ABSTRACT BOOK

5th Symposium of the Belgian Wildlife Disease Society

Spatial Approach of Wildlife Diseases

Friday 18 October 2013
CODA-CERVA - Tervuren

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Spatial Approach of Wildlife Diseases

Fifth symposium of the Belgian Wildlife Disease Society

BELGIAN WILDLIFE
DISEASE SOCIETY
(BWDS)

18 October 2013, CODA-CERVA, Tervuren

Organizing and Scientific Committee:

Paul Tavernier

Stefan Roels

Paul Heyman

Alexandre Dobly

Leen Claes

Kristof Baert

Welcome address: “Spatial approach of wildlife diseases”

This year the Belgian Wildlife Disease Society celebrates its 10th anniversary. We can look back on a sequence of successful activities during the past ten years, including four well-attended biennial Symposia, and each year two to three general meetings covering a wide range of subjects. In 2009, joined to the 3rd BWDS Symposium, we hosted the 2nd EWDA wildlife health surveillance network meeting and a meeting of the European WILDTECH project. We assisted the Belgian veterinary authorities in starting up the yearly notification of wildlife diseases to the World Animal Health Organisation (OIE) and we carried out a Federal Government funded two year project, called WILDSURV (2008-2010), during which a prototype of a first-line risk assessment tool for wildlife-borne diseases was developed. Maybe less visible but not less important are the many spontaneous thematic collaborations that were started up between different institutes and individuals within the BWDS context.

The theme of the 5th BWDS Symposium held today is “the spatial approach of wildlife diseases”. A “spatial” approach means essentially mapping of disease outbreaks and the factors that may be related to an outbreak. Topographical information is usually processed by means of powerful software, called Geographical Information Systems (GIS), allowing to store, analyze, reorder, and access the information.

Mapping is an important aid in identifying the cause and understanding the epidemiological course of a disease outbreak. Strong indications to identify the causes of disease outbreaks can be obtained by describing the spatial pattern of an outbreak (e.g. a random, clustered or regular distribution) or by linking the outbreak location with factors such as vegetation (e.g. *Hantavirus outbreaks with seed fall*), climate *(e.g. vector-borne diseases),* atmospheric pollution *(e.g. presumed links with immune suppression in amphibians),* animal migration patterns *(e.g. pasteurellosis with bird migration flyways),* noise *(e.g. dolphin stranding with acoustic disturbance),* occupational exposure (*e.g. swimmers itch*), management and husbandry practices (*e.g. forest management and tick abundance*). Mapping also helps to predict the occurrence of an outbreak *(e.g. small water bodies in areas with few wetlands could favor avian influenza exposure)*, and to support the management of an outbreak *(e.g. landscape heterogeneity in the US affects rabies vaccination efficiency in raccoons).*

For these reasons the spatial aspects of wildlife diseases are an essential approach to get insight into the interactions between natural and anthropogenic phenomena, of which infectious and non-infectious “diseases” are often only a consequence. Studying wildlife-borne diseases in non-wildlife species cannot disregard the ecological context in which disease problems originate. The “One Health” concept illustrates plainly that medical and veterinary professionals share the same interests with conservationists in advocating ecosystem health at a global scale.

At this 5th BWDS Symposium our invited speakers present a number of wildlife disease problems with a strong spatial rationale. Next to the invited speakers we have for the first time reserved four slots for “free oral communications”, not necessarily reflecting the general Symposium theme. Furthermore, a series of posters is displayed as usual.

At the start of the second decade of existence of the BWDS, the board members confirm their intention to continue enhancing the awareness about threats to biodiversity, impacting on human and animal health. Together with many others worldwide concerned in conservation, we hope this awareness will reach the interest groups behind the main activities implicated in anthropogenic threats to the environment.

We wish you a very instructive, constructive and pleasant Symposium day.

Paul Tavernier

Programme

Moderators: Tim Adriaens (INBO) & Sophie Roelandt (CODA/CERVA)

09:00 - 09:30 Registration & coffee

09:30 - 09:35 Welcome *(P. Kerkhofs - CODA/CERVA - Be*)

09:35 - 09:45 Opening (*S. Roels - CODA/CERVA - BWDS - Be)*

09:45 - 10:15 Spatial organization of badger populations and their influence on disease dynamics (*R. Delahay - FERA - UK*)

10:15 - 10:45 Vector mapping (*E. Ducheyne - AviaGis - Be*)

10:45 - 11:00 Sources of tuberculous-like lesions in Belgian wild boars (M. Govaerts - Ulg - Be)

11:00 - 11:30 Break

11:30 - 12:00 Spatial spread and maintenance of Border Disease Virus Infection in Pyrenean chamois (*Rupicapra pyrenaica*) (*I. Marco - SEFAS,UAB - Sp*)

12:00 - 12:30 Schmallenbergvirus (*B. Cay - CODA/CERVA - Be*)

12:30 - 12:45 Muskrats as wildlife reservoir of human leptospirosis (M. Mori - CODA/CERVA-Be)

12:45 - 14:00 Lunch & poster session

14:00 - 14:30 Ecological factors driving Avian Influenza Virus dynamics in Spanish wetlands (*U. Höfle - SaBio, IREC - Sp*)

14:30 - 15:00 Influence of forest changes on ticks (*W. Tack - AviaGis - Be*)

15:00 - 15:15 E. multilocularis in red foxes in the Netherlands (M. Maas - RIVM - Nl)

15:15 - 15:45 Break

15:45 - 16:15 Spatial approach of pathogen transmission in wild boar (*H. H. Thulke - UFZ - Ge*)

16:15 - 16:45 Predictive model for Hantavirus outbreaks in Western Europe (*K. Tersago - UA - Be*)

16:45 - 17:00 Zoonotic transfer of Chlamydia psittaci (I. Kalmar - UG - Be)

17:00 Closing remarks & poster awards

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Science at the service of safe food production and animal health

In the framework of sustainable and socially acceptable agriculture, the VAR, through scientific research, expert advice, and the provision of services, contributes to a pro-active policy as regards safe food production, animal health, and public health on the federal and international levels.

The CODA-CERVA's core activities consist of policy supporting scientific research, expert advice, and the efficient provision of services in :

- Epidemic, endemic and emerging transmittable diseases in animals
- Zoonotic and emerging infectious diseases threatening public health
- Contaminants and the quality of the environment in the framework of safe food production
- Epidemiology: surveillance, risk analysis, and molecular epidemiology

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The Research Institute for Nature and Forest (INBO) is the Flemish research and knowledge centre for nature and its sustainable management and use. INBO conducts research and supplies knowledge to all those who prepare or make the policies or are interested in them.

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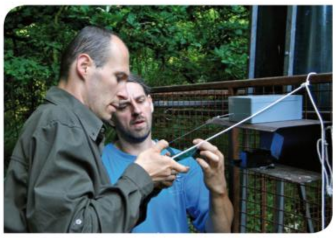
As a leading scientific institute, INBO works for the Flemish government primarily, but also supplies information for international reporting and deals with questions from local authorities. In addition, INBO supports organisations for nature management, forestry, agriculture, hunting and fisheries. INBO is a member of national and European research networks. It makes its findings available to the general public.

