

**Joint Symposium:
Belgian Wildlife Disease Society
&
Dutch Society for Wildlife Health**

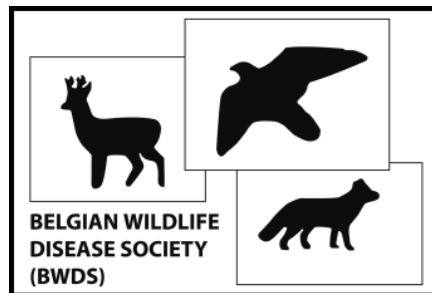
“Wildlife crossing borders”

**Friday the 7th of October 2016
Institute of Tropical Medicine, Antwerp**



Wildlife Crossing Borders

**1st Joint Symposium:
Belgian Wildlife Disease Society
&
Dutch Society for Wildlife Health**



Dutch Society for Wildlife Health

7 October 2016, ITM, Antwerp

Organizing and Scientific Committee:

**Joke van der Giessen, Judith van den Brand, Wim van der Poel, Rogier Bodewes
Paul Tavernier, Stefan Roels, Paul Heyman, Leen Claes, Kristof Baert**

Abstract book edited by Kristof Baert

Introduction: “Wildlife crossing borders”

For the first time, the Belgian Wildlife Disease Society (BWDS) and the Dutch Society for Wildlife Health (DSWH) organize a joint symposium in addition to their respective national initiatives.

This joint BWDS-DWHS symposium is an opportunity to exchange ideas with a wide range of colleagues from the Netherlands and Belgium. The symposium focuses on wildlife-related topics with emphasis on mutual, border-crossing problems for very obvious reasons.

Wild animals are known to be both reservoir and target for pathogens capable of infecting domestic animals and humans, monitoring wildlife thus is a crucial factor for safeguarding animal and public health. This pattern is however known to work both ways, i.e. from humans or domestic animals to wildlife.

At the same time, global change and human activities (global warming, agriculture, invasion of previously undisturbed area for residential purposes, etc) are instrumental in triggering environmental changes that result in more favourable conditions for geographical and inter-species pathogen dissemination. A striking example no doubt is the ability for pathogens and their vectors to travel around the world in a matter of hours or days, “hitch-hiking” on the exponential increase of the transport of people and goods. Pathogens and invasive species act here as the proverbial impurity in the human ecological footprint.

Nowadays, humans and their domestic animals as well as wildlife suffer from these increasingly common events. The past two decades we witnessed the emergence of infectious diseases such as acquired immunodeficiency syndrome, multidrug-resistant tuberculosis, avian influenza and tick- and mosquito-borne diseases, which represent a serious threat to human health globally. These diseases are associated with a range of underlying triggering factors including the interactions of zoonotic pathogens between wildlife, domestic animals and the human population.

Veterinarians and conservation professionals from local and national government, clinical practice, zoos, universities, wildlife sanctuaries and students will hopefully benefit from this information-sharing opportunity, including the latest research and wildlife monitoring efforts from both countries.

Paul Heyman
President of the Belgian Wildlife Disease Society.

Wim van der Poel
President of the Dutch Society for Wildlife Health.

Programme

Moderators: W. van der Poel, T. Adriaens, R. Bodewes & J. van der Giessen

09:30 - 10:00 Registration and coffee

10:00 - 10:05 Welcome (Paul Heyman, BWDS & Wim van der Poel, DSWH)

Keynote speech

10:05 - 10:50 Landscapes and infectious disease - where are the boundaries?
(Richard Kock, RVC - UK)

Session: drivers of emerging diseases

10:50 - 11:10 Invasive species (Tim Adriaens, INBO - BE)

11:10 - 11:30 Avian Influenza in migrating birds (Marjolein Poen, ErasmusMC - NL)

11:30 - 11:50 Rabies in bats and wolves (Steven Van Gucht, WIV - BE)

11:50 - 12:30 Spread of Echinococcus multilocularis in foxes in the Netherlands and Belgium (Joke van der Giessen, RIVM - NL & Leen Claes, ITG - BE)

12:10 - 12:30 Poster pitches

12:30 - 14:00 Lunch/Poster session (on site)

Session: specific habitats in relation to conservation biology/ecology

14:00 - 14:30 Infections in Ecosystems (Hans Heesterbeek, FD - NL)

