in each trapping site. A next step is then to relate the cumulated temperature to entomological trapping data in a degree-day model of emergences.

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Serological and genetical evidence for the presence of Seoul hantavirus in *Rattus norvegicus* in the Flanders, Belgium.

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Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) and nephropathia epidemica (NE) in Europe and Asia, and hantavirus pulmonary syndrome (HPS) in America. In rodent reservoirs, hantaviruses establish a life-long chronic infection. Seoul hantavirus (SEOV), carried by ***Rattus rattus*** and ***R. norvegicus*** is responsible for approximately 25% of the HFRS cases in Asia. Outside Asia there are only a few reports concerning confirmed human SEOV infections. This study aimed to evaluate the presence of SEOV in ***R. norvegicus*** in Flanders and the impact of its presence on a human at-risk group.

From 2004 until 2006, 502 Norway rats were trapped and sampled (476 serum and 338 lung tissue samples). Rat-catchers of the Flemish Government (N = 116) and a control group (N = 121) were screened for the presence of antibodies to hantaviruses. Serum samples were screened using enzyme immuno assay (EIA) tests, immunofluorescence assay (IFA) was applied to confirm EIA findings. Positive samples were subsequently submitted to focus reduction neutralization tests (FRNT) for determination of the causal hantavirus (no results available yet). SEOV genome in rat lung tissue was detected using RT-PCR and subsequently sequencing of the obtained amplicons.

Over the three-year period the average seroprevalence in rats was 27,10%.

In 2004 and 2005 no sex differences in hantavirus prevalence were found, in 2006 twice as much female rats then male rats were infected.

Seasonal changes in seroprevalence were observed throughout the study. In autumn and winter less seropositive rats were captured. The distribution of SEOV-positive brown rats in Flanders is widespread. The human at-risk group presented a significantly higher seroprevalence rate for SEOV and/or Puumala(PUUV)-IgG, i.e. 6.12% (6/98), than normal (1.4%).

Borna virus screening in wild mice in Belgium: preliminary data.

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Borna disease (BD), named after the city Borna in Germany, is a severe immunopathologic disorder of the central nervous system induced by infection with Borna disease virus (BDV), the prototype of a new virus family, ***Bornaviridae***, within the order Mononegavirales. It has been described in many vertebrate species in Europe and around the world, including humans. Whether BDV is zoonotic, easily crossing species barriers, and is transmittable to humans in contact with infected animals has yet to be elucidated.

Understanding the epidemiological processes is further complicated by the fact that not all infected final hosts may develop the disease. This indicates a higher transmission rate from possible reservoir species than assumed by numbers based on BD cases. The number of virus shedders in affected flocks is low and therefore the final hosts seem not to play a crucial role in virus transmission. Intensive experimental work has been conducted in laboratory rodents during the last decades. These investigations revealed that rodents are likely to develop persistent infections when infected with BDV as neonates. Moreover, horizontal and vertical transmission of BDV has been observed in laboratory mice and rats.

Typical robovirus (rodent-borne virus) infections would have disease peaks in late summer and autumn when population growth peaks but not in spring and early summer. The higher frequency of BDV in spring, however, could be related to mating activities of reservoir populations or to very long incubation period in the final host.

Recently, BDV was found in several wild small mammals (shrews, bank voles) in Switzerland and Finland. Cases of BDV infections have also been described in Belgium in a cat and sheep. Therefore the potential of rodent reservoirs can also be present in our country. In this study, we screened a group of 7 different species of wild rodents (Bank voles, Field vole, Wood mouse, Yellow-necked mouse, Pygmy shrew & Common vole & Brown rat) captured in different locations in Belgium during the period 1996-2002, and screened them for BDV using immunohistochemistry on the brain and myocard. None of them proved to be positive.

Frequency of anti-*Brucella abortus* agglutinins in neotropical wild carnivores kept in captivity in the northeast of Brazil.