INBO employs some 250 staff, mainly researchers and technicians. Besides its Brussels head office, INBO has branches in Geraardsbergen, Groenendaal and Linkebeek.



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In Defence of the Environment

What is our point of view?

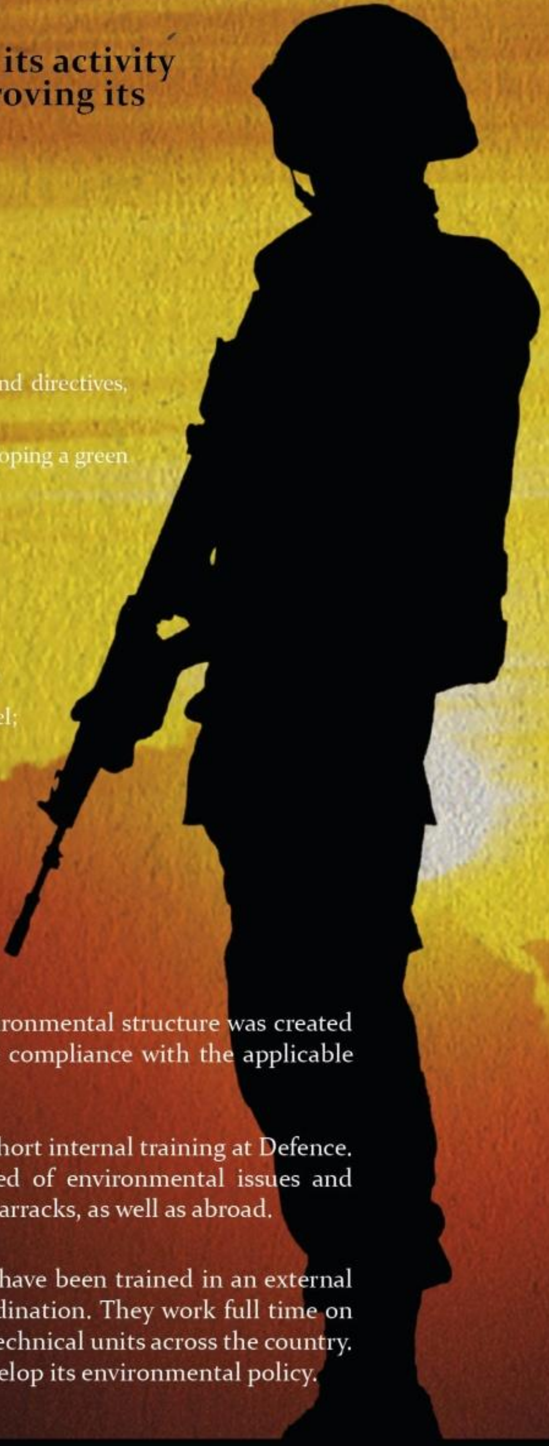
Defence undertakes to prevent and limit the impact of its activity and installations on nature, while continuously improving its environmental performance.

For Defence, achieving this policy involves

- implementing an environmental management system;
- applying the relevant laws and regulations;
- systematically inserting environmental components at the planning stage in programmes and directives, including operations and exercises;
- contributing to the preservation and restoration of biodiversity on its grounds as well as developing a green spaces management focused on minimizing the use of herbicides.

This environmental policy rests on four pillars

- an environmental structure with environmental experts, coordinators and consultants;
- an internal environmental permitting system based on existing systems at regional level;
- continuous training and awareness-raising of personnel;
- communication and reporting.



Who are we?

The environment is everyone's concern at Defence. In 2005 an environmental structure was created in order to get environmental management on track and ensure compliance with the applicable legislation.

The environmental consultants belong to the units and receive a short internal training at Defence. In addition to their primary function, they keep their command advised of environmental issues and environmental policy with regard to activities both inside and outside the barracks, as well as abroad.

For technical advice, they turn to the environmental coordinators. These have been trained in an external institute providing accredited training in the field of environmental coordination. They work full time on environmental issues, and are geographically dispersed in environmental technical units across the country. Some of them are attached to the Defence staff in order to prepare and develop its environmental policy.

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What actions do we take?

Mobility

Based on a preliminary examination, Defence has reduced its vehicle fleet to cut CO_2 emissions. Older, more polluting vehicles are being gradually replaced by "cleaner" ones and transport facilities are used to best advantage.

Soil

Defence has developed a soil management policy to prevent further pollution. Response equipment has also been bought and distributed to enable personnel to react quickly to incidents.
Any existing and well known pollution problem is consistently treated according to the urgency of the matter and the available budgetary means.

Biodiversity

The grounds of Defence amount to acres of fantastic natural areas. Being mostly closed to the public, the grounds are home to many rare species of flora and fauna. After the integration of its parade grounds into the European Natura 2000 network, Defence has committed to preserving the flora, fauna and existing habitats. To that end, it has concluded agreements with regional civilian bodies, namely the Forest and Nature Agency (Agentschap voor Natuur en Bos) and the Directorate-General for Agriculture, Natural Resources and the Environment (Direction générale de l'agriculture, des ressources naturelles et de l'environnement). It has also cooperated in the application of the Walloon river contract and the protection of the North Sea in Flanders.

Energy

Defence wants to reduce its energy consumption (electricity and heating) in a structural way. A study has been made of the use of existing buildings and where possible, buildings with low occupancy rates have been evacuated in order to turn off the heating. Other economy-saving measures have been put into practice: better insulation, phasing out energy-guzzling lighting... True to its commitment to use renewable or sustainable energies, Defence has signed green energy supply contracts and both renovated and new buildings are equipped with solar panels or a cogeneration system.

Waste

Defence puts into practice European, national and regional policies with regard to waste sorting. When elaborating its policy or purchasing new products, Defence also takes into consideration waste generation (including packaging).

Water

Defence is determined to reduce its water consumption by increasing personnel awareness and taking water efficiency measures for the renovation or construction of buildings. In order to comply with the legislation on wastewater treatment, Defence avoids discharging wastewater directly into surface waters or on the land by providing for a separated drainage system (wastewater and rainwater).

Noise

In spite of its frequent use of heavy military vehicles and planes, Defence is trying to control and limit its noise pollution. The important thing here is to maintain good relations with local authorities and inhabitants. Municipalities are kept informed of exercises, and night flights do not take place during exam time. Infrastructure works (e.g. setting up acoustic barriers) are carried out where necessary.

Air

Heating, paint booths, fighting or ordinary vehicles, planes, ships... A wide range of Defence installations and activities release emissions into the air. Defence works towards controlling these emissions according to the standards set by law. All appropriate measurements and tests are carried out and where possible, interventions are made to prevent or reduce emissions (e.g. fitting vehicles with particulate filters).

Awareness-raising, training and information

Looking after the environment is everyone's concern at Defence. Special attention is given to awareness-raising and training of personnel. All trainings received by servicemen during their career include an environmental component. Defence also launches a poster campaign every year to increase personnel awareness regarding the rational use of energy, the reduction of soil pollution and water consumption, waste sorting...

