BELGIAN WILDLIFE DISEASE SOCIETY (BWDS)

First Symposium of the Belgian Wildlife Disease Society

26th of November 2005, Queen Astrid Military Hospital, Brussels

Steering Committee

P. Tavernier (University Ghent)

P. Heyman (Min. Defence, ACOS WB E&B/RLVBD)

S. Roels (CODA/CERVA)

Scientific Committee

A. Linden (University Liege)

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S. Roels (CODA/CERVA)

BWDS on WWW : http://wildlife.var.fgov.be/flashindex.php

Program

08:30-09:15 Registration

09:25 Welcome
Lt.Gen. Devignon (ACOS WB - Be)

09:30 Opening & short presentation
P. Tavernier – (UGent- Be)

09:45 Introduction : EWDA & OIE
M. Artois (ENVL, Fr)

SESSION 1 : Emerging viral diseases in Wildlife
Chairmen : J. Mast & M. Artois

10:20 Emerging viral diseases in Wildlife
A. Osterhaus (Erasmus MC - NL)

10:55 Coffee Break

11:20 Influenza virus
T. Van Den Berg (CODA/CERVA - Be)

11:40 Hantaviruses
P. Heyman (ACOS WB - RLVBD - Be)

12:00 West Nile virus
M. Saggese (Schubot Center - USA)

12:30 Lunch

SESSION 2 : Emerging bacterial diseases in Wildlife

Chairmen : P. Butaye & M. Saggese

14:00 Emerging bacterial diseases in Wildlife
J. Godfroid
(University of Pretoria – Onderstepoort - SA)

14:35 Para TBC
A. Linden (ULG - Be)

14:55 Tularaemia
K. Walravens (CODA/CERVA - Be)

15:15 Coffee Break

SESSION 3 : Other topics in Wildlife

Chairmen : J. De Borchgraeve & E. Claerebout

15:35 Echinococcosis in Belgium
B. Losson (ULG - Be)

15:55 Echinococcosis management
M. Vervaeke (Avia-Gis – Be)

16:15 Trichinella
P. Dorny (ITG - Be)

16:35 TSE in wild ruminants
H. De Bosschere (CODA/CERVA - Be)

16:55 Closing remarks & acknowledgements
S. Roels (CODA /CERVA - Be)

17:15 End of the day & Coffee

Welcome address

Emerging Diseases in Wildlife

Diseases emerging from wild living animals gained worldwide public acquaintance through recent outbreaks of avian influenza, SARS-associated coronavirus and West-Nile virus. Emerging diseases can be defined as mainly, but not exclusively, infectious diseases becoming more apparent through an increasing impact on animal and human health. Their causes are new infectious agents or known agents appearing in new locations, in new hosts or with higher incidences. They declare themselves through unexpected outbreaks, through re-emergence, through continuity from an enzootic area or after being introduced into a population. Actual emergence of diseases has to be distinguished from the detection of pathogens through the availability of better diagnostic methods or through intensified research.

Changes in the infectious agent itself, changes in the susceptible host population, environmental changes and anthropogenic factors have all been implicated as initiating factors for the emergence of diseases. Nevertheless the context in which such changes occur is very complex and is related to profound disturbances in the interaction between pathogenic agent, host and environment. The factors inducing disruptions in the balance of particular ecosystems are poorly known and deserve primary attention.

Recognizing new emerging diseases implicates active monitoring of known pathogens in the environment by using performant diagnostic techniques. Knowledge of the population structure of these pathogens permits differentiating true emerging epizootics from baseline organisms detected through intensified research.

National organisms studying wildlife related diseases are operating in the United Kingdom, France, Spain and Sweden. The European Wildlife Disease Association (EWDA), joining a number of scientists experienced in wildlife disease research, is currently prepared to offer the framework for coordinating the follow-up of emerging diseases at the European level.

In addition to predicting and diagnosing emerging diseases in wildlife, it is indispensable to understand the emergence of diseases in its ecological context. A major challenge is how to manage wildlife related emerging diseases in respect to both human and animal health on the one hand and ecosystem and species conservation on the other hand. The unpredictability of human interventions in natural systems and the value of wild living creatures as sentinels for emerging diseases are important considerations excluding traditional disease management such as stamping out in domestic or industrial kept animals. Hence, a solid base to assess and manage the risks of disease emergence can only be achieved by a multidisciplinary approach including biologists, ecologists, veterinarians and physicians.

The aim of this first Symposium of the Belgian Wildlife Disease Society is to contribute to a broad national and international cooperation in the study and management of wildlife related emerging diseases.

Tavernier P.

Depth. of Pathology, Bacteriology and Poultry Diseases, Veterinary Faculty, Ghent University, Belgium

President of the BWDS

ORAL PRESENTATIONS

09.25

Welcome by Lt. Gen. A. Devignon, Assistant Chief Of Staff - Well Being

09-30

Opening of the symposium by P. Tavernier, President of the Belgian Wildlife Disease Society.

09.45-10.20

Wildlife disease surveillance in Europe : by country and at the continental scale.

Artois M.

Chair of the European section of the Wildlife disease association,
Veterinary School, Lyon, France
m.artois@vet-lyon.fr

Wildlife related disease poses a threat to the human food chain in Europe, human health and can, in some circumstances impede the conservation of endangered populations of wild animal.
Some cases have been documented, but the relevant studies mainly occurred after a disease outbreak.
To implement a global management of the infections and parasites, which are relevant for the various concerns, it is central to be able to have access at surveillance and monitoring data.
Several problems are linked with the quality of this surveillance and can influence the results.
For more than a decade several European countries are now organized to collect data in order to analyse trends over space and time.
Some significant examples can be presented and discussed, in order to try to define what can be the goal to be achieved, both at the regional or continental scale.

10.20-10.55

Emerging virus infections in a changing world

Albert D.M.E. Osterhaus

Department of Virology, Erasmus MC, Rotterdam, The Netherlands
a.osterhaus@erasmusmc.nl

In the past century, pandemic outbreaks of influenza and AIDS have cost the lives of tens of millions of people. These events were all caused by multiple introductions of animal viruses – influenza A viruses and SIV of birds and non-human primates respectively – into the human population. Besides these introductions causing major pandemics in humans, a large number of other virus infections have spilled over from animal reservoirs to humans or other susceptible species, resulting in considerable morbidity and mortality as "virgin soil" epidemics. The most recent examples in humans are the introduction of SARS coronavirus and influenza A viruses (H5N1 and H7N7) from the animal world, which caused global concern about their potential to be at the origin of new pandemics. Over the last decades there seems to be a dramatic increase in the emergence or re-emergence of virus threats in humans and animals worldwide. A long list of exotic names like Ebola, Lassa, Rift-Valley, Crimea-Congo, Hendra, Nipah and West-Nile is the illustration of names of just some of the places associated with the origin of viruses that crossed the species boundary to humans, with dramatic consequences in the last ten years alone. Similarly, recent mass mortalities among wild aquatic and terrestrial mammals caused by previously known and newly discovered morbiliviruses, as well as outbreaks of hog cholera, foot-and-mouth disease and fowl plague among domestic animals, highlight this trend.

Although improved detection and surveillance techniques, as well as increased media attention may have contributed to our perception of an increase in the incidence of outbreaks of virus infections, it is becoming more and more clear that major changes in our modern society increasingly create new opportunities for virus infections to emerge: a complex mix of changes in social environments, medical and agricultural technologies and ecosystems continues to create new niches for viruses to cross species boundaries and to rapidly adapt to new species. In combating this global threat, we should make optimal use of the new tools provided by the unprecedented advances made in the research areas of molecular biology, epidemiology, genomics and bioinformatics. Serious investment in these areas in the future will not only be highly cost-effective but will also save many lives of humans and animals.

11.20-11.40

Avian Influenza

Thierry van den Berg

Avian Virology & Immunology Unit, Veterinary & Agrochemical Research centre (VAR)
Belgium

Avian influenza (AI or « bird flu ») is a highly contagious disease of poultry, which quickly assumes epidemic-like proportions if strict, effective control measures are not implemented. Infections caused by highly pathogenic avian influenza (HPAI) are classified among contagious diseases whose reporting is mandatory. The strategy recommended in case of HPAI consists of avoiding all exposure to the virus and eradication of the disease. Council Directive 92/40/EEC defines highly pathogenic influenza (HPAI) as “infection of poultry caused by any influenza A virus which, in 4-6 week-old chicks, has an intravenous pathogenicity index (IVPI) greater than 1.2 or any infection caused by influenza virus A, subtypes H5 or H7 for which nucleotide sequencing has demonstrated the existence of multiple amino acids in the hemagglutinin cleavage site”.
In fact, viral haemagglutinin is a major determinant of virulence. To penetrate the cell, protein precursor H0 must be cleaved by cellular proteases, otherwise, the virions produced are not infectious and the viral cycle stops. The haemagglutinin in low pathogenic strains (LPAI) contains only one or at most two arginines at the cleavage site of this compound. It can only be cleaved by cellular enzymes such as trypsin present in a limited number of cells restricted to the respiratory and gastrointestinal tracts. This explains why, *in vivo*, infection with non-virulent or moderately pathogenic viruses remains limited. On the other hand, the hemagglutinin in virulent strains presents an accumulation of repeated basic amino acids at its cleavage site. These patterns are recognized by furin proteases that are present in a large number of cells, thus enabling their spread *in vivo* throughout the body of the infected host. Wild birds and mainly migratory waterfowls are the reservoir for the LPAI viruses. Due to the large amount of bases such as purines in viral RNA in the region coding for the haemagglutin cleavage site, viral polymerase may tend to make more errors and the probability of mutations in this region is very high. This is what happens during passage and circulation of LPAI in domestic poultry populations. This mechanism seems entirely unique to avian influenza and only the LPAI of subtypes H5 and H7 have demonstrated as being ascendant forms of HPAI. Consequently, the EU has proposed changing the definition to “infection of poultry caused by any influenza virus of subtype H5 or H7”.
Nevertheless, the outbreak of an epidemic of bird flu remained a rare event but each time had devastating effects for the poultry industry and small poultry breeders. Between 1959 and 1999, a total of 18 HPAI episodes in poultry had been recorded, including seven in the EU member states. The majority of these clinical cases of influenza were observed in turkeys, while chickens were more seldom affected. However, an especially lethal epizootic outbreak occurred in 1983-84 in Pennsylvania, USA (H5N2 virus) and required the slaughter of 17 million poultry. Ten years later, in 1994, the disease was diagnosed in Pakistan (H7N3) and later in Mexico (H5N2). In 1997, Pennsylvania (H7N2) and Hong-Kong (H5N1) in turn were affected. Lastly, an epizootic outbreak raged between 1999 and 2001 in Italy (H7N1). More recently, Chile (H7N3) and Hong Kong (H5N1) were infected in 2002 and an especially serious epizootic outbreak was reported in the

Netherlands and in Belgium in 2003 (H7N7). But, since December 2003, an epizootic outbreak of HPAI (H5N1) rages in Eastern and South-Eastern Asia. This episode is without precedent in terms of virulence, geographic spread and economic consequences for agriculture. In addition, for the first time, effects on public health were recorded in SE Asia where a limited number of infections were reported in humans but with a mortality rate of approximately 75%.