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Habitat destruction results in decreases of wildlife populations, encouraging the adaptation of some species to new ecologic niches. As a consequence, the relationships between humans and animals become progressively closer, posing a potential threat to human health. Wild animals kept in captivity might have a special importance because they are easy to access, they live close to men and also many of these animals come directly from the wild which offers the opportunity to use them as sentinels. The objective of the current study was to determine the antibody prevalence against ***Brucella abortus*** in neotropical captive wild carnivores from three Zoos in the northeast of Brazil. Blood samples were collected from 42 animals, including 17 coatis (***Nasua nasua***), eight crab-eating raccoons (***Procyon cancrivorus***), three crab-eating foxes (***Cerdocyon thous***), three hoary foxes (***Lycalopex vetulus***), two little spotted cats (***Leopardus tigrinus***), five tayras (***Eira barbara***), two greater grisons (***Galictis vittata***) and two neotropical river otters (***Lontra longicaudis***). Two different serological tests were performed; the Rose-Bengal test (RBT) and the Complement Fixation Test (CFT). Our results showed 23 (54.76%) positives in the RBT test. On CFT, 8 (19.50%) of the samples were positive. These were the first reports of these host-pathogen associations. The presence of antibodies suggests that these animals had previous contact with ***Brucella abortus***, and that certain wildlife species are susceptible. We ignore whether wild neotropical carnivores are naturally affected by ***Brucella abortus*** and what is the epidemiological significance of this. On the other hand our results do not permit to extrapolate from carnivores to other wild species, and future studies should be developed to clear the role of wildlife populations. We are far to incriminate any of these species as wild reservoir and as interfering with national control programs. However, the lack of knowledge of epidemiological aspects in wild populations certainly is an obstruction to evaluate the Brucellosis control program in this country.

Status of and threatening factors for the endangered Yellow-shouldered Amazon Parrot (Amazona barbadensis) on the island of Bonaire, Dutch Caribbean

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The Yellow-shouldered Amazon Parrot is a medium sized parrot with a short tail. As is typical of the Amazona genus ***A. barbadensis*** is mostly green with colourful head, wing, thigh, and tail feathers. The extent of this colouration varies between individuals. The subspecies ***A. barbadensis rothschildi*** is described as having less yellow on the head and bend of wing. The validity of this classification however, is much disputed. After examination of skins from all parts of the geographical range Low (1981) concluded the subspecies classification was not valid. More recently genetic analysis of a limited number of birds has been conducted concluding that there is no significant genetic variation between sub populations (Sanz & Grajal 1998).

Habitat loss and alteration have had a negative affect on this species, but the most significant pressure they have faced, and continue to face, is poaching. Trade in parrots for the local market has a long history and keeping a pet parrot is very traditional in the regions were this species lives. On Bonaire in particular poaching has been so intense that there are actually more ***A. barbadensis*** in captivity than in the wild. This species is classified as vulnerable on the IUCN Red list and listed as an Appendix 1 species by CITES (Snyder et al. 2000).

Amazona barbadensis generally inhabit a dry xerophytic thorny scrub. Mangroves may be used during the nesting periods, particularly on Margarita. In this harsh environment, seasonal drought and the subsequent low food availability can have a significant effect on population numbers. On Bonaire there have been drought periods when large numbers of ***A. barbadensis*** have starved despite the efforts of islanders to provide food for these birds (Voous 1983). Less intense dry periods may have a negative effect on reproductive output as well as other natural factors such as predation and parasitism.

Severe alopecia in several Roe deer (*Capreolus capreolus*) due to demodeciosis in Belgium.

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Demodex mites are normal inhabitants of the hair follicles or sebaceous glands in all species of domestic animals, humans and perhaps all mammals. The mites found in the different hosts are regarded as separate species, although they are similar morphologically. Demodex spp. are obligate parasites, completing their life cycle in the hair follicle or its adnexae. They are rapidly killed by desiccation on the surface of the skin, but mites move from follicle to follicle, and it is probably at this time that transmission to another host takes place. Transmission usually occurs by direct contact from the dam to her offspring during nursing in the neonatal period. However, under states of putative immunosuppression these acarids undergo excessive proliferation and cause clinical disease, either localized or generalized. The pathogenesis of demodicosis is not fully understood. This study describes 4 cases of Demodicosis in roe deer (***Capreolus capreolus***) with localized to generalized alopecia. Three out of the four roe deer originated from the same area (Meeuwen – Guitrode)

First diagnosis of Encephalitozoon intestinalis and E. hellem in an European Brown Hare (*Lepus europaeus*) with kidney lesions.