14:30 - 14:50 Toxoplasma in Marine mammals (Norbert Van de Velde, UGent - Be)

14:50 - 15:10 Ecological drivers of Lyme borreliosis (Hein Sprong, RIVM - NL)

15:15 - 15:30 Coffee Break

Session: surveillance WL diseases & public Health

15:30 - 15:50 Wildlife surveillance in Belgium (Annick Linden, Ulg - BE)

15:50 - 16:10 Wildlife surveillance in the Netherlands (Andrea Gröne, DWHC - NL)

16:10 - 16:30 Wild boar: CSF & ASF (Willy Loeffen, CVI - NL)

16:30 Closing remarks, poster awards & drinks

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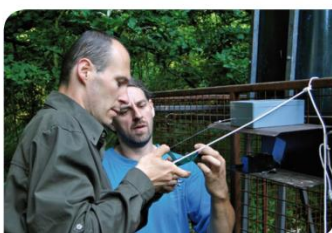
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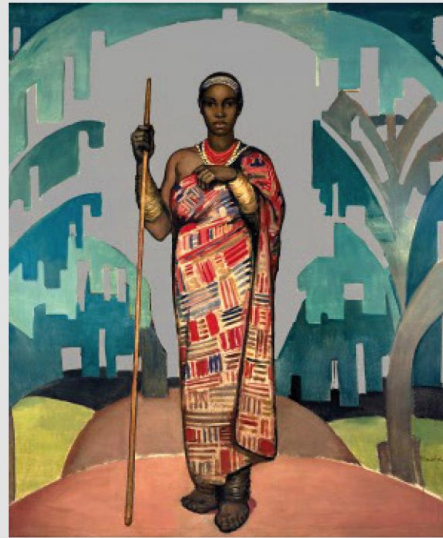
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Oral presentations

Landscapes and infectious disease - where are the boundaries?

Richard Kock

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A decade or more of debate around emergence of infectious diseases and the role of wildlife has helped to grow a discipline hitherto considered esoteric and peripheral in medicine. Can we justify this trend? Can we really aim for a One Health concept with a balance in investment and activity across the health sectors? What is changing in the landscape which predicates this need or desire for more research into the ecologies of diseases and those emerging issues at the interface between species and their environment? Does our current approach to politics, socioeconomics and development and disunion of Nations create or solve disease problems and are secure borders and movement restrictions, the only answer to disease control? These and other critical questions which influence our work in wildlife diseases will be debated in this plenary presentation.

Wildlife pathogens and invasive alien species.

Tim Adriaens

Research Institute for Nature and Forest (INBO), Belgium

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The Convention on Biological Diversity wants priority invasive alien species (IAS) identified, pathways controlled and management strategies deployed. The new EU IAS Regulation provides a framework to protect biodiversity from IAS. For a number of listed species, and more generally at least one quarter of the “world’s worst IAS”, invasion and associated impacts are linked to wildlife diseases. Pathogens may be left behind in the native range, affording enemy release to their host, or they may be introduced and represent a source of pathogen pollution. Risk analysis is used to evaluate the risk associated with species introductions and their pathogens. However, interactions between vectors, hosts, pathogens and the environment are complex. We address gaps constraining our ability to undertake risk assessments and identify risk management options for pathogens of concern. Pathogens need to be better covered in international (IAS) databases and data on prevalence, pathogenicity, virulence and transmission dynamics are needed to assess their risk. Assessing the exposure of native wildlife to novel pathogens is constrained by lack of data on the distribution and likelihood of introduction. Despite their role in mediating invasions, wildlife pathogens are excluded from the IAS Regulation. In contrast with policies on diseases of humans or livestock, policy on pathogens of wildlife is fragmented and characterized by a lack of international cooperation. There is a need to address pathways by increased controls, health surveillance of wildlife imports and wildlife populations, as well as increased biosecurity awareness among all actors in the field. To identify and manage the threats associated with alien pathogens and their vectors, the development of interdisciplinary capacity, expertise and coordination is critical.

Lack of virological and serological evidence for continued circulation of highly pathogenic avian influenza H5N8 virus in wild birds in the Netherlands, November 14, 2014 to January 31, 2016.