Care for the environment during operations and exercises

Having been a routine part of exercises for many years, environmental issues affect operations too. International bodies like the UN, NATO and the EU have a specific environment policy and require armed forces to take appropriate measures concerning environmental management and protection, especially during the stabilization phase of an operation.
Taking care of the environment also offers operational advantages like winning the hearts and minds of a local population.


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Oral presentations

Spatial organization of badger populations: implications for disease dynamics and management.

Richard (Dez) Delahay

Centre for Wildlife Management, Animal Health & Veterinary Laboratories Agency, Woodchester Park, Gloucestershire, UK

Management of disease in wildlife populations is challenging, not least because host behaviour and ecological conditions present substantial practical constraints and can influence the outcome of interventions in unpredictable ways. Understanding the spatial organisation and behaviour of host populations, and of infected individuals in particular, may provide valuable insights into how to more effectively target management interventions.

In parts of the UK European badgers (*Meles meles*) are infected with *Mycobacterium bovis* and constitute an important source infection for cattle. Long term studies indicate how the organisation of badger populations into discrete territorial social groups mitigates disease transmission, with movement of animals being implicated in disease spread. Hence disease appeared to remain spatially aggregated in the population for a period of several years. However, subsequent demographic change and enhanced immigration in part of the study area preceded a departure from this epidemiological pattern. Furthermore, at the individual level intensive radio-tracking studies have revealed variations in socio-spatial behaviour related to infection status. Similar variation in behaviour has also been identified by social network analyses which describe patterns of contact amongst badgers. A better understanding of the relationships between host infection status and social behaviour may provide valuable information on where and when intervention measures are likely to be most effective.

Vector mapping

Els Ducheyne, Veerle Versteirt, Guy Hendrickx

Avia-GIS Belgium

In order to assess the risk of diseases transmitted by vectors such as mosquitoes, ticks and midges, it is important to know the spatio-temporal distribution patterns of these vectors. Remote sensing and GIS tools are now commonly available and permit to map these vectors through the use of either statistical (such as logistic regression) or data mining (such as random forests) techniques.

In the presentation we first show how the Belgian Modirisk inventory of mosquitoes was designed and how this resulted in spatial distribution maps of multiple mosquito species.

In the next part we demonstrate how you can move on from the presence/absence mapping towards abundance mapping showing examples in Spain and the Netherlands. The Central Veterinary Institute in the Netherlands for disease transmission models used the abundance mosquito and Culicoides maps.

All the previous research was combined to generate presence/absence and abundance maps of mosquitoes at the European level for the European Food Safety Agency. These maps served as input data for disease transmission risk models for Rift Valley Fever into Europe.

Sources of tuberculous-like lesions in Belgian wild boars

Marc Govaerts[1], Rosario Volpe[2], Aloïs Vionnet[2], Fabien Gregoire[2], Virginie Ceuleers[2], Ludovic Jouant[2], Julien Paternostre[2], Michael den Haerynck[1], Dominique Cassart[3], Annick Linden[2]

[1] Mycobacteria Unit, CODA-CERVA, Groeselenberg 99, Uccle, Belgium; [2] Wildlife Health and Pathology Unit, Ulg, Sart-Tilman, Belgium; [3] Department of morphology and pathology, Ulg, Sart-Tilman, Belgium

Bovine tuberculosis (bTB) control programmes in cattle are hampered by the presence of wildlife reservoirs. Emerging wildlife maintenance hosts in our neighbour countries, and the associated growing incidence in breakdown herds, sparked interest in a closer surveillance of wildlife for bTB in Belgium.

During the 2011 hunting season, 229 hunter-killed wild boars were sampled in the 4 provinces of Wallonia. Carcasses were subjected to a systematic post mortem inspection, and all sub-mandibular lymph nodes showing gross lesions were processed for bacteriological, histopathological, and mycobacterial examination. 49 out of the 229 submandibular lymph nodes (21 %) showed gross lesions, either abscesses (43) or merely hypertrophy (6). A variety of abscess types were found : purulent (8), caseous (33), calcified (1), fibrous (11), multifocal (12), or any combinations thereof. Microscopically, lesions of necrosis (21), mineralisation (14), encapsulation (18), fibrosis (8), and the presence of eosinophils (16), macrophages (2) or giant cells (1), were found. Besides commensal bacteria isolated on generic media (9 x Staphylococcus aureus, 8 x coliforms, 5 x Proteus spp., 4 x Streptococcus porcinus etc.), no Mycobacterium bovis were isolated from the lesioned organs. Using selective media, M. avium subsp. avium was isolated from 1 among the 8 multifocal caseous lymph nodes, M. celatum from 4 out of 14 necrotic, mineralised and encapsulated lymph nodes, and a co-infection with Rhodococcus equi was found in one of the latter. These 3 actinomycetal species are known to induce granulomatous adenitis lesions in slaughter pigs, undistinguishable from true M. bovis lesions.

The prospective cross-reactivity to be expected from such atypical infections in the serosurveillance of bTB in wild boar, and the challenges of the competitive isolation and correct identification of M. bovis in wild boar will be discussed.

Spatial spread and maintenance of Border Disease Virus Infection in Pyrenean chamois (Rupicapra pyrenaica)

Ignasi Marco

Servei d'Ecopatologia de Fauna Salvatge (SEFaS),Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, 08193-Bellaterra, Spain

Since 2001 several outbreaks of a previously unreported disease associated to Border Disease Virus (BDV) infection have been described in Pyrenean chamois (Rupicapra pyrenaica pyrenaica) in Spain, France and Andorra. This process has decimated several Pyrenean chamois populations, with mortalities ranging from 42% to 86%. Unexpectedly, however, the epidemic waves have followed an erratic pattern that may be explained by ecological and epidemiological factors.

A retrospective study described the detection and isolation of a BDV in two chamois sampled in 1996 in the Eastern Pyrenees, 5 years before the first outbreak of disease. These two chamois did not show clinical symptoms and lesions consistent with BDV infection. Interestingly, the Eastern Pyrenees comprises one of the few chamois populations that have not been affected by the severe epidemic episodes associated to BDV infection.

After the severe BDV outbreaks, different epidemiological scenarios have appeared in the Pyrenees, some of which are having a negative impact on host population dynamics. Thus, management of chamois populations affected by these epizootics should be designed according to the epidemiological status and no generalizations should be made.

Schmallenberg virus

Brigitte Cay[1], Nick De Regge[1] and Thierry van den Berg[1]

[1]CODA-CERVA, Groeselenberg 99, 1180 Brussels, Belgium

After the emergence of bluetongue virus serotype 8 in 2006, another vector-transmitted pathogen was introduced in Northern Europe in 2011. This pathogen was characterized as an novel orthobunyavirus and named "Schmallenberg virus" (SBV) after the town where it was first discovered. An acute SBV infection causes no or only mild disease in adult cattle including fever, reduced milk production or diarrhoea for a few days. However, infection of naïve cows, ewes and goats during a critical phase of pregnancy can provoke abortions and severe foetal malformations.

Since 2011 SBV has spread extremely rapid all over Europe and has reached the Asian borders. This fast spread is probably caused by the transmission of the virus by insect vectors. Based on the detection of SBV RNA in biting midges, several *Culicoides* species are regarded as putative vectors although other unknown vectors may not be excluded.