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Encephalitozoon intestinales and ***E. hellem*** were diagnosed in the kidneys of a free –ranging European brown hare (***Lepus europaeus***) with multifocal wedge-shaped chronic interstitial neprhitis using real-time PCR and microarray. The detection of ***E. intestinalis*** and ***E. hellem*** in the kidney lesions of this hare was rather unexpected, as more likely ***E. cuniculi*** was assumed to be the etiology of these lesions, as the lesions caused by ***E. cuniculi*** in rabbits are very similar. In animals***, E. hellem*** has only been described in birds and fruit bats. In contrast, ***E. intestinalis*** seems to be a more mammalian parasite as it has been diagnosed in goat, pig, cow, dog, donkey and free-ranging gorilla. This is the first description of these microsporidia species in a European brown hare, which are both potential zoonotic agents.

Based on literature, ***E. hellem*** seems to be more pathogenic than ***E. intestinalis***. It would be interesting to know if ***E. hellem*** infections in mammalian species result in more pathogenic lesions compared to avian ***E. hellem*** infections and if the co-infection with ***E. intestinalis*** has an importance in the outcome of the pathology.

Anaplasmosis in Belgium; a six-year surveillance.

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Background: Human Granulocytic anaplasmosis (HGA) is a recently recognized tick-borne infectious disease. The causative agent of Human Granulocytic Anaplasmosis (***Anaplasma phagocytophilum***) is a Gram-negative obligate intracellular bacterium that invades granulocytes. Since 1995, serological evidence for HGA infection has been demonstrated in several European countries. ***Ixodes*** ticks are the main vectors for the HGA agent.

Methods: From 2000 to 2006, 420 serum samples were tested with an HGA IFA IgG and IgM antibody test kit. For IgG antibody detection a reciprocal titer of 64 was withheld as cut-off value, for IgM this was 20. A reciprocal titer higher than 64 for IgG, or higher than 20 for IgM was, according to the manufacturer's protocol, considered as positive.

Results: In total 186 samples (44.3%), out of 420, were found positive for either IgG or IgM antibodies. The 186 positive serum samples represented 143 patients. Seroconversion was observed in 5.6% (2/36), 6.1% (2/33), 9.7% (3/31), 22.2% (18/81) and 8.1% (18/223) of the patients from the years 2001 to 2005 respectivelyl. The sum for the period 2000-2005 brings the total number of confirmed cases in Belgium -according to the proposed case definition of Brouqui et al. to 43 cases

Conclusion: Our surveillance for HGA started in 2000, after we established the presence of ***A. phagocytophilum*** in Belgium. The results of this study probably still only show a tip of the iceberg; only 223 samples were submitted in 2005 while for LB, approximately 4.000 to 8.000 samples were tested. Considering that both LB and HGA are transmitted by the same tick species, it would be warranted to test febrile ill patients with a history of tick bite for both LB and HGA. Whether or not human granulocytic anaplasmosis is a true emerging disease in our country, rather than a persisting problem, can then be established.

Haemorrhagic Fever with Renal Syndrome outbreak during 2005 in Belgium, France, Germany, The Netherlands and the Grand Duchy of Luxembourg.

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Background: Hantavirus, a genus of the ***Bunyaviridae*** family, comprises the etiological agents of haemorrhagic fever with renal syndrome (HFRS). HFRS occurs in Europe and Asia, causing a disease characterised by fever, headache, gastrointestinal symptoms and renal dysfunction, the more severe forms with haemorrhagic manifestations. Nephropathia epidemica (NE), the milder form of HFRS, caused by Puumala (PUUV) hantavirus, is a viral infection present in most countries of North-Western Europe.

Methods: The National Reference Laboratories report data to the Public Health authorities.

Results: The 2005 HFRS epidemic in Belgium, France, Germany, The Netherlands and the Grand Duchy of Luxembourg resulted in a grand total of 1115 cases. Belgium, France, Germany, The Netherlands and the Grand Duchy of Luxembourg were respectively responsible for 31.4%, 22.7%, 40.2%, 2.5% and 1.26% of the grand total of 1086 cases. The main feature of the 2005 epidemic was the extension of the historically known endemic area in, at least, Belgium, France and Germany. In Belgium, the Liege province figured as a new hot spot area, in the Jura region in France a significant increase of human hantavirus cases was noted and in Germany an unexpected outbreak occurred in Lower Bavaria. The age distribution curb shows for all five countries an increased risk for hantavirus infection in the age range from 20 to 60 years, with the peak in the 41-50 years category.