Marjolein J. Poen*¹, Josanne H. Verhagen*¹, Ruth J. Manvell², Ian Brown², Theo M. Bestebroer¹, Stefan van der Vliet¹, Oanh Vuong¹, Rachel D. Scheuer¹, Henk P. van der Jeugd³, Bart A. Nolet³, Erik Kleyheeg³, Gerhard J.D.M. Müskens⁴, Frank A. Majoor⁵, Christian Grund⁶, Ron A.M. Fouchier¹

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In 2014, H5N8 clade 2.3.4.4 highly pathogenic avian influenza (HPAI) viruses of the A/Goose/Guangdong/1/1996 lineage emerged in poultry and wild birds in Asia, Europe and North America. To improve our understanding of the role of wild birds in the epidemiology of this virus, more precise knowledge on potential key host species is required. Here, wild birds were sampled extensively for HPAI H5N8 virus (RT-PCR targeting the matrix and H5 gene) and antibody detection (haemagglutination inhibition and virus neutralisation assays) in the Netherlands in a 15-month period following the first virus detection in Europe.

Between February 21, 2015 and January 31, 2016, one HPAI H5N8 virus infected Eurasian wigeon (*Anas penelope*) sampled on February 23, 2015 was detected in the Netherlands (1/7,337). Serological analyses revealed the absence of HPAI H5 clade 2.3.4.4-specific antibodies in wild bird sera obtained before 2014 and the presence of such antibodies in sera obtained during and after the HPAI H5N8 emergence in Europe, with antibody incidence declining after the 2014/2015 winter. Our results indicate that the HPAI H5N8 virus has not continued to circulate extensively in wild bird populations since the winter of 2014/2015 and that independent maintenance of virus in these populations appears less likely.

Rabies in bats and wolves in Europe.

Steven Van Gucht

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Statistics on rabies cases in Europe show a remarkable dichotomy. Whereas the number of cases in foxes and racoon dogs is clearly going down, the number of cases in wolves seems to increase. This probably reflects the fact that the wolf population is expanding since the eighties. About 20-30 rabies cases in wolves are reported per year. Wolves are highly susceptible to spill-over infections from foxes or racoon dogs, but are not a reservoir of rabies.

Rabies in wolves is however not without risk. Wolves develop the furious form of the disease, lose their fear for humans and typically attack numerous people. They travel long distances and can introduce the virus in an unaffected region. Rabies in wolves has not yet been detected in our neighbouring countries, so the risk that wolves introduce the disease in Belgium is low for now.

In Europe, different lyssaviruses circulate in bats of which European Bat Lyssavirus-1 (EBLV-1) and -2 (EBLV-2) are most common. EBLV-1 typically occurs in serotine and EBLV-2 in myotis bats. Affected bats are disoriented, uncoordinated, but only rarely aggressive.

Between 1977 and 2014, 1085 cases of bat rabies were reported in Europe, with 30-40 new cases per year. Belgium seems a lone wolf, since no case has been detected so far. Each year, about 30-40 bats are examined in Belgium, but most of these are from species which are not typical reservoirs.

Spill-over infections from bats to other animals or humans are rare. EBLV-1 has been found in sheep and in a stone marten, but more worrisome were two cases of EBLV-1 in cats in France in 2003 and 2007. In humans, four lethal cases of EBLV-1 or-2 have been diagnosed. Continuous surveillance is warranted since these viruses share similar virulence characteristics with the classic rabies virus and every spill over in another species can be the beginning of a new epidemiological cycle.

Studies on *Echinococcus multilocularis* in a border area of Belgium and the Netherlands: a new hot spot area in Europe?

Joke van der Giessen¹, Leen Claes²

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Echinococcus multilocularis is the cause of alveolar echinococcosis, a severe disease in humans. Increase in red fox populations in many parts of Europe, due to the successful rabies control campaigns, might have extended the parasite distribution. In the Netherlands, *E. multilocularis* was detected for the first time from a population of foxes in 1997 in the northern region of Groningen adjacent to Germany and in the southern region of Limburg adjacent to Belgium. Studies in Belgium have shown a low prevalence of 0-1.7% in the Flanders and Brussels areas of the North, while in Wallonia, the Southern part of the country, the prevalence was found between 20 and 25%. These areas are considered the geographical westernmost border area of the parasite distribution in Europe. Studies in the border areas in Europe might be very interesting to elucidate the dynamics of the infection. Previous studies in foxes in the Netherlands and the Northern part of Belgium showed a spatial distribution in both countries with a comparable prevalence across the borders. In the Netherlands, results of the last survey in foxes east of Maastricht in 2013 showed that there is a steep increase in prevalence compared with a survey in the same region in 2006 from 11% to 59%.