In Belgium more than 99 % of the cattle herds were serological positive after the first year. Although the genome of SBV has also been found in bull semen, until today the possible role of semen in the dissemination of the virus is still unclear. Besides the susceptibility of domestic ruminants to SBV, different species from wildlife such as wild red deer (*Cervus elaphus),* roe deer (*Capreolus capreolus*) and mouflon were found to be serological positive for SBV, while the presence of SBV viral genome was demonstrated in a bison and elk (*Alces alces*). Antibodies to SBV have also been found in New World camelids (alpacas and lamas) and a dog. The role of these species in the SBV epidemiology is unknown.

Two years after the introduction of this new virus into Europe, the exact origin and way of introduction remains unknown, but the region of first detection and initial spread was highly similar for SBV and BTV. What's next ?....

Role of ondatra zibethicus as wildlife reservoir in an outbreak of human leptospirosis following a boy scout camp, Belgium summer 2012

Marcella Mori[1], Marjan Van Esbroeck[2], Raïssa Bakinahe[1], Sylvia Depoorter[3],Wim Decaluwe[3], Stefaan Vandecasteele[3], David Fretin[1], Marijke Reynders[3]

[1]Veterinary and Agrochemical Research Center, VAR-CODA-CERVA, Brussels, Belgium; [2]Tropical Medicine Institute, ITM, Antwerp, Belgium; [3]AZ Sint-Jan Brugge-Oostende AV, Campus Sint-Jan, Bruges, Belgium

Leptospirosis is a pandemic zoonosis largely distributed in tropical areas, where warm climate conditions are favorable for Leptospira spp. maintenance in the environment. People may contract the disease by direct or indirect contact with infected urine of reservoir animals and show signs of Weil's disease. In Europe, the last famous endemic case dates back to 2010 with the death of the double Olympic champion Andy Holmes, who contracted Weil's disease during canoeing (prolonged exposure to contaminated waters).

In Belgium, reported cases are associated with outdoor activities, travelling abroad or farming. We here report upon an outbreak of leptospirosis that occurred in the South of Belgium, during the month of August 2012, involving 10 teenagers who participated to Scout camps near the Semois River (Luxembourg province). Clinical symptoms included general malaise, fever, vomiting, myalgia and bilateral conjunctivitis. Three of the Boy Scouts had severe complications, including compromised renal function.

The three severe cases were laboratory confirmed by serology or Lipl32 RT-PCR. Risk factors were direct contact with *Ondrata zibethicus* (muskrat) and daily bathing in the river. Environmental investigation included water analysis and capturing of wild muskrats from the three nests located in the area. Five out of 9 captured muskrats (56%) presented antibodies against pathogenic *Leptospira* serovars. Kidney and liver tissue from 2 muskrats were additionally found positive with Lipl32 RT-PCR. The present study highlights the role of wild animals in the maintenance and spreading of *Leptospira* spp. in the environment. It is therefore important to monitor wild animals as carriers for leptospirosis as a preventive measure for public and veterinary health.

Ecological factors driving avian influenza virus dynamics in Spanish wetland ecosystems

U. Höfle[1], E. Pérez-Ramírez[1], P. Acevedo[2], N. Busquets[2], N. Majó[2,3]; A. Allepuz[2, 3], A. Alba[2], X. Gerrikagoitia[4], M. Barral[4],

[1]Instituto de Investigación en Recursos Cinegéticos IREC (CSIC-UCLM, JCCM), Ciudad Real, Spain; [2]Centre de Recerca en Sanitat Animal CReSA, Universitat Autónoma de Barcelona, Barcelona, Spain; [3]Departament de Sanitat i Anatomia animals, Facultad de Veterinària (UAB), Barcelona (Spain); [4]Instituto Vasco de Investigación Agraria, NEIKER, Derio, Spain

Objectives:

There is currently limited scientific information about the basic epidemiology of avian influenza viruses (AIV) in wild birds in natural ecosystems, including the role of the environment itself and the effect of bird population ecology on transmission and persistence of AIV. The aim of this work was to study the contribution of environmental and host factors in the occurrence of the low pathogenic AIV (LPAIV) in Spanish wetlands under field conditions.

Methods:

For this purpose five different Spanish wetlands were studied during two consecutive years. Fresh faeces and water were periodically collected for viral detection, and the association of LPAIV prevalence with climatic conditions, vegetation, physical properties of surface water and density and diversity of wild birds of the wetlands was assessed.

Results:

Mean LPAIV prevalence detected in fresh faeces was 1.7%±0.4, with significant temporal and spatial variation. Using variation partitioning procedures it could be concluded that the combination of space and time variables (locality, year and season), independently to other factors, explained the largest proportion of the variation (36.8%), followed by the meteorological factor (21.5%), and finally the combined effect of vegetation structure and wild bird community data, accounting for 21% of the variation.

Conclusion:

The results obtained are useful to generate hypotheses about ecological factors that may be associated with a higher occurrence of LPAIV in wetlands. This work may contribute to identify more efficient strategies of surveillance that target hotspots thus favouring early detection of AIV.

The impact of forest changes on ticks

Wesley Tack[1], Maxime Madder[2], Kris Verheyen[1]

[1]Ghent University, Belgium; [2]Institute of Tropical Medicine Antwerp, Belgium,

By altering the tree species composition and vertical structure, forest management may have a considerable impact on the suitability of forests for ticks and their hosts, thus influencing the dynamics of tick-borne diseases.This is particularly important in the context of ongoing projects that aim to convert pine plantations into semi-natural deciduous forests to optimize their economic, ecological and social/recreational functions.

The impact of forest conversion on Ixodes ricinus populations was investigated in the Campine region (BE). Two observational studies – a large-scale survey and a local longitudinal study – were carried out in forest stands varying in tree species (pine vs. oak) and shrub cover to investigate the effect of landscape and local habitat on tick abundance.

Also, a moderate thinning and shrub removal experiment were carried out to verify the influence of vertical forest structure on ticks. Finally, we studied the effect of a seed-addition experiment and an oak mast year on the abundances of rodents and their tick parasites to assess the importance of abundant food resources.

At the landscape level, a positive effect was found for forest edge length (i.e. fragmentation) on the abundance of nymphs and adults. At the local level, the abundances of larvae, nymphs and adults were higher in oak stands compared to pine stands, and increased with increasing shrub cover. Thinning had no effect on tick abundances, while shrub clearing had an adverse affect on the abundances of all life stages up to two years post-clearing. Finally, rodent abundance and the abundance of questing nymphs showed a lagged positive response to acorn mast.

Our results provide more insight into the role of forest composition, forest structure, and landscape configuration on the spatiotemporal variation in the abundance of I. ricinus ticks. We conclude that forest conversion might create suitable habitats for ticks by altering the dynamics between ticks and their key hosts.