Following the major epidemics of avian influenza over the last few years, current methods to fight them have been called into question. The main opposition of such measures involves the large number of animals slaughtered preventively in an effort to contain the spread of the epidemic. In addition to the ethical aspect related to animal welfare, there are social aspects to be taken into consideration (animal breeding for a hobby, zoos, rustic races and rare genotypes etc…) and economic aspects (costs of a crisis associated with direct and indirect losses related to world trade interruption). Prophylaxis programs that tolerate a low incidence of infection are not an acceptable method to deal with such cases of highly pathogenic influenza and currently available vaccines are effective against the disease but not against the infection. Therefore, they must be reserved for specific circumstances. Conversely, epidemiological surveillance should be reinforced. In this context, in addition to yearly evaluation of its reference laboratories (proficiency tests), the EU has planned a yearly serological surveillance program of poultry and virological survey in wild birds for all member states as well as the development of Early Warning Systems, crisis scenarios and practice drills. The Asian epidemic also highlighted the essential role of world veterinary and medical health information systems.

11.40-12.00

Hantaviruses.

Heyman P.[1], Cochez C.[1,2], Van Mele R.[1], Vandenvelde C.[1,2]

[1] Research Laboratory for Vector-borne Diseases, Queen Astrid Mil. Hospital, Brussels, Belgium
[2] Reference Laboratory for Vector-borne Diseases, Queen Astrid Mil. Hospital, Brussels, Belgium
paul.heyman@mil.be

The hantavirus genus is a member of the family *Bunyaviridae.* The viruses are spread and carried worldwide by wild rodents. In Europe, Puumala (PUUV), Tula (TULV), Seoul (SEOV), Saaremaa (SAAV) and Dobrava (DOB) serotypes can be expected to cause human disease. Humans are infected with hantaviruses by inhalation of aerosolised, virus-containing particles of rodent excreta. The main risk for acquiring hantavirus infection is intensive physical activity in rural and forested area where the virus is endemic. Mortality rates vary from less than 1% for PUUV, over 5-20% for SEOV, HTNV and DOBV to up to 40% for the North- and South-American *Sin Nombre*-like viruses.

In Belgium and France, the first reports on wild-type human hantavirus infections date from 1978. Epidemics in Belgium show a major summer peak between June and September and a minor spring peak from January to March, a shift is however observed between the north and the south of the country. Human hantavirus epidemics also coincide with rodent populations peaks.

The prevalent hantaviral serotype in Belgium is Puumala virus, carried by *Clethrionomys glareolus* (bank vole). We also demonstrated the presence of Tula virus (*Microtus arvalis*, field mouse) and, recently, Seoul virus (*Rattus norvegicus*, brown rat).

Seroprevalences in the human population in Europe range from 0.9% in Austria, over 1.5% in Belgium, France and Germany, up to 17% in some hyper-endemic area in Scandinavia, demonstrating that hantavirus infections are by no means rare on this continent.

Epidemics were observed in Belgium in 1993 (174 cases), 1996 (224 cases), 1999 (124 cases), 2001 (110 cases), 2003 (122 cases) and an ongoing epidemic in 2005. For the 2005 epidemic, of which we have yet only seen the minor spring peak, we detected already more than 200 cases (01/01/2005 to 31/07/2005). As the major summer peak is yet to come, we expect this epidemic to be the largest since 1978.

Epidemics coincide with important increases in rodent population densities and increased seroprevalences in the population. Trapping indexes can, in epidemic years, be four- or five times higher than normal and seroprevalences can be as high as 70% in certain *C. glareolus* sub-populations.

12.00-12.20

WEST NILE VIRUS INFECTION IN NORTH AMERICAN RAPTORS

Miguel D. Saggese, DVM MS

The Schubot Exotic Bird Health Center, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A & M University

Birds of prey have been particularly susceptible to West Nile virus infection in the mid-western states of North America during the last four years. Species commonly affected included great horned owls, Cooper's hawks and red-tailed hawks among wild birds, and Gyrfalcons and Goshawks in captive collections. Clinical signs in these birds included altered mental status, head tremors, seizures, paresis and paralysis, retinal degeneration and detachment, anorexia, weight loss, dehydration, anemia and leucocytosis. Pathological findings included lymphoplasmacytic and histiocytic encephalitis, endophthalmitis, and myocarditis mainly, but other organs were also affected. The distribution and severity of histological lesions, the antigen distribution in the various organs and the amount of antigen varied among the species. WNV infection appears to be capable of causing fatal disease in most raptors species, usually after a course of several weeks post infection. The impact that WNV infection could have on populations of threatened or endangered birds of prey may be detrimental. Vaccination has been suggested to prevent this disease in valuable or endangered captive raptors but currently there are not specific vaccines for birds of prey and the use of different horses vaccines in birds are still being investigated.

14.00-14.35

Emerging bacterial diseases in Wildlife : Wildlife tuberculosis in South African conservation areas: implications and challenges.

Michel AL[1], Bengis RG[2], Keet DF[2], Hofmeyer M[3], de Klerk LM[3], Cross PC[4], Jolles AE[5], Cooper D[6], Whyte IJ[3], Buss P[3], Godfroid J[7].

[1]Dpt. of Bacteriology, ARC-Onderstepoort Veterinary Institute, South Africa;
[2]Directorate Veterinary Services, Skukuza, South Africa;
[3]South African National Parks, Skukuza, South Africa;
[4]U.S. Geological Survey, Northern Rocky Mountain Science Center, Bozeman MT, USA;
[5]Dpt. of Ecology & Evolutionary Biology, Princeton University NJ, USA;
[6]Chief Veterinarian, Ezemvelo KwaZulu/Natal Wildlife - KZN Wildlife, St Lucia, South Africa;
[7]Dpt. of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa
jacques.godfroid@up.ac.za

Tuberculosis, caused by *Mycobacterium bovis*, was first diagnosed in African buffalo (*Syncerus caffer*) in South Africa's Kruger National Park in 1990. Over the past 15 years the disease has spread northwards leaving only the most northern buffalo herds unaffected. Evidence suggests that ten other small and large mammalian species, including large predators, are spill-over hosts. Wildlife tuberculosis has also been diagnosed in several adjacent private game reserves and the Hluhluwe-Imfolozi Park, the third largest game reserve in South Africa.

Potential negative long-term effects of tuberculosis on the population dynamics of certain social animal species and the direct threat for the survival of endangered species pose particular problems for wildlife conservationists. On the other hand, the risk of spillover infection to neighbouring communal cattle raises concerns about human health at the wildlife-livestock-human interface, not only along KNP's western boundary, but also with regards to the joint development of the Greater Limpopo Transfrontier Conservation Area (GLTFCA) with Zimbabwe and Mozambique. From an economic point of view, wildlife tuberculosis has resulted in national and international trade restrictions for affected species. The lack of diagnostic tools for most wildlife species and the absence of an effective vaccine make it currently impossible to contain and control this disease within an infected free-ranging ecosystem. Veterinary researchers and policy-makers have recognized the need to intensify research on this disease and the need to develop tools for controlling this disease, initially targeting buffalo and lion (*Panthera leo*).

14.35-14.55

Paratuberculosis in wild red deer (*Cervus elaphus*) in Southern Belgium

Linden A.[1], Grégoire F.[1], Hoyoux A.[1], Mousset B.[1], De Rijk P.[3], Rigouts L.[3], and Desmecht D.[2]

[1] Dpt. of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege
[2] Dpt. of Morphology and Pathology, Faculty of Veterinary Medicine, University of Liege, Belgium
[3] Mycobacteriology Unit, Institute of Tropical Medicine, Antwerp, Belgium
a.linden@ulg.ac.be

In 2001, a Health Surveillance Program for Wild Cervids was created in Southern Belgium. One of the objectives was to evaluate the importance of paratuberculosis (*Mycobacterium avium* subspecies *paratuberculosis* - *Map*) in wild cervids (*Cervus elaphus*). This mycobacterial infection is responsible for chronic granulomatous enteritis and lymphadenitis leading to diarrhoea, in some species, weight loss and death. *Map* has been isolated in ruminant and non-ruminant wildlife species, leading to the problematic of wildlife reservoirs for these mycobacteria.

A total of 661 red deer were analysed (593 hunter-killed during seasons 2002-2003 and 68 found dead animals). A complete necropsy was conducted on each animal, which allowed sampling of serum, spleen, mesenteric lymph nodes, ileocaecal junction and feces. Direct smears of tissue and feces were stained by the Ziehl-Neelsen method and examined for acid-fast organisms. Formalin-fixed and frozen tissues were conserved for histopathology and bacteriologic examination. Serum samples were tested by use of a commercial ELISA (HerdChek^{R} MptAb, IDEXX). Direct diagnostic (IS*900* PCR on ileocaecal lymph nodes, spleen and /or feces) was performed on all suspected cases (either suggestive macro/microscopic lesions or positive serological results or both). Moreover, culture was performed on 50 samples to evaluate the sensitivity of IS*900* PCR.

In the group of hunter-killed cervids (n = 593), overall seroprevalence was 2.15 % (95IC = 0.54 – 3.76). For direct diagnostic, 64 of 593 animals were considered as suspected cases (see above) and submitted to further analysis. Amongst them, 21 were confirmed *Map* positive by IS*900* PCR. So, these results indicated a bacteriologic prevalence of 3.54 %. For samples for which PCR and culture were performed, the sensitivity of IS*900* PCR compared to culture was 84%.

In the group of found dead cervids (n = 68), the 3 major mortality causes were paratuberculosis (21/68), traumatic lesions (16/68) and polyparasitism (9/68).

To propose a control strategy regarding paratuberculosis, an official decree was published in 2003, according to which any wild red deer showing obvious signs suggestive of emaciation and/or diarrhoea could be culled (even outside hunting seasons). Within 2 years, 42 wild cervids were culled according this decree and 19 of them were *Map* positive (by PCR).