Conclusion: The 2005 HFRS epidemic in Belgium, France, Germany, The Netherlands and the Grand Duchy of Luxembourg resulted in a grand total of 1115 cases. The main feature of the 2005 epidemic was the extension of the historically known endemic area in, at least, Belgium (the Liege province), France (the Jura region) and Germany (Lower Bavaria). The 2005 epidemic was the so far most important epidemic since 1994.

Puumala hantavirus, *Myodes glareolus* and their habitat preferences.

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Background: In Europe, Puumala hantavirus (PUUV) is the etiological agent of the mildest form of HFRS, Nephropathia epidemica. The bank vole (***Myodes glareolus***) is the carrier rodent and fluctuations in its population density correlate with epidemics in the human population. The aim of the study was to determine habitat preferences of ***M. glareolus***.
Methods: Rodents were captured during the years 2001 to 2003 in Belgium. The test site was an, approximately, 170-hectare terrain that showed a variety of significantly different habitats. It was known to harbour various PUUV-positive rodent species. Habitat properties, trapping indexes, rodent characteristics and seroprevalences were obtained. Rodent sera were screened for PUUV IgG and HTNV IgG positivity, the species and density of trees, understore vegetation, scrub wood, grasses and mosses were determined and soil humidity was determined.

Results: Habitat 1 : 39.6% of the ***M. glareolus*** and 39.3% of the ***A. sylvaticus*** were IgG positive. Traps in a biotope with a vegetation index of over 6 were significantly more successful.
Habitat 2 : 10.9% of the ***M. glareolus*** and 60% of the ***A. sylvaticus*** IgG positive. Traps in a biotope with a vegetation index of over 6 were, as in habitat 1, still successful.
Habitat 3 : 11.7% of the ***M. glareolu*** and 0.0% of the ***A. sylvaticus*** IgG positive. Trapping success was no longer linked to the vegetation index and appears random.

Conclusion: A decreasing diversity in food-producing plants and trees (represented by the vegetation index), significantly decreased the absolute numbers of ***M. glareolus.*** A determining factor for ***M. glareolus*** abundance was the presence of mosses for nesting. In a habitat where abundance (food, shelter) is the rule, ***M. glareolus*** and ***A. sylvaticus*** closely interact as evidenced by the similar PUUV antibody seroprevalences in both rodent species, this dramatically changes in favour of ***M. glareolus*** when habitat conditions change.

Host-parasite interactions between great tits (*Parus major*) and sheep ticks (*Ixodes ricinus*).

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Many ornithological studies have addressed questions on the fitness costs of nest-dwelling ectoparasites and their hole-breeding hosts. During a 10-year period we recorded infestation of sheep ticks ***(Ixodes ricinus)***, an ectoparasite which on the contrary has a complete life-cycle outside nestboxes of birds, in a Belgian population of great tits ***(Parus major)***. All ticks found on the birds were immatures. The prevalence on parents trapped during nest provisioning varied significantly between years from 16 to 50%, while young were rarely infected with this tick species. Parasite mean intensity on the parents was low (2.5 ticks per individual) but severe infections may occur (up to 25). A nonlinear increase in prevalence with relative date of hatching could be a consequence of changes in host's foraging behaviour. Also the positive association between the parasite loads of both parents during each breeding season and spatial clusters with high infection risk suggest a non-random infestation process.

Physiological effects of the sheep tick were studied by observational and experimental data. Artificially infected birds had a lowered hematocrit, and an elevated sedimentation rate of erythrocytes. The total leukocyte count increased with number of ticks in males, while a decrease was found in females. No effect of the ticks was found on residual body mass.

In summary, our data suggest sheep ticks play a part in the ecology of a small passerine as a harmful parasite.