Notable increase of E. multilocularis in red foxes (Vulpes vulpes) in South Limburg, the Netherlands

Miriam Maas[1], Cecile Dam-Deisz[1], Katsuhisa Takumi[1], Annika van Roon[2], Joke van der Giessen[1],

[1]RIVM, The Netherlands; [2]WUR, Wageningen, The Netherlands

Echinococcus multilocularis, the small fox tape worm, is considered the most pathogenic zoonotic parasite in Europe. If eggs of E. multilocularis are ingested by humans the disease alveolar echinococcosis (AE) can develop, which may be fatal if left untreated. E. multilocularis was first detected in the Netherlands in 1996 in the south of Limburg and the east of Groningen.

Subsequent studies detected no other infected areas in the Netherlands. However, a spread northwards of E. multilocularis infection was found in Limburg (2.7 km/year), as well as an increase in worm burden of infected foxes. R0 was 1.6 (95% CI 1.1–3.5). Regular monitoring of E. multilocularis in this area is important to assess public health risks.

To determine the current prevalence of E. multilocularis in the eastern suburban and rural areas of the city Maastricht (South Limburg), 37 foxes in this area were hunted in the winter of 2012-2013. They were examined by the Intestinal Scraping Technique and by a nested PCR on colon contents. Foxes were considered infected when either or both of the tests was/were positive.

A prevalence of 57% (21/37; 95% CI 41-71%) was found. This is a notable increase compared to a similar study in 2005-2006, when 11% ((13/115; 95% CI 7-18%) of the foxes was infected. Worm burdens of foxes ranged from 1 to 500-1000 worms per fox, with four foxes having worm burdens higher than 100 worms per fox.

The current prevalence in South-Limburg is comparable to the prevalence found in endemic areas in Central Europe, where the parasite has been present for many decades. The high prevalence will increase the zoonotic risk. Predictive models about the number of cases of AE will be adapted with this new prevalence to estimate the public health risks. Interventions measures need to be studied to determine the best control strategy for E. multilocularis in South Limburg.

Spatial approach of pathogen transmission in wild boar

Hans-Hermann Thulke

Helmholtz Centre for Environmental Research - UFZ, Leipzig, Germany

The pragmatic approach to infectious diseases of wild boar populations refers to large numbers, proportions thereof being infected, and quotas of the population for removal or protection to achieve control aims. This simplified world comprises few dynamics and spatial heterogeneities. Under field conditions the world often is less simple and research is forced to consider the spatially dynamic nature of disease transmission and host ecology. The wild boar occurs ubiquitous across the continents and may harbour a list of pathogens and parasites. Thus the wildlife host allows elaboration of spatial approaches using lessons learned from various incidents in European populations.

We combined GIS data of landscape determinants of wild boar habitat with spatially structured population models to assess the risk associated with reintroduction of the species into depopulated areas in Denmark. We show how the static spatial approach was incomplete when understanding the very nature of Aujeszky infection spreading within East German populations. Using a dynamic approach we predict the most rewarding spatial design for wild boar vaccination against Classical Swine Fever. We illustrate how disregarding the spatial dimension in disease data was misleading our model validation. We highlight how the spatial view on African Swine Fever outbreaks in Russia, together with simulation of hypothetic spread of an infection in real landscapes, may be applied to determine characteristics of an emerging disease.

The spatially explicit and dynamic approach will be visualised with appropriate means.

Nephropathia epidemica in Western Europe: can we improve predictions by including space-time dependence?

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The most common hantavirus in Europe, Puumala virus (PUUV), is specifically hosted by bank voles (Myodes glareolus) and causes a mild form of haemorrhagic fever with renal syndrome, called nephropathia epidemica (NE) in humans. In the last decade, significant changes in incidence patterns of NE have been described in Western Europe. A sudden increase in NE incidence and changes in epidemic periodicity ask for models allowing early prediction of local NE outbreaks in this region. There is however explicit space-time variation in terms of local clusters with varying NE incidence in humans.

Spatial variation in NE risk has been associated mainly with human behavioural patterns, preferred bank vole habitat and local physicochemical or climate conditions that affect both vole ecology and virus survival.

Temporal variation is driven by bank vole population dynamics where peaks in bank vole abundance and consequent PUUV epizootics are followed by NE epidemics in humans.

In the presented study we hypothesize that a single set of explanatory variables can describe the observed space-time patterns found in Western Europe. Hence, we aim to construct an ecological-based regression model for NE, allowing prediction of local NE outbreaks in the coming year throughout the West-European region. A Bayesian Mixed model is used, which is based on long-term NE occurrence datasets from Belgium, France, Germany and the Netherlands where geo-referenced NE cases were registered at NUTS3 or NUTS4 resolution, with varying time series between 2000 and 2012. Explanatory variables (climate, land use, soil texture variables and remote sensing derivatives) have been selected based on previous modelling work and explored in their spatio-temporal dependence structure. First results point out a single set of explanatory variables for the observed spatio-temporal trend in NE occurrence in Western Europe.

Zoonotic transfer of *Chlamydia psittaci* from avian wildlife to staff of a Belgian bird rehabilitation centre

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Chlamydia (C.) psittaci, a gram-negative obligatory intracellular bacterium, is a zoonotic pathogen with a wide host range and worldwide geographical spread. The objective of the current study was to investigate the occurrence of *C. psittaci* infections in Belgian avian wildlife and its zoonotic transmission to staff of a bird rehabilitation centre.

Throat swabs of 10 human caretakers and pharyngeal swabs of 42 randomly sampled birds of 8 orders were examined for presence of viable *C. psittaci* by isolation in buffalo green monkey cells and direct immunofluorescene staining (IMAGEN, DK). All isolates were characterized using an ompA based genotype-specific real-time PCR.

Eleven of 42 wild birds were culture positive. Isolates were characterised as genotype A, B, D, and E/B. Only four infected birds were generally ill and two of seven infected but clinically healthy birds were being trained for release. Viable *C. psittaci* genotype B was detected in 3 of 10 sampled caretakers. A 66-year-old male was sub clinically infected, a 31-year-old male reported sneezing for the past two weeks and a 33-year-old male suffered from sneezing, sore throat, headache, arthralgia, fatigue, fever and light-headedness for 3 days during the last two weeks.

Heightened surveillance and control measures should be implemented in bird rehabilitation centres to prevent zoonotic transmission and pathogen (re)-introduction into the wild. In addition, increased general awareness of the zoonotic risk of *C. psittaci* through contact with avian wildlife is warranted to facilitate early diagnosis and adequate treatment of psittacosis.

Poster presentations

Brucella surveillance in stranded marine mammals from the North Sea

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Brucella infections have been recognized in sea mammals. Two different species, *B. pinnipedialis* and *B.ceti*, have been described in pinnipeds and cetaceans, respectively. Brucellosis in marine mammals is recognized as a zoonotic disease. Three cases of marine *Brucella* infection in humans have been reported without any direct contact with marine mammals. This suggests that the infection may have been acquired by the consumption of see food.

Transmission of *Brucella* in marine mammals is poorly understood, but both vertical and food chain transmission have been suggested. The aim of this study was to evaluate the prevalence of *Brucella* infection among marine mammals, in order to assess the potential zoonotic risk of marine mammal *Brucella* in the North Sea.