In a wildlife health perspective, it is hoped that, in addition to classic control strategies (decrease of cervids densities and management of winter feeding) a selective culling, by eliminating the potential shedders, will improve the control of the disease.

14.55-15.15

Tularemia, an emerging zoonosis in Belgium?

K. Walravens[1] , J. Bughin [2] , S. Roels[3] , C. Sion[4] , A. Linden[5] , M. Reginster[6] , J. Godfroid[1] , M. Govaerts[1] , F. Grégoire[5] , J.-M Troussart[4] , & C. Saegerman[5].

[1] Department of Bacteriology and Immunology, Veterinary and Agrochemical Research Centre (CODA/CERVA), Groeselenberg 99, B- 1180 Brussels,

[2] Pathologie générale, ARSIA, Allée des Artisans 2, B-5590 Ciney.

[3] Department of Biocontrol, CODA/CERVA,

[4] Service de Biologie clinique Centre Hospitalier Hutois (CHH) rue Trois Ponts, 2 - 4500 HUY,

[5] Department of Infectious and Parasitic Diseases, Epidemiology and Risk analysis, Faculty of Veterinary Medicine, University of Liege Boulevard de Colonster, 20, B43b, B-4000 Liege,

[6] Hémato-oncologie CHH.

Francisella tularensis is a gram-negative coccobacillus and the etiologic agent of the zoonotic disease tularaemia. First described in 1911 in Tulare County, California, it has since been reported throughout the Northern Hemisphere, with natural infections reported among a wide range of vertebrates and invertebrates. Human infection mostly occurred after arthropods bites or following direct contact with contaminated animals or dust. In recent years, tularaemia has emerged in new geographic locations, populations, and settings.

In Belgium, the latest isolation of *Francisella tularensis* from wildlife has been reported in 1981 in the province of Liège. The Public interest to this disease re-emerged in 2002, when 250 - wild black-tailed prairie dogs (*Cynomys ludovicianus*) were imported from the USA. Before shipment, the wild caught animals were hold in an animal export quarantine where an outbreak of tularaemia due to infection with *Francisella tularensis* Type B was notified. The Belgian authorities informed by WHO and OIE took initiatives to trace the animals to inform the owner of the risk and to define measures to be taken in case of mortality of the animals. The epidemiological inquiry and diagnostic tests made on traced Prairie dogs concluded that the animals imported in Belgium from the infected animal facility in Texas were free of *F. tularensis* infection. During summer 2003, the European Commission bans the import of prairie dogs from the United States, because of the risk of Monkey Pox virus transmission to human. These episodes highlight the need for control measures on international transport of wild animals susceptible to carry human pathogens.

During the first quarter 2003, F. tularensis was isolated from a dead hare (*Lepus capensis*) found in the Province of Namur. Biochemical and PCR typing of this isolate showed that the strain belonged to the subspecies *holarctica* (Type B) commonly isolated in Western Europe. This subspecies is less pathogenic (lethality rate lower than 1% in case of a early treatment to 5% in case of late treatment) than strains belonging to the subspecies *tularensis* (Type A) highly pathogenic for human and only limited to Slovakia in Europe but commonly isolated in North America. Genotyping of this strain by Multiple-Locus Variable-Number Tandem Repeat Analysis suggested that this strain belongs to a lineage closely related to French isolates (M. Forsman personal communication). Since 2004 a wildlife surveillance program has been implemented in the Walloon region. Until now, 30 spleens from hares and rabbits presenting suspected

macroscopic lesions at necropsy were tested negative in culture and PCR. This surveillance is ongoing and will be intensified with the aim to improve the early detection of this emerging disease.

Two human cases of tularaemia were diagnosed by serum agglutination tests in 2003. The two patients were in close contact with a dying hare. One patient suffered of a flu syndrome with fever (38°C), headaches and arthralgia, followed by the appearance of axillary adenopathy and lymphangitis. The patient reacted positively to fluoroquinolones treatments. The second patient did not show any sign of disease. An enzyme-linked immunosorbent assay (ELISA) to detect human antibodies against *Francisella tularensis* based on partially purified lipopolysaccharide (LPS) was developed. The sera of the two patients were classified as positive. The specificity of the test was assessed on control human sera and human sera from individuals infected by smooth *brucellae.* No cross-reactivity was observed. Thus, this newly developed iELISA (using a G-protein peroxydase conjugate) will be suitable for laboratory confirmation of tularaemia as well as for large-scale epidemiological studies in different animal species.

15.35-15.55

Echinococcosis in Belgium.

Losson B, Hanosset R

Dpt. of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Belgium
blosson@ulg.ac.be

Echinococcus multilocularis, a small tapeworm belonging to the family of the Taenidae, is responsible in man for a rare but potentially lethal liver condition known as alveolar echinococcosis (A.E.) . This cestode uses a fairly complex life cycle in which different species of foxes and small rodents play the role of final and intermediate hosts, respectively. In Western and Central Europe the red fox (*Vulpes vulpes*) is by far the most important final host. Rarely, the dog and the cat can harbour the intestinal adult stage. Human contamination takes place through the ingestion of *E. multilocularis* infective eggs (direct or indirect contact with an infected carnivore, ingestion of raw contaminated vegetables or fruits). Geoclimatic conditions are known to play a significant role on the distribution of *E. multilocularis*. In Central Europe, the parasite is most prevalent in cold hilly areas. The first record of *E. multilocularis* in Belgium was reported by Brochier et al. (1992) who found a 15.3% prevalence in red foxes in the province of Luxembourg (maximum altitude 700 m above sea level). In 1997, Losson et al. reported a 51.0% (74/145) prevalence in the same host and area. Between 1998 and 2002, the parasite was found in red foxes killed in other areas of Wallonia; the highest prevalence (33%) was found in the Ardennes and the lowest (0%) on the Plateau de Herve. Additionally Vervaeke et al. (2003) identified the parasite in Flanders although the mean prevalence in this region was much lower (1.7%). In Brussels area Brochier et al. (submitted for publication) failed to demonstrate the presence of the worm in 160 available foxes. In 2003-2004 the prevalence of *E. multilocularis* carriage in the red fox in different areas of Wallonia was assessed together with the role of different species of rodents or insectivores as intermediate hosts. A total of 990 foxes were available during the study period. The prevalences were found to be highly variable from one province to another (from 1.92% in Walloon Brabant to 41.67% in Luxembourg). During the same period, 914 *Microtus arvalis*, 39 *Microtus agrestis*, 55 *Apodemus sylvaticus*, 215 *Sorex araneus*, 1 *Sorex minutus,* 23 *Cletrionomys glareolus* and 2 *Arvicola terrestris* were captured in a highly endemic area (Luxembourg). Only one *M. arvalis* (0.11%) and 1 *C. glareolus* (4.34%) were found to be infected by the metacestode. As far as the muskrat was concerned, 1728 animals originating from Wallonia were available. Four hundred and ninety five (28.6%) were found to be infected by the metacestode. Since 1999, 9 cases of A.E. were diagnosed in man of whom 4 were published. The infections were most probably acquired in Belgium. Finally, larval forms of the disease were described in other aberrant host such as the dog. All these data indicate that *E. multilocularis* is widely distributed in Belgium especially in the Southern part of the country and represents a potential problem for public health.

1. Losson B et al. Prevalence of *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in southern Belgium. Vet. Parasitol. 2003, 117, 23-28.

2. Vervaeke M et al. *Echinococcus multilocularis* (Cestoda, Taeniidae) in Red foxes (*Vulpes vulpes*) in northerne Belgium. Vet. Parasitol. 2003, 115, 257-263.

15.55-16.15

Implications of increased susceptibility to predation for management of the sylvatic cycle of *Echinococcus multilocularis*.

Vervaeke M[1], Davis S[1,2], Leirs H[1], Verhagen R[1]

[1] University of Antwerp, Department of Biology, Evolutionary Biology Group, Groenenborgerlaan 171, B-2020 Antwerp, Belgium

[2] Danish Pest Infestation Laboratory, Skovbrynet 14, DK-2800 Kgs. Lyngby, Denmark

muriel.vervaeke@skynet.be

The ability to increase the chances that infectious prey are taken by predators is an observed feature of many parasites that rely on one or more predator-prey relationships to complete their life cycle. In the sylvatic life cycle of *Echinococcus multilocularis* – the causative agent of human alveolar echinococcosis – foxes are the final host, with voles acting as intermediate hosts. Here we use a general mathematical model to show that increased susceptibility to predation reduces the sensitivity of a parasite population to adverse conditions, and hence we provide a plausible explanation for the observed resilience of *Echinococcus multilocularis* during and following field trials of praziquantel baiting. We also note that with increased susceptibility to predation, there is no critical density of foxes below which the parasite is expected to die out, even if the effect on infected prey is very small. If the anecdotal evidence that *E. multilocularis* infection in voles increases the chance of being taken by a fox is confirmed by field work then our results add to the growing body of literature that suggests management of *E. multilocularis* will not be achieved by eradication.

16.15-16.35

Trichinellosis in Belgium

Dorny P[1, 2], De Borchgrave J[1]

[1]Dpt. of Animal Health, Institute of Tropical Medicine, Antwerp, Belgium
pdorny@itg.be

Trichinellosis is virtually absent in Belgian domestic livestock. Since systematic controls of pigs and horses have become compulsory at slaughter (EU Directive 92/45/EEC, 1992) no positive case was found. The last outbreak in humans in Belgium occurred in 1979 following the consumption of meat from wild boar. Increased monitoring in the last decade has shown that *Trichinella* spp. still circulate amongst wildlife, although both the prevalence and the intensities of infection are low. No infection was detected in muscles of Belgian foxes between 1996 and 2000; however, circulating antibodies against Trichinella were found by ELISA in 164 foxes (20%). In 2003-04, 199 red foxes, 32 badgers, 44 beech-martens and 52 polecats from Belgium were examined by artificial digestion of 25-33 g of tongue, diaphragm and hind leg muscles. Trichinella larvae were detected in only one fox (0.5%) from Wallonia; however, larvae were not identified at the species level. Since 1992 the EU Directive requires that also wild boars hunted in the EU for commercial purpose are examined for Trichinella. In Belgium each year about 8000 sport-hunted wild boars are tested. Until now, only one animal, in 2004, originating from Mettet (Namur), was found to harbour a light infection (0.7 larvae per gram). The larvae, isolated by artificial digestion were identified by PCR to be *Trichinella britovi*, a species previously not demonstrated in Belgium. Sylvatic carnivores are the main hosts of *T. britovi*. Even if wild boars are not the preferred hosts they can acquire the infection and consequently pass it to humans. Both *T. spiralis* and *T. britovi* have been associated with human infection. About 20% of the human cases that occurred in France, Germany, Italy and Spain, were caused by the consumption of wild boar.