In Belgium, results of a survey in foxes in Flanders in 2011 to 2014 show that the situation has remained unchanged in most of this region with occasional infections in the whole area; however, in Voeren, at the eastern border area with Wallonia and South Limburg in the Netherlands, a very high prevalence (>50%) was found. Combining the results from the Dutch and Belgian studies, there is evidence of the emergence of a new hot spot area of *E. multilocularis* in the eastern border zone of these two countries.

Infections in Ecosystems.

Hans Heesterbeek

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Empirical ecological studies that take parasites and pathogens into account are rapidly increasing in both number and diversity. It is becoming clear that infectious disease agents are influenced by the ecology of all species in their ecosystem, hosts and non-hosts, and their interaction, and that vice versa, the ecosystem is influenced by the dynamics and effects of the pathogens and parasites it contains. There are, for example, effects on biodiversity, ecosystem structure, ecosystem stability and resilience. Despite the many and diverse studies and results, a theoretical context in which to study and understand infectious disease agents in ecosystems and food webs is still in early days of development. I will give a number of examples of ecosystem-infectious agent interaction and give a description of initial attempts to study the interaction between ecology and epidemiology. The ultimate aim of the research is to obtain insight into the role that pathogens and parasites play in shaping and maintaining ecosystems as we observe and experience them.

Toxoplasma in marine mammals.

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Toxoplasma gondii is a zoonotic protozoan parasite which presence has been demonstrated in various marine mammal species. In this study, samples from 631 marine mammals covering 17 species and 34 European otters (*Lutra lutra*), which stranded on the coasts of Scotland, Belgium, the Netherlands and Germany were tested for the presence of *T. gondii*. Brain samples were analysed by Polymerase Chain Reaction (PCR) for detection of parasite DNA. Blood and muscle fluid samples were tested for specific antibodies by the Modified Agglutination Test (MAT), a commercial multi-species Enzyme-Linked Immuno Sorbent Assay (ELISA) and an Immunofluorescence Assay (IFA). Out of 213 animals tested by PCR, two harbour porpoise (*Phocoena phocoena*) cerebrum samples gained from animals stranded on the Dutch coast tested positive. Serology showed a wide variation depending on the test used. Using a cut-off value of 1/40 dilution in MAT, 141 out of 292 animals (41%) were positive. Using IFA, 30 out of 244 tested samples (12%) were positive at a 1/50 dilution. The commercial ELISA yielded 7% positives with a cut-off of the S/N ratio ≥ 50 ; and 12% when the cut-off was set at S/N ≥ 20 . It is possible that the high number of positives in MAT is an overestimation due to the high degree of haemolysis of the samples and/or the presence of lipids. The ELISA results could be an underestimation due to the use of a multispecies conjugate. Our results confirm the presence of *T. gondii* in marine mammals in the Netherlands and show exposure to the parasite in both the North Sea and the Eastern Atlantic Ocean. We also highlight the limitations of the tests used to diagnose *T. gondii* in stranded marine mammals.

Ecological drivers of Lyme.

Hein Sprong

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In order to develop sustainable approaches to minimize the risk of Lyme borreliosis, we aimed to identify environmental drivers which affect the enzootic cycles of ticks and *Borrelia burgdorferi s.l.*. Although *Ixodes ricinus* is a generalist, we found that only a few abundant vertebrate species dominate its life cycle. Whereas roe deer act as its main propagation host, only three rodent species contribute for more than 90% to the feeding of larvae, and for ~40% to the feeding of nymphs. Thrushes contribute for ~40% to the feeding of nymphs. These common rodent and bird species are not only the main contributors to the tick cycle, but also of the *B. afzelii* and *B. garinii* cycles, making the tick and pathogen cycles inseparable. Long-term trend analyses indicated that the length of the annual tick questing season has increased with one month annually since 1985. The surface area of tick-suitable habitats in the Netherlands has increased with ~20% over the last decades as well. Forest conversion from production forest to natural/recreational forest, not only increased the density of infected nymphs, but also the genetic diversity of the Lyme spirochete. Epidemiological and experimental analyses revealed that rodents contribute most to the infection of ticks with *B. afzelii*, *Neoehrlichia mikurensis* and *B. miyamotoi*. Birds contribute most to the infection of ticks with *B. garinii* and *Rickettsia helvetica*. As a result, preferential co-infections occur in ticks, which in turn present co-exposure to, and possibly cause infections in humans. Indeed, we found *B. garinii* and *R. helvetica* co-infections in patients suspected for neuroborreliosis. The vertebrate hosts responsible for the maintenance of *B. spielmanii*, and *B. bavariensis*, have not been identified yet. The development and future implementation of interventions in the tick or *Borrelia* cycle requires long-term and fruitful collaboration with nature reserve owners.