Persecution of predators in Flanders, January 2003 to March 2006

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Malicious poisoning was confirmed in 66 (42%) and death from shooting injuries in 19 (12%) of 157 submissions of predators, both birds and mammals, presented for examination between early January, 2003 and the end of March, 2006. Each submission of one or more found-dead animals corresponded to one incident. Out of 242 subjects, 98 (40%) were poisoned and 20 (8 %) were shot. The number of submissions in which poisoning was confirmed gives a better indication for the amount of offences than the number of killed animals.

The percentage of confirmed poisoning incidents was 45 % for the period 2003-2005, 43 % for the first three months of 2003-2005 joined and 40 % for the first three months of 2006. When correcting for the high amount of winter mortality (30%) in the first three months of 2006, a poisoning percentage of 56 % of the submissions was obtained, coinciding with the gradual increase in poisoning percentages that were calculated from year to year between 2003 and 2005 (i.e. 30 %, 46 % and 56 %).

The most frequently detected poison in poisoned animals and in baits found in the field was carbofuran. Other organocarbamates, organophosphates, and strychnine were less frequently recorded. Rodenticides including coumarines and indandion derivates were recorded sporadically.

Ignoring the target species for malicious poisoning and not taking into account the poisoned animals that were not recovered, buzzards (***Buteo buteo***) were the most frequent victims of fatal poisoning incidents. Out of 162 buzzards, 91 (57 %) were deliberately killed from which 79 (49 %) were poisoned and 12 (7 %) were shot. The buzzard is a regular carrion eater and it is amongst the most common raptor species in Flanders. The current data do not allow to estimate possible effects of persecution on the population of buzzards in Flanders

Results of 2 years sampling for avian influenza in free ranging geese in Belgium.

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During two consecutive years Canada geese (***Branta Canadensis***) and small numbers of cross-bred geese were caught at 3 different locations in Flanders from the end of June to the beginning of July, in their moulting period. Cloacal swabs (n = 564) were collected and stored in Hank's solution at -80°C for the 2005 samples and at -4°C for the 2006 samples.The samples were analysed by real-time reverse transciption-polymerase chain reaction (RRT-PCR) for the detection of influenza A viruses. Serum samples (n = 423) were collected from the V.basilica and specific antibody titers against H5 and H7 subtypes of avian influenza A virus were determined by a hemagglutination inhibition (HI) test following standard procedures (Council directive 92/40 EEC). Additionally 325 serum samples were subjected to an IgG ELISA test for the detection of antibodies against avian influenza M2e antigen. M2e is an influenza A virus induced cell membrane protein that is abundant on virus infected cells.

All of the cloacal samples were negative for influenza A viruses. No HI antibody titers were detected in none of the sera. Fifteen sera (14 from Canada geese and 1 from a cross-bred goose) showed moderate to high titers of anti M2e IgG, ranging from 100 to 8100. Though until now no high pathogen (HP) avian influenza viruses have been found in free ranging birds in Belgium, the detection of anti M2e antibodies in 15/325 (5 %) sera from geese proves the circulation of unidentified influenza viruses. Since Anseriformes constitute a natural reservoir for influenza A viruses, and the breeding and wintering populations of feral Canada geese are steadily increasing, he detection of seropositive Canada geese offers a good yet incomplete reflection of the avian influenza A virus prevalence. Sampling should be intensified and extended to the migration periods and to other species.

Absence of blood parasites in feral geese in Flanders

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Blood parasites in Anseriformes have been investigated extensively in North America and in Canada, but little is known about their prevalence in Europe. Environmental changes and the translocation of avian species may increase the opportunities for interactions between blood parasites, their hosts and their specific vectors. Blood parasites known to occur in Anseriformes include ***Leucocytozoon simondi, Haemoproteus nettionis, H. greineri and Plasmodium sp.*** Blood smears (n = 268) from mainly Canada geese (***Branta canadensis***) and small numbers of cross-bred geese from three different locations in Flanders were stained with Hemacolor® (Merck) and examined under immersion light microscopy (magnification x1000) in order to detect blood stages of hematozoa.

No blood parasites could be found in none of the smears. The number of smears per geese (one), the minimum examination time of each smear (5 minutes), and the period of sampling may have influenced this result. Sampling was performed within a two weeks period from the end of June to the beginning of July, corresponding to the annual mute period in which the geese could be caught. Due to the existence of seasonal peak parasitemia periods our sampling period may have missed these peaks.