The routine examination of wild boars devoted to the market has proved to be a good measure to protect the consumer against sylvatic trichinellosis. In addition, monitoring of infection through examining sentinel animals, such as the fox, is recommended to assess the prevalence of trichinellosis and to follow trends in time. Serological examination might be an alternative for muscle digestion but needs further evaluation. An extra measure to protect the consumer against trichinellosis but also against other food-borne infections is to eat meat of wild boar “well done”, or to freeze the meat at -20°C for 4 weeks. An important measure to avoid spreading of the infection among wildlife is not to leave offal of animal carcasses in the field after skinning.

16.35-16.55

Screening for chronic wasting disease – specific prion protein in Belgian free-ranging cervids.

[1]Roels S., [2]C. Saegerman, [1]De Bosschere H., [3]Berkvens D., [4]Gregoire F., [4]Hoyoux A., [4]Mousset B., [4]Desmecht D., [1]Vanopdenbosch E., [4]Linden A.

[1] National Reference Laboratory for veterinary TSEs (Be & Lux) Veterinary and Agrochemical Research Centre (CODA/CERVA), Section of Pathology, Department of Biocontrol, Groeselenberg 99, B-1180 Brussels (Ukkel), Belgium

[2] Department of Infectious and Parasitic Diseases, Epidemiology and Risk analysis, Faculty of Veterinary Medicine, University of Liege Boulevard de Colonster, 20, B43b, B-4000 Liege, Belgium

[3] Department of Tropical Animal Health and Production, Epidemiology and Statistics, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium

[4] Department of Infectious and Parasitic Diseases, Bacteriology, Faculty of Veterinary Medicine, University of Liege Boulevard de Colonster, 20, B43a, B-4000 Liege, Belgium

hedeb@var.fgov.be

Chronic wasting disease (CWD) has not been reported in Europe, whereas it is considered enzootic in free-ranging mule deer, Rocky mountain elk and white-tailed deer in the area of Colorado, Wyoming, and Nebraska, and new foci of CWD have been detected in other parts of the United States. However, no large-scale active epidemiosurveillance of European wild cervids is installed in Europe. In accordance with the opinion of the European Scientific Steering Committee, a preliminary (active) surveillance scheme was installed, in order to improve the knowledge of the CWD status of the Belgian free-ranging cervids (roe deer and red deer). Spleen samples (n = 866) of roe deer and red deer collected in the Southeast part of Belgium, were examined for CWD using an enzyme-linked immunosorbent assay (ELISA) of Bio-Rad. Afterwards, the ELISA was systematically confirmed by immunohistochemistry (IHC) using 3 antibodies, namely R524, 2G11 and 12F10. There were no indications on the occurrence of TSE in any of the samples. A Bayesian framework was used for the estimation of the true prevalence of CWD in South-East part of Belgium was estimated to have a median value of 0% with a 95% percentile value of 0.115%.

POSTERS

POSTER 1

Occurrence of *Echinococcus multilocularis* in Flanders

Breyne P, De Pauw W, Halfmaerten D, Van Den Berge K.

Institute for Forestry and Game management, Gaverstraat 4, 9890 Geraardsbergen, Belgium

Peter.breyne@inbo.be

The recent expansion of the distribution range of foxes in Flanders increases the risk of human alveolar echinococcosis, a lethal disease caused by the metacestode of the fox tapeworm *Echinococcus multilocularis*. Infected foxes shed the eggs in the environment and oral uptake by humans can cause this zoonotic disease. Recent monitoring programmes in Europe indicate that the parasite may be spreading to new regions. While the prevalence of *E. multilocularis* in the southern part of Belgium is well documented, only limited information is available for the Flemish part. In order to obtain more detailed knowledge about the occurrence and distribution of the parasite in Flanders, a non-invasive monitoring analysis was performed.

Faecal samples were collected all over Flanders and investigated for presence of *E. multilocularis* eggs using PCR-based detection of the 12S ribosomal DNA. As direct PCR on DNA extracted from faecal samples may be hampered due to inhibition, it is difficult to get a clear view on the exact number of infected foxes. Nevertheless, several of the samples were positive showing that the parasite is present in Flanders. Moreover, the infected samples originate from geographically separated regions demonstrating that the tapeworm is not restricted to particular locations.

POSTER 2

Antibiotic resistance in faecal *Escherichia coli* isolates of hare.

Casteleyn C[1], Dewulf J[2], Catry B[2], de Kruif A[2], Maes D[2].

[1]Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Belgium
[2]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Belgium
Christophe.Casteleyn@UGent.be

Faecal samples of hare were collected immediately after hunt in three different regions of Flanders, Belgium. Per region, seven animals were sampled twice, resulting in a total of 42 samples. After isolation and identification of the indicator bacterium *E. coli*, growth inhibition diameters were measured for 14 antibiotics, namely ampicillin, amoxicillin-clavulanic acid, ceftiofur, sulfamethizole, trimethoprim-sulfa, oxytetracycline, gentamicin, neomycin, spectinomycin, streptomycin, enrofloxacin, flumequine, nalidixic acid and florfenicol by means of the disk diffusion method.

In general, resistance was low and was most frequently seen against sulfamethizole (16.7%), neomycin (19.7%) and streptomycin (13.6%). In spite of the fact that wild hares are not treated with antibiotics, 21.2% of the isolates were resistant to one antibiotic, 12.1% showed resistance against two antibiotics and 6.1% of the isolates showed resistance against three or even six different antibiotics. Faecal samples of swine, broilers and dairy cattle were collected during the same study too. Compared to these domestic animal species, antibiotic resistance in wild animals was very low.

These results show that antibiotic resistance in hares is limited and thus cannot be considered as a major problem. The resistance we have noticed could be due to the presence of natural produced antibiotics or the dissemination, e.g. through surface water, of synthetic antibiotics administered in agriculture. In this way, resistance in livestock could influence the prevalence of antibiotic resistance in wildlife.

POSTER 3

Determination of the age of the fox on the basis of the closure of the growth plates.

Cools M, Simoens P[1], Vandenberghe K[2].

[1]Dpt. of Morphology, Faculty of Veterinary Medicine, University of Gent, Belgium.
[2]Instituut voor Bosbouw en Wildbeheer, Geraardsbergen.
marleencools@skynet.be

In this study, 17 wild red foxes were examined. The foxes came from the IBW (Instituut voor Bosbouw en Wildbeheer), located in Geraardsbergen. Every year the IBW marks (with 2 earmarks) and weighs the juvenile foxes when they leave their holes in April-May. At that moment they are approximately 1 to 2 months old. Their date of birth is estimated on the basis of their weight at capture. Some foxes are killed during the hunting season and brought back to the IBW. This way, 17 foxes were available for the study. From nearly each fox, one forelimb, one hind limb and a part of the spine were obtained. The youngest fox was 25 weeks old, the eldest 3 years. The closure of the growth plates was evaluated by performing radiographs (at 55 KV, 50 mA and 0.06 sec (3 mAs)) and by maceration of the bones (2 weeks in a bath of 0.4% solution of Biotex® at 56°C).

Before the age of 6 months the epiphysis of the manus of for- and hind limbs are closed. The supraglenoïd tubercle is also fused with the scapula. At 6 months the proximal and distal epiphysis of the radius and ulna are fused, there is still a fraction of the growth plate visible. At 7 months the distal growth plate of the fibula is closed and the distal epiphyse of the femur and the proximal epiphysis of the tibia and fibula are starting to melt with the respective diaphysis. At 8 months the proximal growth plate of the humerus is only slightly open. At 8½ months the growth plates of the spines are fully closed. At 9 months there was still a fraction visible of the proximal growth plate of the humerus. The proximal growth plate of the tibia was still slightly open at that age. The distal growth plate of the radius stays visible (radiographically as well as macroscopically) for a long time, and therefore cannot be used as an accurate criterion.

Our conclusion is that the proximal growth plate of the tibia closes last (with a fraction still being visible at 9 months), and growth plates of the forelimb are closed earlier than the hind limb. As far as the forelimb is concerned: the proximal growth plate of the humerus is the last one closing, at 7½ months.

Evaluation of the closure of the growth plates is an adequate method, but it can only be used for foxes under 9 months. Unlike maceration, radiography can be used on live animals; but it is expensive and difficult to perform in the field.

This study was limited because of the small number of foxes and because of the fact that none of the foxes was younger than 25 weeks.

POSTER 4

Persistent pollutants in the European hedgehog (*Erinaceus europeaus*) in Flanders: Brominated flame retardants, organochlorinated compounds and heavy metals.

D'Havé H[1,*], Scheirs J[2], Covaci A[3], Verhagen R[2], Schepens P[3], Blust R[1], De Coen W[1].

[1]Ecophysiology, Biochemistry and Toxicology Group, Dpt. of Biology, University of Antwerp, Belgium
[2]Evolutionary Biology Group, Dpt. of Biology, University of Antwerp
[3]Toxicological Centre, University of Antwerp
helga.dhave@ua.ac.be

Until now no data were available on persistent pollutants in European hedgehogs, although this species may be especially vulnerable to pollution. First, hedgehogs are abundant in (sub)urban areas, which are often characterized by a high degree of pollution. Second, hedgehogs mainly prey on invertebrates – such as earthworms, slugs, beetles and caterpillars - some of which are known to accumulate high pollutant levels. Last, their relatively high life expectancy may imply age-dependent accumulation possibly resulting in chronic toxicological effects.

In the present study we investigated persistent pollutant levels in 42 hedgehog road kills and carcasses from wildlife rescue centres all over Flanders. The investigated compounds were polybrominated diphenyl ethers (PBDEs) and 11 heavy metals (Ag, Al, As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Zn). Targeted organochlorine compounds were: polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), octachlorostyrene, and organochlorinated pesticides: dichloro-diphenyl-trichloroethanes (DDTs), hexachlorocyclohexanes (HCHs) and chlordanes. Pollutants were quantified in liver, kidney, muscle, adipose tissue, hair and spines, but only results for liver are presented here.