A *Brucella* surveillance program of stranded marine mammals on the coast of Belgium, France and Netherlands has been monitored since 1999. A total of 341 organ samples from 131 marine mammals, recovered between 1999 and 2011, have been cultured in Farrell medium for *Brucella* detection. Isolates were identified and typed by MLVA (Multi Locus Variable Tandem Repeats Analysis) on the basis of 16 different markers.

Brucella spp. was isolated in 8.4%, (11/131), of the stranded animals. The isolates were recovered from harbor porpoises (*Phocoena phocoena*) (n=6), harbor seals (*Phoca vitulina*) (n=3) and grey seals (*Halichoerus grypus*) (n=2). As expected, *B. pinnipedialis* and *B.ceti* were detected in pinnipeds and cetaceans, respectively. *Brucella* was mainly found in lungs (n=7) and bronchial lymph nodes (n=4) among the positive animals, showing the importance of these organs as targets for *Brucella* detection. Different genetic profiles were identified by MLVA, demonstrating strain variability of *Brucella* spp. circulating in marine mammals in the North Sea.

Swimmer’s itch in Belgium: first recorded outbreak at the Lacs de l’Eau d’Heure

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Swimmer’s itch is a skin condition in humans due to the larval forms of the bird schistosomes of the genus *Trichobilharzia*. The life cycle of these schistosomes requires freshwater snails and waterfowls. In humans engaged in water activities, repeated exposures to cercariae can lead to skin sensitization with the induction of pruritic skin lesions. Thus swimmer’s itch is considered essentially not harmful but very unpleasant. Consequently, outbreaks can lead to financial losses for touristic recreational areas.

We describe here an outbreak of cercarial dermatitis at the Eau d’Heure dams, Belgium. The Eau d’Heure and Platte Taill dams were built in the 70’s. The Platte Taille dam is used for power supply and consequently the depth of the lake can vary considerably which is not the case at Falemprise. In the early 2000 a recreational area comprising a series of cottages and touristic facilities was built up at the Platte Taille. Two small shallow swimming areas are available for the tourists. On July 27th 2012 the weather was hot and sunny and the swimming area at Platte Taille was crowded. A total of 78 people reported a sudden skin rash accompanied by itching. Initially cyanobacteria (algal bloom) were suspected as aetiological agents and the swimming area was closed.

However, the samples were fond negative for cyanobacteria toxins and the swimming area was reopened on August 2nd. On August 19th there was a second outbreak involving 10 people. No cases were reported at Falemprise. On September 21th several thousand of snails (mainly of the genus *Lymnaea* and *Radix*) were collected, transported to the lab and cercarial shedding was stimulated through light exposure. A massive emergence of ocellate cercariae was recorded at the Platte Taille indicating that the skin condition was due to cercarial exposure. Infected snails and cercariae were kept for molecular identification.

Exotic vector and pathogen surveillance programme in Belgium

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Several vector-borne diseases (e.g. Babesiosis, Bluetongue Virus (BTV), Schmallenberg Virus (SBV), West-Nile Virus (WNV)) are emerging in Belgium or in other European countries and some are now endemic. The increase in international transport, the removal of quarantine measures within the EU zone and the growing tourism contribute to the increasing risk of spread of pathogens and vectors. Strengthening surveillance of exotic vectors (mosquitoes, *Culicoides* and ticks) and their pathogens in areas at risk of importation or spread and risk of virus transmission is therefore required.

Following the implementation of invasive mosquito surveillance in Belgium in 2012, to evaluate the guidelines of the European Centre for Disease Prevention and Control (ECDC), the surveillance continued in 2013 and also included that of exotic biting midges and ticks, and their most important pathogens:

Passive and active sampling of mosquitoes and passive sampling of *Culicoides* from April till November 2013 at shelters for imported cutting plants, fruits and vegetables, main parking lots along highways originating from colonised areas, ports, airports and/or platforms of imported used tyres.

Passive sampling of endemic *Culicoides* from April till November 2013 at a private site in rural area (Betekom).

Active sampling of the invasive tick *Dermacentor reticulatus* (and also of endemic tick species) from January till June and from September till December 2013 at two colonised sites (De Panne & Moen) and at sites where its presence is suspected (Nature Reserves where cattle or horses are imported from colonised sites).

Screening for pathogens in invasive mosquitoes (WNV), exotic and endemic Culicoides (BTV, SBV) and invasive and endemic ticks (*Theileria equi*, *Babesia canis*, *Babesia caballi*, *Borrelia*, Tick Borne Encephalitis Virus (TBE)).

Until now only endemic mosquitoes and biting midges were collected at the points of entry, while the tick *Dermacentor reticulatus* was caught between December 2012 and May 2013 in De Panne and Moen. The screening for pathogens is ongoing. Seven pools (2 to 5 ticks/pool) of *Dermacentor reticulatus* were negative for *Babesia* and *Theileria*.

The Belgian protocol for ranking alien animals: the role of wildlife diseases

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Invasive alien species are species that newly enter a region and spread so rapidly that their presence becomes problematic. The pathogens they carry play an essential role in such invasion events. First, pathogens can act as a driver contributing to the host species' success. Second, the pathogens themselves can directly lead to harm of human health, production loss of livestock, or decline of wildlife animals. Tools that wish to screen the invasion potential of newly emerging alien species therefore need to take wildlife diseases into account. The Belgian HARMONIA system makes such an attempt, but also calls for an increased effort in studying wildlife disease dynamics for alien species.

Transmission dynamics of Borrelia bacteria in an European bird tick community

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We examined the *Borrelia burgdorferi* s.l. circulation in a tick community consisting of three species (*Ixodes ricinus*, *I. frontalis*, *I. arboricola*) with contrasting ecologies, but sharing a common host: the great tit (*Parus major*), one of the most common birds of gardens and woodlands. While this bird is the principal host for the specialized nidicolous tick *I. arboricola*, it is one of many hosts for the generalists *I. frontalis* and *I. ricinus*.

We found evidence that all the three tick species have a potential to contribute to the maintenance of *Borrelia* infections in *P. major*. Field data show that the birds hosted *Borrelia*-infected larvae of both *I. frontalis* and *I. ricinus*, indicating the facilitation of *Borrelia* transmission by the bird. The low, but significant numbers of *Borrelia* in unfed *I. arboricola* ticks collected from bird nest boxes, provide the first field data showing that it is competent in maintaining *Borrelia* over long periods of time. Aside from the known avian genospecies (*B. garinii* and *B. valaisiana*), several less dominant genospecies were observed in all three ticks, including mammalian genospecies (*B. afzelii, B. burgdorferi* s.s. and *B. spielmanii*) and a first record of *B. turdi* for North-Western Europe.

In a laboratory experiment, we show that *P. major* selectively facilitates the transmission of different *Borrelia* strains. We imitated the natural situation during the bird's post-fledging period, in which juvenile birds are repeatedly exposed to infected *I. ricinus* nymphs. Birds developed systemic infections of the avian genospecies, while they showed a low transmission competence for the mammalian genospecies. However, a low number of birds were permissive for *B. afzelii* strains.