The Canada goose is an exotic species that was introduced in Europe in the 17th century. In Flanders the numbers are increasing excessively since the seventies. Moreover litlle is known about the prevalence of the specific vectors of hematozoa of Anseriformes in Flanders. Consequently evolutionary balances between parasites, hosts and vectors, may not have been established yet, which could have determined our results. Investigating the prevalence of hematozoa in native and domestic species would therefore be appropriate.

Molecular characterization of *Pseudomonas aeruginosa* animal isolates

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Introduction. ***Pseudomonas aeruginosa*** is emerging as one of the most problematic nosocomial pathogens in hospitalized patients mainly due to increasing levels of antibiotic resistance and a multiplicity of virulence factors. Not surprisingly, ***P. aeruginosa*** is also associated with infection and even outbreaks in animals (sheep, goats, cows, dogs, seals, ..).

Materials and Methods. We characterized 60 ***P. aeruginosa*** animal isolates (dogs, horses, sea turtles, dolphins, cats, birds, canguru,..) using, amongst others, serotyping and fAFLP (fluorescent Amplified Fragments Length Polymorphisms) fingerprinting and compared them to 250 human clinical isolates using biological data analysis software (BioNumerics).

Results and Discussion. Most ***P. aeruginosa*** animal isolates clustered into successful clonal complexes also harboring human isolates, including some renown human epidemic strains. These findings illustrate the significance of animals (domestic and wild) as a reservoir of potentially human pathogenic ***P. aeruginosa*** strains and could have repercussions on antinosocomial infection strategies.
In a constant evolving world, medical epidemiology should strive for a broader perspective, consisting of a global approach including the study of bacteria isolated from humans, the environment and last but not least animals from different geographical locations at different points in time. This should result in a better understanding of bacterial evolution and its impact on human health.

Genetic variability in the European brown hare *(Lepus europaeus)* population in Flanders.

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The European brown hare (***Lepus europaeus***) is an important game species. The numbers of European brown hare have declined throughout Europe since 1960 (1). Although different causes for the decline were suggested, none of them could fully explain the disappearance of the species (2,3). The loss of genetic variability and the inbreeding depression were rarely mentioned.

The aim of this study was to investigate a microsatellite marker based genotyping test and the application of this test in screening genetic variability in Flanders.

Firstly, eight pairs of previously described microsatellite primers of related species were tested on DNA of the European brown hare. Seven of them turned out to be useful in a screening protocol. SOL28 never gave any result.

Secondly, the Flemish hare population was sampled by the prelevation of ear tissue of 598 recently shot hares on 12 different sites across Flanders. After PCR, the specific allele combination for SOL08, SOL30, SOL33, SAT02, SAT05, SAT08 and SAT12 for each sample was determined according to the earlier derived screening protocol.

Each of the microsatellites showed polymorphism with 4 to 16 alleles. Few of the alleles were unique for 1 site. Heterozygosity reached a normal level in most of the cases. In the other cases a low effective number of alleles was responsible for the heterozygote deficiency. So the obtained results suggest the absence of a severe depression of the genetic variability or inbreeding in the Flemish hare population.

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First clue of circulation of Lyssaviruses in bat population

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Scarce data about bats were available before 2005 in Belgium. Conventional dead bat collecting did not bring significant results because of few samples in bad conservation condition. During 15 years, about 30 brains were tested by IFI or PCR and none were positive.

To improve our knowledge, an active survey was undertaken since 2005. In order to cause minimal disturbances to the reproductive and social behaviour of bats (protected species), the captures were carried out after the weaning of offsprings and before the hibernation, from mid august to the first cold weather period, in front of the entrance of natural cavities which were not used as nursery.

In 2005, bats were captured in 6 cavities from Thierache and Haute Ardenne. They belonged to 6 different species (***Myotis myotis***, ***Myotis mystacinus brandtii***, ***Myotis naterreri***, ***Myotis bechsteinii, Myotis daubentonii*** and ***Plecotus spp***) ; 24 sera from 40 bats could be analysed for EBL1.

High titres of EBL1 antibodies (>100) were found in 1 ***myotis myotis*** out of 8 and 1 ***plecotus spp*** out of 8. Antibodies were also found at lower levels in 1 more ***myotis myotis*** and in 2 ***Myotis natererri***.