Hedgehogs showed considerably high PBDE levels (Min-Max: 1-1178 ng/g liver wet weight (ww)), however, few data are available on terrestrial wildlife for comparison. Accumulation of organochlorine compounds in hedgehogs was substantial compared to other terrestrial mammalian wildlife, especially for PCBs (2-5910 ng/g liver ww), chlordanes (0.2-76 ng/g liver ww), DDTs (0-750 ng/ g liver ww) and HCB (0.02-248 ng/g ww). Ten hedgehogs showed PCB concentrations that might have induced reproductive impairment, while the hepatic PCB level might have been lethal to one hedgehog. Concentrations of HCHs (0-9 ng/g liver ww) and octachlorostyrene (0.03-3 ng/g liver ww) were low to moderate. Our results indicate that the Flemish terrestrial environment is still considerably polluted with PCBs and DDTs despite the declining use of these compounds since several decennia. Metal pollution levels were moderate; however, individual hedgehogs accumulated high concentrations of certain metals (ranges for Cadmium: 0.05-124 µg/g liver dry weight (dw), Lead: 0.7-71 µg/g liver dw, Copper: 3-200 µg/g liver dw, Zinc: 50-747 µg/g liver dw). Fourteen hedgehogs showed cadmium concentrations, which may have induced a decline in normal renal functioning, while 15 hedgehogs had lead levels associated with toxicosis.

Our results demonstrated that mean persistent pollutant levels of a random sample of hedgehogs in Flanders are moderate for most compounds. However, individual hedgehogs accumulated pollutant concentrations above critical concentrations and may have suffered adverse effects.

POSTER 5

Hantavirus infection dynamics and spatial organisation of bank voles and wood mice in southern Brussels

Dobly A[1], Cochez C[2] and Heyman P[2]

[1] Unit of Social Ecology, Université Libre de Bruxelles – CP 231, 1050 Brussels, Belgium

[2] Research Laboratory for Vector-borne Diseases, Queen Astrid Military Hospital, 1120 Brussels, Belgium

adobly@ulb.ac.be

The rodent-borne hantaviruses are important emerging diseases. They cause potentially deadly human infections while the reservoir species apparently remains symptom-free. In Belgium, bank voles, *Clethrionomys glareolus*, is known to carry Puumala hantavirus and to transmit a mild form of hemorrhagic fever with renal syndrome to humans (Nephropathia epidemica). The sympatric wood mice, *Apodemus sylvaticus*, usually do not carry any hantavirus. We first tested the presence of hantavirus in both species, then we tested the hypothesis that infected animals showed spatially interconnected home ranges (supporting transmission through contacts) and if, consequently, seroprevalence was density-dependent. We also checked the absence of behavioural differences between negative and positive rodents by comparing home ranges within each sex and maturity-class. From 2003, we trapped in Autumn and Spring in three 0.75 ha grids in the Sonian Forest, south to Brussels. On each site, 60 traps were set for 11 nights each season. Trapped animals were weighed and sex was determined. We checked the presence of Puumala IgG by rapid field tests (Reagena, Finland). In voles, DNA samples were taken for a microsatellite study. Rodents were then individually marked and released. Hantavirus was detected not only in voles but also in wood mice. The seroprevalence varied from 6% to 59% in adults with comparable seroprevalence between sexes and species. In voles, males had larger home ranges than females. At low density, when positive voles were present, they had interconnected home ranges forming clusters of very high seroprevalence. This observation was confirmed by the microsatellite analysis and suggests a core of new infections. The vole genetic analysis also showed that two populations separated by a 4-lane road (approximately 100 m) were as isolated as populations that lived six kilometres apart. Finally the vole seroprevalence was density-dependent both at low and high density but with two different dynamics. We discuss differences in individual strategies and (horizontal) virus transmission in light of these data.

POSTER 6

The situation of the rabbit in the Netherlands in relation to RHD

J.M. Drees,

Community and Conservation Ecology, University of Groningen, P.O. Box 14, 9750 AA haren, The Netherlands (marijke.drees@home.nl)

A nature conservation lobby in The Netherlands has pressed for the closing of the hunting on the rabbit, because of its decline. The rabbit is valued by nature managers, especially in the coastal dunes, for its impact on flora and fauna. The ministry of Agriculture, Nature management and Food has given me an assignment to study the situation of the rabbit in The Netherlands.
The report (in Dutch) provides information on the recent status of the rabbit in the different regions and gives proposals for measures that could be taken to promote the rabbit. No data on the prevalence of RHD are collected.

Trends in population numbers
Three series were used for the analysis. Numbers of the hunting bag were available from 1980-1999. Since 1985 nature managers in the coastal dunes count rabbits along transects. Since 1995 bird watchers who map breeding birds in 50 ha plots also count rabbits. Also some data were collected about individual areas by interviewing hunters.

Rabbit numbers have decreased with 90% between 1990 and 2003. Rabbit numbers went down with 10-30% per year. The first RHD epidemic occurred in 1990 in the coastal dunes and other regions, but Zeeland, the north of Noord-Holland and the Wadden isles were reached later

<u>Regional and local differences</u>
The following regional and local differences were found:
a) A much stronger decrease north of the river Rhine than in the south.
b) More fluctuations in the agrarian areas than in the forests, heather or dunes
c) Recovery in the 'urban' sphere: sports fields, urban parks, industrial areas.
These differences might be related to differences in the prevalence or virus strain of RHD. For the recovery in the urban sphere the explanation is sought in the lower number of predators (foxes, birds of prey) and facilitation by mowing and disturbing the soil

Measures that could be taken
To help recovery of rabbit numbers facilitation by larger herbivores (cattle) and mowing could help.
Vaccination is not advised. Reintroduction has to be done very carefully. The social behaviour has to be taken into account.

POSTER 7

Hemangiosarcoma in a wild red deer (*Cervus elaphus*).

Gregoire F[1], Mousset B[1], Hoyoux A[1], Desmecht D[2], Linden A[1].

[1]Dpt. of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liege, Belgium
[2]Dpt. of Morphology and Pathology
f.gregoire@ulg.ac.be

A 14-yr-old female red deer (*Cervus elaphus*) was found dead in March 2003 in the region of Bièvre (Southern Belgium). At necropsy, numerous nodular, well-demarcated, 0,5-3 cm in diameter, red-black, soft to firm masses were scattered throughout the lungs. On section, the masses were dark red and oozed blood. On the left thoracic flank of the animal, a soft invasive sub-cutaneous mass (approximatively 15 cm in diameter) was adhering to the 7th rib, with bone lysis. Section of the mass revealed large areas of necrosis and hemorrhage.

At histopathology, the paracostal mass and the pulmonary masses displayed a similar morphologic pattern. The latter showed conspicuous accumulation of blood, either filling small clefts or giant cavernous channels, or freely dissecting the tissues (hemorrhages). The clefts or channels were clearly delineated by endothelial cells, some of them were visibly ruptured or thrombosed. The stroma interspersed between channels and clefts was obviously constituted by neoplastic cells, varying in size and shape, but being usually elongated. The nuclei of these cells were round or ovoid, very hyperchromatic and commonly displayed mitotic figures. All the masses comprised numerous macrophages filled with large amounts of hemosiderin. The paracostal mass also displayed very large areas of necrosis, with foci of neutrophilic accumulation. These histological features are compatible with a diagnosis of cavernous hemangiosarcoma, a malignant tumor of vascular endothelial cells. The primary tumor probably developed within the 7th left rib, then extended to the pleurae and the thoracic muscles and, finally, showered the lungs with metastases.

Besides this report, a few cases of neoplasms have been documented in wild cervids. Only one case of hemangiosarcoma has been previously reported in deer, involving a captive aged Père david's deer.

POSTER 8

Serological and genetic evidence for the presence of Seoul hantavirus in *Rattus norvegicus* in Belgium.

Heyman P.[1], Baert K.[2], Cochez C.[1], Vandenvelde C.[1], Stuyck J.[2].

[1] Research Laboratory for Vector-borne diseases, Queen Astrid Military Hospital, Bruynstraat, B-1120 Brussels, Belgium

[2] Rodent Management Research Group, Institute for Forestry and Game Management, Gaverstraat 4, B-9500 Geraardsbergen, Belgium

The hantavirus genus is a member of the family *Bunyaviridae.* The viruses are spread and carried worldwide by wild rodents. In Europe Puumala (PUUV), carried by *Clethrionomys glareolus*, Dobrava (DOBV), carried by *Apodemus flavicollis*, and Seoul (SEOV), carried by *Rattus norvegicus* and *R. rattus*, can be expected to cause human disease. Humans are infected with hantaviruses by inhalation of aerosolised virus-containing particles of rodent excreta. In Belgium the presence of PUUV is well established as the causal agent for the majority of human hantavirus infections. Also hantavirus antibody positive brown rats were reported by Verhagen and co-workers in the eighties, but SEOV as such was never reported from Belgium.

In order to address the question whether SEOV is circulating in local rat populations and assess the health risk for employees that possibly come into contact with the virus, the Rodent Management Research Group of the Institute for Forestry and Game Management provided the Research Laboratory for Vector-borne Diseases with sera and tissue samples from brown rats captured in Flanders during 2004. The serum samples were tested for the presence of hantaviral IgG antibodies in an ELISA test specific for SEOV. When available, tissue samples belonging to SEOV IgG antibody positive rats were tested by species-specific nested RT-PCR for the presence of SEOV genome.

Out of the 194 serum samples that were tested, 51 (26.3 %) were SEOV IgG antibody positive. Nine tissue samples from antibody positive rats were so far tested by RT-PCR and 4 out of 9 (44 %) were found positive.

Although this study is still ongoing, the preliminary data demonstrate that 26.3 % of the rats captured in Flanders in 2004 have been in contact with SEOV and have seroconverted after infection. The presence of the SEOV genome in lung tissue of antibody positive rats indicates that the virus is actively circulating in local rat populations.

POSTER 9

Use of Seprion Capture Technology for the Detection of PrP^{sc} in Chronic Wasting Disease (CWD) Infected Tissues

V. Leathers, K. Velek, S. Koller, L. Plourde, R. Toomik

IDEXX Laboratories, One IDEXX Drive, Westbrook, Maine 04092
Valerie-Leathers@idexx.com

Misfolding of the normal host prion protein (PrP^{C}) and its subsequent accumulation as a protease-resistant conformer (PrP^{sc}) is a well-documented correlate of transmissible spongiform encephalopathies (TSEs). Proteinase K resistance is commonly leveraged as a method for distinguishing PrP^{sc} from PrP^{C} in most TSE diagnostics on the market. The IDEXX TSE post mortem diagnostics (BSE, CWD and scrapie) do not require proteinase K digestion, but instead use Seprion-capture technology applied to a microtiter plate format. This method utilizes a nonbiological PrP^{sc}-specific ligand that selectively binds PrP^{sc} in the presence of excess PrP^{C}. Captured PrP^{sc} is then detected using an anti-PrP antibody-HRPO conjugate. Sample preparation is limited to homogenisation of tissue and the addition of diluent; no other processing is required before applying samples to the assay plate. Assay run time is 3–4 hours, depending on the tissue type under evaluation.