In a second experiment, we also show successful *Borrelia* infection and trans-stadial transmission in the bird-specialized *I. frontalis* and *I. arboricola*, however, the infected ticks were not able to transmit the *Borrelia* to a next host.

Seroprevalence of *Mycoplasma gallisepticum* in wild crows and geese in Belgium

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Mycoplasma gallisepticum is the causative agent of chronic respiratory disease (CRD) in chickens. CRD is characterized by coughing, nasal discharge, sinusitis and severe lesions on the air sacs. Economic losses, due to a decrease in weight gain and egg production, are significant. Moreover, breeder flocks that are infected with *M. gallisepticum* have to be culled.

Mycoplasma species are host restricted but infections with *M. gallisepticum*, which occur mostly in chickens, also have been detected in other bird species (reservoirs) such as pheasants, partridges, peacocks, ducks, wild turkeys, and house finches. The aim of this study was to investigate whether there were other reservoirs for *M. gallisepticum* in wildlife. Therefor we analyzed serum samples of wild crows and geese for the presence of antibodies against *M. gallisepticum.*

In 2012, blood samples of wild geese were collected in Belgium. Samples from Canada geese (*Branta canadensis*) and Greylag geese (*Anser anser*) were tested. Samples from carrion crows (*Corvus corone*) were also collected in 2012. We obtained a total of 192 blood samples of acceptable quality to analyze the presence of antibodies against *M. gallisepticum*. Ninety-six samples were from crows and 96 were from geese. Antibodies were detected using a Blocking Enzyme Immunosorbent Assay (Svanovir® MG-Ab). Results were read using a photometer at 450 nm. All samples of both wild crows and geese were negative.

In this study, it seems like wild crows and geese do not play an important role in the spread of *M. gallisepticum* in Belgium. However, further investigation in other wild birds is ongoing. Acks: T. Michiels is supported by the Federal Public Service of Health, Food Chain Safety and Environment (RCO-6752 MYCOPLASMA). The study was funded by the Federal Agency for the Safety of the Food Chain. The authors thank Didier Vangeluwe (Belgian Ringing Centre, Royal Belgian Institute for Natural Sciences, Belgium) for providing the samples.

Spatial descriptive analysis of 2012 winter colony mortality in Belgian honeybees

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Over the last 6 years, increases in honeybee mortality have been reported in several countries within and outside the EU. In 2011, the EC set up and co-financed a standardized and Europe-wide voluntary surveillance program to obtain reliable and accurate measures of honeybee colony losses and health.

The Belgian surveillance program was based on the study by Nguyen et al. (2011), which documented recent Belgian bee-pathogen prevalence. The EU guidelines were adapted for a small-scale Belgian beekeeping scenario. The sampling strategy was multi-stage (apiary/colony levels) and stratified by province: 150 apiaries were selected from a sampling frame of 3000 registered beekeepers. These apiaries were visited twice - in autumn 2012 and spring 2013 - and questionnaires were completed, collecting info on potential risk factors and mortality. Honeybee samples, systematically taken at the first visit, were screened for *Varroa destructor* by mite counts. Subsequently, the samples were then stored (frozen at -80°C) until spring 2013. Once winter colony mortality was recorded at the second visit, stored samples were selected in a case control design (dead versus live colonies) and screened for 6 bee-specific RNA-viruses by MLPA-PCR, as developed by De Smet et al. (2012).

Within ArcGIS® software, several data layers from the apiary visit questionnaires (such as mortality, management practices, and surrounding landscapes) as well as the obtained laboratory test results, will be visualized on maps in relation to the associated spatial data (GPS coordinates, postal codes). Within SatScan® software, the spatial scan statistic will be used to highlight potential clusters in these spatial data.

The available data, procedures and maps will be presented. This descriptive spatial analysis of honeybee winter colony mortality will generate hypotheses for in depth risk factor and GIS analyses.

ESBL-producing *Enterobacteriaceae* isolated from the faeces of Falconidae, Accipitridae and Laridae in bird rescue centres in Belgium

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Although Extended-Spectrum-Beta-Lactamases (ESBLs) and *Klebsiella pneumoniae* Carbapenemases (KPCs) have been investigated widely, little is known about their presence in *Enterobacteriaceae* isolated from wild birds in bird rescue centres in Western Europe.

Faeces, choanal and cloacal swabs of 181 birds (*Columbiformes*, *Passeriformes*, *Charadriiformes*, *Falconiformes*, *Accipitriformes* and *Strigiformes*) were inoculated onto MacConkey agar supplemented with 8mg/l ceftiofur. Colonies suspected to be *Escherichia coli* or *Klebsiella pneumoniae* were purified and identified using biochemical tests and sequencing of the 16S rRNA-gene. To confirm resistance, a disk diffusion test was done using 30µg ceftiofur, 30µg-15µg amoxicillin-clavunate and 15µg imipenem tablets. PCR and sequencing was performed to detect the genes coding for the CTX-M, TEM, SHV and CMY enzymes.

E. coli resistant to ceftiofur, was isolated from the faeces of two *Falco tinnunculus*' and a *Buteo buteo*. A ceftiofur resistant *Citrobacter freundii* was isolated from a pooled faecal sample from a group of gulls (including a *Larus marinus, Larus fuscus* and *Larus argentatus'*). *Bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTX-M-32}}$ genes coded for the resistance. *Bla*$_{\text{TEM-1}}$ and *bla*$_{\text{TEM-135}}$ genes, beta-lactamases, although no ESBL's, were also recovered. Imipenem resistant bacteria were not isolated. ESBL-producing *Enterobacteriaceae* were found in the faeces of a small number of hospitalised birds. Spread of the genes encoding this type of resistance among the enteric microbiota of birds in these centres appears to be limited.

Racing Pigeons: a Reservoir for Nitro-Imidazole-Resistant *Trichomonas gallinae*

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Trichomonas gallinae, the cause of avian trichomonosis, is most commonly found in the order of the Columbiformes. Racing pigeons are often treated preventively with nitro-imidazoles which could result in the emergence of resistant strains. These strains can be a threat to wildlife when exchange of strains would occur.

The sequence type of 16 *T. gallinae* strains isolated from racing pigeons and 15 strains from wild pigeons was determined based on the ITS1/5.8S rRNA/ITS2 region sequence. Besides, the resistance profile of these strains against five different nitro-imidazoles (metronidazole, dimetridazole, ronidazole, tinidazole and carnidazole) was determined.

Two different *Trichomonas* sequence types were isolated. Sequence type A strains were isolated out of racing and wild pigeons, in contrast to sequence type B which was only recovered from wild pigeons. Strains with sequence type B were all susceptible to the tested nitro-imidazoles, except for tinidazole where three resistant isolates were isolated. Resistance to the nitro-imidazoles was more frequently observed in strains isolated from racing pigeons than from wild pigeons, with most strains belonging to sequence type A. A higher percentage of the sequence types A isolated from racing pigeons, in comparison with those isolated from the wild pigeons, were resistant to the nitro-imidazoles and displayed higher MLC values. Two isolates belonging to the same sequence type A, one recovered from a racing pigeon and one from a wild pigeon, displayed a similar resistance pattern. This could be an indication of exchange of resistant strains between racing pigeons and wild pigeons.