Those preliminary results underlined that serology is more sensitive than virology to control bat exposition to rabies virus and to have an index of virus circulation among bats. A better epidemiological description of the viral circulation(s) is necessary to assess the risk exposure of humans and domestic animal to bat rabies virus.

Serological investigation of brucellosis in wild boars in Southern Belgium : comparison of four tests

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Brucellosis is endemic in wild boar populations of many European countries. The causative agent is ***Brucella suis*** biovar 2. This biovar is not zoonotic, but it represents an important threat for outdoor-reared pigs, as it is observed in France with a spillover from wild boar. The serological diagnosis of brucellosis remains difficult as no test, taken individually, offers optimal intrinsic values. The diagnosis gets even more complicated in wildlife surveys due to the poor quality of serum sometimes reported.

To survey brucellosis in wild boars in Wallonia, blood samples from 1006 animals have been collected from 2002 to 2006. Four serological tests have been used in parallel : an indirect ELISA test using protein G (iELISA), fixation complement test (CFT), rose bengale test (RBT) and slow agglutination test (SAT).

The highest seroprevalence was obtained with iELISA (49,5 %), then with CFT (36,3 %), RBT (31,4 %) and SAT (23,1 %). The relative specificity to iELISA reached 99,42 % for CFT, 90,09 % for RBT and 97,29 % for SAT.

These results denote a chronic pattern for brucellosis amongst wild boars populations. The iELISA gave the highest seroprevalence rate, which was expected because iELISA is considered as a high-sensitivity screening test. Moreover this test showed to be very robust towards haemolytic and/or contaminated samples, as not all samples were able to give a result by the three other tests. Our results confirm also that CFT is very specific. SAT is known for having a low sensitivity; nevertheless it has the advantage to detect IgM and thus acute infections.

The serological profile of the boars will be compared with the results of the direct tests (PCR and culture on spleen and tonsil). Some of cultures have already been proceeded and all the ***Brucella*** isolated showed to be ***B. suis*** biovar 2. The eventual cross-reactivities with ***Yersinia enterocolitica*** O:9 will be investigated by the way of an ELISA YOP (***Yersinia Outer Proteins***).

Vector-borne diseases: Recrudescence of Canine Leptospirosis in Belgium?.

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Leptospirosis - also known as Weil's disease – is a well-known zoönotic bacterial disease, which can affect several species of mammals, including cattle, pigs, dogs and men. Leptospira are long helical spirochetes that thrive in a moderately warm, watery environment. However, draught and subtle changes in humidity and temperature can be responsible for short survival times outside hosts. Leptospira use reservoirs such as all sorts of rodents, which carry the bacteria in the kidney and other organs for long periods. The Leptospira can be intermittently shed in the urine. Men and other mammals can become infected by contact with infected urine or urine-contaminated water. Mainly the strains or serovars of ***Leptospira interogans***, which are divided into more then 20 serogroups, can cause fever-like symptoms, hepatic disease of kidney failure. This poster will discuss and present some observational data of the occurrence of Leptospirosis in Belgium. Data were gathered in the Belgian veterinary diagnostic laboratory (CODA-CERVA). In 2000-2001, low-titre leptospirosis mainly occurred in cattle (serovars Sejroe and Grippotyphosa) and pigs (serovars Pomona and Australis). At the same time, the most common found serovars in dogs and horses were Icterohemorrhagiae and Grippotyphosa. Since 2002, seropositivity in cattle and pigs became very rare and dropped to less then 1% of the suspected cases. Sensitization and increased vaccination could be responsible for the apparent disappearance of the serovars Icterohemorrhagiae and Canicola In dogs since 2002. A sudden increase of cases in dogs and horses with serovars Australis and Pyrogenes was observed in 2006 and 2007. The number of positive cases doubled since 2005 - 5% of the submitted sera of dogs and horses with hepatic of renal failures, combined with fever, were positive, compared to less then 2% before. Especially in the case of serovar Australis in dogs, acute kidney failure was prominently present and the infection frequently ended fatally. In the case of Pyrogenes, hepatic disease dominated and most animals recovered. The source of infection could in most cases be related to contact with water, watery soils and high presence of rodents. It should be considered that dogs and horses could be a source of infection to their owners.