The USDA approved IDEXX Herdchek Chronic Wasting Disease Antigen test kit (IDEXX CWD-EIA) has been used to detect PrP^{sc} in characterized white-tailed and mule deer lymph nodes samples. The specificity of the IDEXX assay for the combined population of white-tailed deer and mule deer lymph node tissues is 99.8% (1364/1367) as compared to IHC. Sensitivity of the IDEXX assay for detection of CWD in white-tailed deer and mule deer lymph node tissues is 98.8% and 100% respectively as compared to IHC.

The IDEXX CWD test, with its absence of a proteinase K digestion step and minimal handling during sample preparation, provides a sensitive, rapid and easy-to-use method for identifying CWD positive samples. The simplicity of the method allows straightforward adaptation to automation, making it an ideal tool for screening large numbers of samples. The IDEXX CWD EIA is under European Commission evaluation required for EU approval as a CWD surveillance method.

POSTER 10

Electron microscopic diagnosis of a lethal reovirus-like agent in carrion crows (*Corvus corone*) associated with clinical symptoms similar to West Nile disease

J. Mast, T. van den Berg, C. Letellier, P. Kerkhofs and G. Meulemans
Veterinary and Agrochemical Research Centre, Groeselenberg 99, B-1180 Brussels, Belgium
jamas@var.fgov.be

During the first two weeks of March (2004), in total 80 crows were found dead in three parks in the center of Brussels. Two times five birds originating from different parks were autopsied. All crows demonstrated splenomegaly, 60 % of the birds showed hemorrhagic enteritis with extensive lesions at the level of the duodenum and 50 % of the birds demonstrated cerebral or meningal hemorrhagies.

Toxicological examination revealed no specific toxins, but inoculation of specific pathogen-free (SPF), fertilised eggs with spleen extract by yolk sac injection killed the embryos. Chlamydia psitacii was isolated. Because the crow is an indicator species for West Nile disease (WND) and because these lesions are entirely compatible with WND, virus isolations (Prof. Zeller, National Reference Centre for Arbovirus & Hemorragic Fevers, Lyon, France) and WND-specific RT-PCR were done. All results of these tests were negative.

Transmission electron microscopy (EM) demonstrated groups of few intracytoplasmic particles in ultra-thin epoxide sections of the spleen of two of six affected birds and several groups of large numbers of identical particles in sections of two of three duodenum samples. The observed particles had the size and morphological characteristics of reovirus-like virions. They were not enveloped, had an isometric, round appearance and comprised of a core and an outer capsid. This capsid shell was composed of two layers, had a regular surface structure and was approximately 75 nm in diameter.

Proliferation of virus extracted from spleen in chicken embryonic hepatocytes was evidenced by its cytopathogenic effect and by EM demonstration of identical reovirus-like particles.

In the field, no other avian species seemed to be affected while experimentally infected specific pathogen free chickens did not show clinical signs, indicating a limited host range.

This case report illustrates how the ‘non-directed, open-view approach’ of EM efficiently allowed coming to a positive diagnosis in a critical situation (big city – public place – suspicion of emerging pathogen). Because the carrion crow is an indicator species for WND, it appears advisable to include diagnosis of reoviruses in the differential diagnosis of WND.

POSTER 11

ESTIMATING THE PROBABILITY OF FREEDOM OF CLASSICAL SWINE FEVER VIRUS OF THE EAST-BELGIUM WILD-BOAR POPULATION

Mintiens K[1], Verloo D[2], Laevens H[1], Dufey J[3], Dewulf J[4], Kerkhofs P[1], Koenen F[1].

[1]Veterinary and Agrochemical Research Centre (CODA-CERVA), Brussels, Belgium
[2]European Food Safety Authority, Parma, Italy
[3]The Food Agency, Brussels, Belgium
[4]Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium
koen.mintiens@var.fgov.be

A report of the Scientific Committee on Animal Health and Animal Welfare of the European Commission [1] includes recommendations for setting up monitoring programmes for classical swine fever (CSF) infection in a wild-boar population, based on the assumption that one would detect at least 5% prevalence in a CSF-infected wild-boar population. This assumption, however, is not science based. We propose an alternative method to provide evidence for a wild-boar population being free of CSF and evaluate the efficiency of a surveillance programme that was implemented in Belgium in 1998.

In our study, the probability of freedom of CSF-virus was estimated based on 789 samples; these were collected from wild boars within the surveillance programme (within the three provinces which include 95% of the Belgian wild-boar population) and examined by 3 diagnostics methods (antibody detection, virus detection and virus RNA detection). A Bayesian framework was used for the estimation, accounting for the diagnostic test characteristics without the assumption of the presence of a gold standard. The median probability of freedom of CSF-virus was estimated at 0.970, with a 95% credibility interval of 0.149 to 1.000. Independent on the choice of the prior information, the posterior distributions for the probability of freedom of CSF-virus were always skewed close to the upper boundary of 1. This represents a big gain of knowledge since we did not use any prior information for the probability of freedom of CSF-virus and took the uncertainty about the accuracy of the diagnostic methods into account.

1. CEC, *Classical swine fever in wild boar*. 1999, European Commission: Brussels, Belgium.

POSTER 12

Anti-influenza function of wild-boar- and domestic pig-restricted alleles at the *Sus scrofa* Mx1 locus

Palm M., Pire G., Leroy M., Baise E., Thomas A., Desmecht D.

Laboratory of Pathology, Faculty of Veterinary Medicine,
University of Liège, Sart Tilman B43, B-4000 Liège

Pigs are supposed to play the role of intermediate host –mixing vessel- for the reassortment of influenza A viruses of avian and human origin since their respiratory epithelium allows productive replication of the two types of viruses. Several MX proteins (IFN-induced GTPases) are known to confer an innate resistance against influenza A viruses. In laboratory mouse strains, allelic polymorphisms at the *Mx1* locus affect the probability of survival after experimental influenza infection, which raises the possibility that identification of an antiviral MX isoform in pigs might allow selection programmes aimed at improving their innate resistance. Concerning the porcine *Mx1* locus, three alleles coding for two distinct proteins (poMX1α and poMX1β) are known. We first investigated the distribution of the non deleted and deleted alleles and noticed an extreme allelic imbalance between European wild boar and domestic pig populations: 100% of the wild boars genotyped were homozygous for an allele that was detected in only ~ 50 % of domestic pigs. In the latter, the "wild" allele is replaced by an allele in which a small 11-bp deletion is predicted to result in a frameshift that should yield a significantly altered MX1 protein. We then started a comparative study of the antiviral function of both isoforms and the human MXA taken as a reference. Specific expression vectors were constructed, Vero cells were transfected before infection, and the success of viral replication was assessed by IF. The three MX proteins conferred quantitatively similar resistance to the virus. The results gathered here suggest that marker-assisted selection based on the discrimination between *poMx1*α and *poMx1*β alleles would not improve the innate resistance of pigs to influenza A viruses.

POSTER 13

Ranavirus associated mass mortality in imported red tailed knobby newts (*Tylototriton kweichowensis*)

Frank Pasmans [1], Silvia Blahak [2], An Martel [1], Nikola Pantchev [3], Filip Boyen [1], Peer Zwart [4]

1 Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium
2 Chemisches und Veterinäruntersuchungsamt, Westerfeldstr. 1, 32758 Detmold, Germany
3 Intervet Innovation GmbH, Profiling, Zur Propstei, 55270 Schwabenheim, Germany
4 Department of Veterinary Pathology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, PO box 80.158, 3508 Utrecht, The Netherlands

Wild caught salamanders and newts are still imported to Europe in large numbers, especially from Asia and North America. This might compromise the survival of wild populations, the status of which is often very poorly known. Besides, imported animals may introduce diseases to the native amphibians.
In June 2004, a mass mortality event occurred in 100 red tailed knobby newts (*Tylototrion kweichowensis*) imported in Belgium. Symptoms included skin ulceration, anorexia, apathy and occasionally oedema. Fecal examination and necropsy showed massive infections with lung worms (*Rhabdias tokyoensis*). Oral administration of fenbendazole cured the *Rhabdias* infections but the animals continued dying. From three newts examined, an *Iridovirus* isolate was obtained. The sequence of the major capsid protein gene of the isolate was determined and revealed 99.8% similarity with the published sequence of Frog Virus 3. An infection experiment with the Ranavirus isolate will be carried out to establish a causal relationship with the disease.
Necropsy revealed few macroscopic lesions: skin ulceration, large numbers of *Rhabdias* nematodes in the lungs and migrating larvae in the body cavity of non-dewormed animals and occasionally oedema. Fat bodies were absent. Most of the females contained large numbers of eggs. Histological examination showed few microscopic lesions: presence of nematode worms in the lungs and degeneration of secondary egg follicles. The latter was characterized by macrophage infiltration, angiogenesis, loss of vitelline membrane integrity and clumping of vitelline.
Salamanders and newts often are sold by garden centers for garden ponds (which is illegal) and thus may transfer imported diseases to native amphibian species. Preventive measures might include the obligatory testing of imported amphibians for the presence of pathogens such as iridoviruses or chytrid fungi.

POSTER 14

Prevalence of Liver Flukes *(Fasciola jacksoni)* in Wild Elephants (*Elephas maximus maximus*) of Sri Lanka

Perera, B.V. P.[1], Rajapakse, R.P.V.J.[2]

Department of Wildlife Conservation, Sri Lanka[1], Department of Veterinary Pathobiology, Faculty of Veterinary Medicine, University of Peradeniya, Sri Lanka[2],

Abstract

This study was carried out from January 2001 to April 2004 (39 months). The liver was examined for the presence of adult liver flukes in 47 elephants and these postmortems were performed within 48 hours from the time of death. Adult Flukes were identified visually and the species was determined through laboratory investigations. The severity of the parasitic infestation in the affected animals was accessed by considering the total number of flukes in the liver. In addition, laboratory investigations for the presence of fluke eggs were carried out for 48 dung samples from the living wild elephants.