This study shows that resistant T. gallinae strains occur frequently in racing pigeons and exchange of these resistant strains between domestic and wild birds can occur.

Antibodies against Schmallenberg virus in Flemish Roe Deer (*Capreolus capreolus*)

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Schmallenberg virus (SBV) infection in domestic ruminants, causing atypical disease in adults and congenital malformations in newborn animals, was first recorded in Belgium at the end of 2011, after the causative agent of the emerging epidemic had been identified in Germany.

SBV is transmitted by insect vectors, biting midges of the genus *Culicoides*. The massive circulation of the virus in 2011 in Belgium was evidenced by a between-herd seroprevalence of almost 100 % in sheep and cattle at the end of the first vector season. In wild cervids, a high seroprevalence was found in Wallonia and in the Netherlands using ELISA and virusneutralization (VNT) tests respectively. Signs of disease are difficult to observe in wild cervids but have not been reported so far.

In Flanders, 112 roe deer sera, collected from 02/2009 to 09/2011 (period 1) and from 01/2012 to 03/2013 (period 2), were tested for SBV antibodies with VNT. All the sera from the period 1 were negative. In the sera from period 2, the percentage of positives (63 %: 95% confidence interval 48-77%) was comparable to that from roe deer sera collected from October to December 2011 in Wallonia (46 %). In Flanders, the circulation of SBV in its *Culicoides* vectors was reported from September 2011 on.

It appears probable that seroconversion in Flemish roe deer took place between October and December 2011, corresponding to the Walloon observations. However, due to the legal hunting periods in Flanders, roe deer sera from October to December 2011 were not available.

Serologic findings in Roe Deer in Flanders

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Roe deer numbers have increased steadily in the last twenty years in Flanders and are estimated at 20,000 with the highest densities in the eastern provinces. Contacts between roe deer and domestic ruminants are probable, due to the small-scale Flemish landscape structure.

A preliminary serologic screening on hunter-collected roe deer sera (N = 131) was carried out from May 2008 to September 2011, in order to detect the circulation of relevant viruses, bacteria and parasites in roe deer. Using different methods, the sera were examined for antibodies to twelve infectious agents (percent positives and suspects in brackets): *Mycobacterium avium* subsp. paratuberculosis (5.22 %; susp. 2.61 %), *Brucella abortus* (0 %), *Coxiella burnetii* (0.87 %), Leptospira sp. (2.54 %), *Chlamydia abortus* (1.65 %; susp. 1.65 %), *Anaplasma phagocytophilum* (56.1 %), Bovine Viral Diarrhoea virus (BVD) (1.85 %), Infectious Bovine Rhinotracheitis virus (IBR) (0 %), Bluetongue virus (BTV) (0%; susp. 3.48 %), Tick-borne Encephalitis virus (TBE) (0 %), *Toxoplasma gondii* (47.50 %) and *Neospora caninum* (6.72%).

Like in Belgian cattle and southern Belgian cervids, paratuberculosis appears enzootic in Flemish roe deer. Except for *A.phagocytophilum ,T.gondii and N.caninum*, exposure to the other pathogens is low or non-existent. No difference in seroprevalence was found between sexes, ages and geographical origin of the sera. The quality of hunter-collected samples and the determination of the cut-off values in roe deer are main challenges for the interpretation of the results. The samples will be further tested for Schmallenberg virus antibodies.

Emergence of the deadly Middle East Respiratory Syndrome coronavirus: are bats to blame?

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The Middle East Respiratory Syndrome (MERS) coronavirus (CoV) is a newly emerging beta-coronavirus that causes a severe acute respiratory infection. The first human cases were identified in April 2012. Up to the 4th of September 2013, 111 cases were identified, with 51 deaths (46%). All cases originate directly or indirectly from the Middle East. Sporadic cases continue to emerge, family or hospital clusters occur, but there is no evidence of sustained transmission. The virus has several characteristics in common with the SARS CoV, which emerged in China in 2002.

It is not possible to predict the future epidemiology. Considering the continuous emergence of sporadic cases, the high genetic variability of coronaviruses and the occurrence of milder or subclinical cases, there seems to be some epidemic potential. Thanks to lessons learned from the SARS pandemic, affected countries have adopted appropriate surveillance, infection prevention and control measures.

The epidemiological pattern suggests a zoonotic source, but the animal vector or reservoir remain unidentified. If the animal source can be located, sporadic transmission to humans may be controlled, reducing the epidemic potential. Hence, the importance to invest in surveillance systems in wildlife and domestic animals.

Data on MERS CoV in animals remain scarce. Cross-neutralizing antibodies have been detected in dromedary camels in Egypt, Oman and Spain, but the virus was not demonstrated. Bats are recognized as the ancestral host of many mammalian coronaviruses, SARS CoV had a bat reservoir and a close relative of MERS CoV was isolated from a *Pipistrellus* bat in the Netherlands in 2008. Related viruses were detected in 15% faecal samples of *Pipistrellus* bats in Ukraine, Romania, Germany and the Netherlands. MERS CoV might therefore have diverged from European bat coronaviruses. A CoV with 100% homology in a 181 basepair fragment was recently detected in faeces of 1 insectivorous bat (*Taphozous perforatus*) in Saudi Arabia.

In Belgium, sick or dead-found bats are collected for rabies analysis. The National Reference Centre of Rabies (WIV-ISP, Brussels) holds a collection of cadavers since 2006. Lungs and intestines are currently being tested for the presence of MERS-like CoV. The work is ongoing, but no positive bats have been found thus far in Belgium.

Pseudorabies virus isolates originating from Belgian domestic pigs and wild boar: genetic characterization and evaluation of their in vitro virulence

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Aujeszky's disease is an economical important disease caused by the porcine alphaherpesvirus, pseudorabies virus (PRV). The disease has been eradicated in domestic pigs in a large part of Europe due to vaccination programs. Serological studies however show that the virus is still present in wild boar.

To get first insights in the variability and virulence of Belgian wild boar strains, five PRV strains were genetically characterized and their in vitro virulence was compared with strains isolated from Belgian domestic pigs and the virulent NIA3 strain.

Genetic characterization by BamHI restriction fragment length polymorphism (RFLP) analysis showed that all wild boar isolates and three of the oldest domestic swine isolates have RFLP pattern type I, typically seen in wild boar and older PRV strains. The other domestic pig strains were characterized as type II, commonly found in European domestic pigs. Phylogenetic analysis based on the sequence of a fragment of the glycoprotein C gene revealed that four wild boar isolates are identical apart from one nucleotide substitution and belong to clade B (Muller et al. 2010). The other wild boar isolate and all domestic pig isolates belong to clade A.

Concerning the in vitro virulence, one step growth kinetics on continuous swine testical (ST) cells and primary porcine cells showed no obvious differences in the in vitro growth capacity between isolates from wild boar and domestic pigs. Also, no difference in sensitivity to interferon alpha and gamma was observed between the different isolates on ST cells.

Our results indicate that Belgian wild boar strains show many similarities with other European isolates and that the in vitro virulence is not reduced when compared to isolates from domestic pigs.