Age categories of study population were 04 calves (1-5 yrs), 01 juvenile (5-10 yrs), 08 sub-adults (10 – 20 yrs), 29 adults (20 – 40 yrs) and 05 prime adults (>40 yrs). Sex ratio male:female was 35:12. Causes of their death were gun shot associated wounds leading to septicemia (23), gun shot associated severe injuries to the brain or lung (07), deaths due to electrocution (09), incidences of drowned animals (01), obstruction of airways 01, presence of severe pneumonia (02), fasciola associated parasitism (02), old age with severe debility (01) and a dead animal in which a cause could not ascertained (01). Presence of liver flukes in the elephants was determined in the laboratory as the *Fasciola jaksoni*, of which the average size 12 – 14 mm x 9 – 12.5 mm. There were 27 animals infected with the fluke from all the age categories. The severity of the infestation was very varied as the most severe – 02 (>100 flukes), moderate – 04 (50 – 100 flukes), mild – 07 (10 – 50), and low – 14 (<10) animals. It was clear that the severity of the infection was higher in weaker animals rather than in healthy animals. Histopathological changes in the affected liver tissue were cholangitis and fibrous tissue proliferation of the wall of the bile duct. Sixty percent of examined dung sample were harbouring the eggs of *F. jaksoni*.

POSTER 15

AVIAN MYCOBACTERIOSIS IN WHITE-WINGED DUCKS (*CAIRINA SCUTULATA*)

Miguel D. Saggese[1], DVM MS, Gary Riggs[2], DVM, Dipl ABVP and David N. Phalen[1], DVM, Ph. D, Dipl. ABVP (Avian)

[1]The Schubot Exotic Bird Health Center, Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A & M University, College Station, Texas, 77843-4475, USA.
[2]Akron Zoological Park, Norton, Ohio, 44203, USA

Avian mycobacteriosis is a relatively common cause of disease in pet, avicultural, wild and zoo birds. It can be a particularly devastating disease in certain species and has seriously impacted the captive propagation of an endangered species, the South-East Asian White-winged wood duck (*Cairina scutulata*). More than 80% of White-winged ducks submitted for necropsy at The Schubot Exotic Bird Health Center, from a captive population of this species in North America, were diagnosed with avian mycobacteriosis. Significant lesions were found in liver, spleen, lungs and air-sacs. The majority of these birds presented chronic moderate to severe granulomatous hepatitis, splenitis, airsaculitis and pneumonia. Acid-fast bacilli numbers present in lesions were numerous. Polymerase chain reaction results and posterior sequencing revealed no differences between the isolates of *Mycobacterium avium* from these birds. Poor genetic diversity and management factors have been suggested for this high susceptibility to avian mycobacteriosis in captive White-winged ducks. Further studies are currently undergoing at The Schubot Exotic Bird Health Center on the ante-mortem diagnosis and associated risk factors of avian mycobacteriosis in this species.

POSTER 16

Rodent control and wildlife diseases

Stuyck J, Verbeylen G, Baert K, Lens B

Rodent Management Research Group, Institute for Forestry and Game Management, Gaverstraat 4, B-9500 Geraardsbergen, Belgium (goedele.verbeylen@inbo.be)

The Rodent Management Research Group advises the Water Division of the Flemish government on how to control brown rats *(Rattus norvegicus)*, muskrats (*Ondatra zibethicus)* and coypus (*Myocastor coypus)* in the best way. Recently also beavers *Castor fiber* have appeared in Flanders and are studied because of their interaction with the muskrat and coypu control.

In order to optimise control methods and minimize ecological side effects, basic ecological research is carried out including parameters such as space use and dispersal. The occurrence of resistance to anticoagulant rodenticides is checked. Trapped animals are examined post-mortem taking into account both biological and pathological parameters. Since rodents are known to be important vectors for a number of diseases including hantavirus, EMCV, leptospirosis and rodentiosis, samples are collected and preserved. These samples are partially examined in collaboration with Jan De Borchgrave (Institute of Tropical Medicine, Antwerp), Paul Heyman (Research Laboratory for Vector-borne Diseases, Brussels), Paul Tavernier (Dept. of Bacteriology, Pathology & Poultry Diseases, Ghent University), Karl Walravens (Veterinary & Agrochemical Research centre, Brussels) and Peter Breyne (Molecular-genetic Research Group, Institute for Forestry and Game Management). Hopefully future collaborations initiated through the Belgian Wildlife Disease Society will lead to further research.

A survey of more than 16.000 muskrats revealed seasonal fluctuations in the infestation incidence and intensity of *Taenia taeniaeformis*. Several hundreds of coypu livers were examined macroscopically for the presence of parasites, resulting in only two cases of *Fasciola* sp. and one case of *Echinococcus granulosus*. Norway rats were screened for *Trichinella spiralis* and none of them were found positive. Lung and serum samples are collected from brown rats and muskrats for hantavirus screening. Some of the few dead-found beavers were checked for infections such as giardiasis, tularaemia and toxoplasmosis, with only negative results yet. In one case eggs of *Fasciola* sp. were detected in the caecum and one liver was infected with protoscolices of *Echinococcus multilocularis*.

These results will be discussed more detailed on the poster.

POSTER 17

Distribution of K88 *Escherichia coli* resistance/susceptibility haplotypes at the *mucin 4* locus among cohorts of wild boars and domestic pigs

Thomas A., Broers A., Linden A.*, Desmecht D.

Departments of Pathology and Infectious and Parasitic diseases*,
Faculty of Veterinary Medicine, University of Liège, Sart Tilman B43, 4000 Liège.

Two haplotypes were recently described in the region exon 5 to exon 8 of the porcine *mucin 4* gene (1), one being associated with innate resistance to enterotoxigenic *Escherichia coli* (ETEC), more particularly to K88 ETEC-associated neonatal diarrhea. As European wild boars were found to resist adherence by K88 ETEC (2) and because the natural selection should indeed have resulted in the selection of resistant animals, this study was undertaken to confirm that the K88 receptor is absent from wild boar populations.
We first validated a PCR procedure amplifying an intronic fragment homologous to the 7th intron of the human *mucin 4* gene. After PCR product sequencing, the two expected haplotypes were found, the resistant phenotype being associated with haplotype g1849-t2129 and the susceptible with c1849-c2129. We then genotyped 2 cohorts of 30 wild boars -originating from southern Belgium- and 30 domestic pigs from different breeds (Piétrain, landrace, Large White, Yorkshire, Berkshire, Duroc, I'pig, and Meishan). Both alleles were found in the two cohorts. Domestic pigs were either homozygous resistant/susceptible, or heterozygous. The wild boars were either homozygous resistant or heterozygous, but never homozygous susceptible. These results will be discussed in terms of marker-associated selection of ETEC-resistant pigs and in terms of natural selection.

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POSTER 18

Population dynamics of Red foxes (*Vulpes vulpes*) in Flanders

Van Den Berge K, Quataert P.

Ministry of the Flemish Community, Institute for Forestry and Game Management, Geraardsbergen, Belgium
koen.vandenberge@inbo.be

After 150 years of absence in a large part of Flanders, the fox filled up this gap in his distribution area since about half the eighties (1,2). Simultaneously, some problems have risen or are aggravated, a.o. the public health risk of *Echinococcus multilocularis* (1). Since 1996, i.e. one decennium after the start of the re-colonisation, an intensive fox population research on landscape level proceeds (3) in an optimal fox biotope with a surface of 100 km² (+ 44 km² buffer zone).
In this intensively monitored area, an annual average of 12.3 (± 2.4) successful litters was recorded, with a maximum of 15. When the unsuccessful litters are added, the average rises to 15 (± 1.4), with a maximum of 17. The counting of placental scars or embryo's in dead females revealed a mean neo-natal litter size of 6.64 (± 1.71), which is high. An extensive capture-mark-recapture experiment (309 juveniles) confirms this in the field. In a maximum scenario, fox abundance in autumn mounts up to 2 animals / km², while in spring it does not reach 1 animal / km². This density remains quite stable during recent years, and has to be considered as relative low. Both spot light counts and radio telemetry data support this statement : home ranges are very large, indicating a limited intern population pressure. For the first time, it could also been proved that also females contribute in long distance dispersion – giving a possible explanation for the quick re-colonisation of Flanders.

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POSTER 19

ECHINOCOCCUS MULTILOCULARIS, INCREASING PRESENCE IN THE SOUTHERN BORDER AREA IN THE NETHERLANDS.

J.W.B. VAN DER GIESSEN[1], A. DE VRIES[1], M.L.CHU[1], J. MULDER[2], P.TEUNIS[1], K. TAKUMI[1]

1. National Institute for Public Health and the Environment, Netherlands
2. Alterra, Wageningen and Mulder-natuurlijk, de Bilt

A base line prevalence study was carried out in the province of Limburg between 2002-2003 to get a better insight in possible spread of the parasite after its first recognition in 1996 in the Netherlands. In an area of approximately 800 skm, with an average prebreeding fox population of 2000 animals, a total of 196 animals were investigated for *E. multilocularis* by microscopical examination of the jejunum and PCR method of colon contents. In addition, the same foxes were examined for ecological factors, such as sex, age, condition and stomach contents, to get a better insight into the interaction between the parasite and the fox population dynamics. Of the 196 foxes examined, 25 were positive by either microscopy or by PCR. Most positive foxes were identified during the winter period of January and February 2003. Worm burdens were significantly higher in this study compared to the previous study. In addition, spatial analysis using GIS and kriging shows that the regional parasite prevalence increased in the period between 1996 and 2003. This is the first time an increased infection pressure has been described for humans in the Netherlands.

POSTER 20

Of fox and hound: age determination by counting dental cementum growth lines

Van Lancker S[1], Van Den Broeck W[1], Van Den Berge K[2], Simoens P[1].

[1]Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Belgium
[2]Institute for Forestry and Game Management (IBW), Geraardsbergen, Belgium
sofie.van.lancker@telenet.be

Counting of dental growth lines is a useful method for age determination in feral animals that are subject to seasonal environmental variations. To assess whether or not this technique could also be used in domestic animals, the cementum growth lines of 7 domestic dogs (*Canis familiaris*), kept in housing conditions, were compared with those of 4 red foxes (*Vulpes vulpes)* living in the natural habitat of Flanders. Dates of birth of all animals were registered. In the foxes a good correlation was found between the number of growth lines and the age of each animal. In contrast, in domestic dogs the number of cementum lines was variable and could hardly be determined because the difference between primary and secondary lines was often indistinct. It was concluded that the count of cementum growth lines could not be used for age assessment of dogs that are living in domestic conditions devoid of seasonal variations in food accessibility and weather conditions.

POSTER 21

Cyanobacterial blooms as a potential threat for waterfowl in Flanders.

Van Wichelen J[1], De Coster S[1], De Ruysscher F[1], van Gremberghe I[1], Van der Gucht K[1], Vyverman W[1], Tavernier P[2], Boutte C[3], Descy J-P[4], Wilmotte A[3].

[1] Laboratory of Protistology and Aquatic Ecology, Ghent University
[2] Department of Pathology, bacteriology and poultry diseases, Faculty of Veterinary Medicine, Ghent University
[3] Center for Protein Engineering, Institute of Chemistry, University of Liège
[4] Laboratory of Freshwater Ecology, Facultés Universitaires Notre-Dame de la Paix, Namur
jeroen.vanwichelen@UGent.be

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, 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, pets and even humans. In the framework of the national research-project B-Blooms (http://www.bblooms.ulg.ac.be) about 60 different algal bloom events, mainly in ponds and small lakes, were sampled in order to reveal their distribution and nature in Flemish surface waters. We therefore isolated and cultivated the dominant bloom-forming taxa and microscopically assessed the species composition, which was complemented by the analysis of the photosynthetic pigment composition, the concentration of cyanotoxins, and the genotypic and molecular diversity.

Cyanobacteria dominated in 90 % of all blooms, with *Microcystis* (40 %) and *Planktothrix* (20 %) being the most dominant genera. Blooms of *Aphanizomenon*, *Anabaena* and *Woronichinia* were less frequently observed. Molecular analysis revealed that two microcystin synthetase genes (*mcyB* and *mcyE*) were present in almost every sample, which is indicative of a widespread potential for toxin production among Cyanobacteria. Two 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 Lake Leeuwenhof (Drongen, August 2004) and Lake Tiense 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, even 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.

POSTER 22

Human-beaver (*Castor fiber*) conflicts in Flanders?

Verbeylen G[1]

[1]Rat Control Research Group, Institute for Forestry and Game Management, Geraardsbergen, Belgium
goedele.verbeylen@inbo.be

After a long period of absence, beavers reappeared in Flanders in spring 2000, originating from the unofficially reintroduced Walloon population. A feasibility study for a future reintroduction project to restock this not yet reproducing population was conducted at the request of the Flemish government. This concluded that the 2 studied river basins could carry a viable beaver population of at least 40 families that were all expected to stay in the same area and cause no problems in the wide vicinity. Even before the Flemish government had decided whether or not to proceed with an official reintroduction, 20 Bavarian beavers of unknown age and sex were released in April 2003 along the rivers Dijle and Laan. This happened unofficially, without any scientific support and without preparing or informing the local population or other interest groups. Since 2002 beavers are also entering Flanders from the officially reintroduced (but without consulting Flanders) Dutch population and probably by some natural dispersal from the German Eiffel area.
Now, after 2 years of reproduction, beavers are permanently present along the rivers Dijle and Laan south of Leuven and along the river Maas, and also start to turn up in other parts of Flanders. Complaints are coming in about damage to private as well as to public property, and up till now no preventive measures are taken. An adaptation of rat control methods is required, but this will also require more manpower. Budgets are limited and may not be sufficient to avoid serious problems. Some of the few actions taken at the moment are some curative measures in places where damage (to trees, dikes, …) occurs and a very limited monitoring of the Dijle-population by the Nature Division of the Flemish government. The Institute for Forestry and Game Management advises the Water Division of the Flemish government on the situation by studying literature, continuously mapping the Flemish beaver distribution and autopsying dead-found animals to find out mortality causes. Since in the densely populated Flanders many human-beaver conflicts can be expected, the pros and cons of beaver presence should be weighed up carefully. An integrated vision on how to continue with beavers in Flanders should be developed as soon as possible.

POSTER 23

The coypu (*Myocastor coypus*) in Flanders: eradicated but still re-immigrating

Verbeylen G[1]

[1]Rat Control Research Group, Institute for Forestry and Game Management, Geraardsbergen, Belgium
goedele.verbeylen@inbo.be

Coypus originate from South America and were imported to Europe at the end 19th-beginning 20th century for their fur, meat and as 'weed cutters'. They escaped or were released and could establish vital populations in places where the climate was not too cold. In Flanders populations of coypus were only present since the seventies in the province of Limburg. Here they enter Flanders from The Netherlands along the river Maas, where they in their turn immigrate from Germany. A second isolated and probably introduced population in Limburg could be found in the pond area of Hasselt-Zonhoven. During cold winters, the coypus survive in marshes and near the power plants that discharge warm water into the river Maas. In other provinces incidental observations are made over the years, most likely of escaped individuals.

A few years ago numbers in Flanders were estimated at several hundred individuals, and they were increasing due to the warm winters of the last few years. Experiences from other countries show that high densities of coypus can seriously damage – among other things – dikes, crops and natural plant communities. That's why the Flemish government started a structured coypu control in 2002, by intensive campaigns with life traps and large conibear traps in spring and continuous trapping in places where coypu signs were found during the rest of the year. All trapped coypus are autopsied at the Institute for Forestry and Game Management, to look at – among other things – sex, age, reproduction and parasites. Based on these data, the Institute advises the government on how to adjust the control methods.

Now, three years later, coypus are very much under control. The isolated population in Hasselt-Zonhoven seems to be eradicated and along the river Maas coypus are stopped at the Dutch border. This success is – besides the intensive trapping – probably due to the lack of a high water level on the river Maas in spring 2004, which led to a much lower influx of animals from The Netherlands. Also the structured coypu control in The Netherlands, followed by Germany from 2005 onwards, will lower immigration and facilitate coypu control in the future.

LEAD INTOXICATION BY INGESTION OF LEAD SHOT IN RACING PIGEONS (*Columba livia*)

P. Tavernier [1, 2], S. Roels[1,3], K. Baert[1, 4], K. Hermans[2], F. Pasmans[2]

[1] Belgian Wildlife Disease Society (BWDS); [2] Bird Clinic, Veterinary Faculty, Ghent University; [3] Unit Pathology, Department of Biocontrol, Veterinary and Agrochemical Research Centre (CODA/CERVA); [4] Institute for Forestry and Game Management (IBW).

Introduction

Lead intoxication in birds by ingestion of lead shot has been recognised in many countries since Bellrose (1959) first described the long-term devastating effects on wild waterfowl populations. Secondary intoxication occurs in raptors predating on waterfowl (Müller et al., 2001). A wide variety of other bird species have been found susceptible to lead intoxication by mistaking lead shot for grit or food particles (Scheuhammer and Norris, 1995).

Only few descriptions of clinical cases of lead intoxication in pigeons are available. We comment a case of lead intoxication by ingestion of lead shot in a loft of racing pigeons. Similar casualties in domestic birds might reflect a more widespread intoxication problem in wildliving birds in Belgium.

Case report

Two pigeons of about 8 months old were presented in august 2004 with complaints of a distended crop and regurgitation of liquids. They belonged to a loft of 20 young pigeons, which during their daily flights alighted and fed on a nearby meadow where frequent shooting activity was noticed during and beyond the hunting season. The owner reported having seen similar cases in former years.

On clinical examination the two pigeons were thin and anaemic and displayed an unusual behaviour characterised by lethargy and a backwards stretching of the neck. Simultaneously lateral to and fro rolling movements of the distended crop could be seen under the skin. Deep palpation of the crops induced regurgitation of foul smelling liquid crop contents. In one pigeon ptosis was obvious. The urate fraction of the excreta was watery in one pigeon and a soft consistency of the faeces was noticed in both.

In the differential diagnosis we retained crop infection by trichomoniasis or candidiasis, adenovirus infection, salmonellosis and heavy metal intoxications including lead and zinc. Newcastle disease (paramyxovirus 1) was not considered because all the pigeons on the loft had been vaccinated secundum artem.

Microscopical examination of a wet crop swab (magnification x100) was negative for *Trichomonas gallinae.* A second crop swab was rolled on a glas slide, stained with Hemacolor® (Merck, Darmstadt, D.) and examined under immersion microscopy (x1000). No trichomonads nor *Candida albicans* could be detected but numerous lactobacilli were present on the smear. A whole body radiograph of one pigeon confirmed the wide distention of the crop, which contained grit and food particles. In the gizzard one round radiopaque pellet, probably lead shot, could be easily distinguished from the grit particles. A heparinised blood sample was taken from the V. basilica in the same pigeon. Haematological examination of a blood smear stained with Hemacolor® showed numerous erythroblasts, polychromatic erythrocytes and reticulocytes, indicating a severe regenerative anaemia. Atomic absorption spectrophotometry performed in a commercial laboratory showed a blood lead concentration exceeding 7 ppm (7000 µg/l).

The two pigeons were treated by I.M. injection of 40 mg/kg calcium disodium EDTA twice daily in intermittent 5 day courses. No grit was offered because the grinding action of grit favours the fragmentation and ionisation of lead in the gizzard. Mineral oil was administered orally instead of attempting surgery to eliminate the lead shot.

The pigeon in which the high blood lead concentration was found, died after 5 days of treatment. A post-mortem radiograph and a necropsy revealed 11 lead pellets in the gizzard and in the intestine, whereas only the biggest one had been seen on the first radiograph. An impression smear of the kidney showed numerous intranuclear inclusion bodies.

Discussion

The cytotoxic nature of lead and its capacity of inhibiting important enzymatic processes are responsible for the wide variety of symptoms observed in cases of lead intoxication. The most typical clinical picture is a combination of anaemia with neurologic, most often paralytic signs. Anaemia is partly regenerative, due to the destruction of red blood cells, and partly non-regenerative because of interference of lead with heme-synthesis through inhibition of δ-aminolevulinic-acid-dehydratatase (ALAD) and of heme-synthetase. In waterfowl and raptors, blood lead levels above 0.4 ppm reflect abnormal exposure to lead and levels above 1 ppm are indicative of acute clinical poisoning (Pain et al., 1993).

The use of lead shot for hunting waterfowl has been banned and replaced by the use of steel shot in a number of countries. In the Netherlands and in Denmark the use for any kind of hunting, as well as the possession of lead shot, is forbidden. In Belgium, though lead shot is still used for hunting, no information is available on the impact of lead intoxication on wild or domestic birds. Due to the high density of building in this country, hunting is often taking place in the close proximity of backyards in inhabited areas. Lead poisoning was diagnosed in 16.6 % living (n = 30) and in 4 % dead (n=99) domestic anseriformes presented at the Bird Clinic of the Veterinary Faculty of Ghent University. These percentages were considered as minimal estimates (Tavernier et al., 2004).

Conclusion

Casualties of lead intoxication by ingestion of lead shot in domestic birds might indicate similar effects on wildliving birds. A systemic approach is needed to evaluate the impact of the use of lead shot on wild bird populations in Belgium.

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