Wildlife surveillance in Belgium.

Annick Linden¹, Muriel Vervaeke²

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In Belgium, wildlife disease surveillance is a regional competence and 3 networks are operative in Flanders, Wallonia and Brussels. These networks aim at (i) detecting new pathogens in wildlife to take adequate control, (ii) evaluating the true extent of present infections to advise the authorities in the decision making process, (iii) evaluating control plans and (iv) providing data for conducting research.

Targeted populations concern mainly wild mammals and birds. Studies are carried out on prioritised pathogens on the basis of impact on public health, rural economy and wildlife conservation. The decision process leading to prioritization and associated surveillance funding are shared by regional and federal authorities. Some targeted studies are regional and other are carried out at a national level (e.g. avian influenza or bovine tuberculosis).

Over the last 10 years, several new or exotic pathogens were detected in Wallonia or Flanders such as bluetongue and Schmallenberg virus in wild cervids, EBHS in hares, RHDV2 in rabbits and *Batrachochytrium salamandrivorans* in amphibians. Our country is actually free of rabies, HPAI, CSF and ASF. A large program of surveillance of bovine tuberculosis is currently in progress in Flanders and Wallonia.

Besides epidemiovigilance, studies are also carried out to evaluate the true extent of present pathogens in wildlife (e.g. Aujeszky disease, brucellosis, paratuberculosis, trichinellosis or echinococcosis). Results are transmitted to regional authorities to implement eventual control measures and notifiable data are transmitted to federal (FASFC) and international (OIE) institutions.

In the future, the array of partnerships should be extended and some priorities have to be targeted to anticipate new infectious zoonotic threats, especially in the context of vectorial diseases.

Wildlife surveillance in the Netherlands.

Andrea Gröne

Dutch Wildlife Health Centre (DWHC), the Netherlands
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The Dutch Wildlife Health Centre (DWHC) was founded in April 2002 and is since 2008 located at the Department of Pathobiology of the Veterinary Faculty of Utrecht University. It coordinates surveillance of wildlife diseases and pathogens in the Netherlands and thus plays a central role in early warning of wildlife disease. Non-targeted surveillance based on thorough pathological examination is aimed to determine the possible causes of unexplained or unusual mortality of wildlife followed by initiation of further testing, if needed at affiliated institutions (incident investigation) such as the CVI in Lelystad, the RIVM in Bilthoven or Erasmus MC in Rotterdam. In addition, important tasks of the DWHC include collection of all wildlife surveillance information from the Netherlands and provide it to authorities for use in decision-making at a national and international level or for reports to the OIE or EU. Non-targeted surveillance can result in targeted surveillance research projects, often in collaboration with other organizations.

Wild boar: CSF & ASF.

Willie Loeffen

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Classical and African swine fever (CSF, ASF) are both virus diseases exclusive to pigs. In Europe, domestic pigs and wild boar (both *Sus scrofa*) are equally susceptible. In Africa, other pig species, mainly warthogs, are the main drivers of this infection in the wild.

In Europe, between circa 1990 and 2012, CSF was endemic in wild boar in several countries at some time (e.g. Germany, France, and Slovakia). Eradication was ultimately successful with the help of bait vaccination. For the last few years, CSF has not occurred in any wild boar in the EU, with the exception of a few outbreaks in Latvia in 2015.

ASF was endemic on the Iberian peninsula between 1960 and 1995. In this period outbreaks occurred in several European countries, including one in Belgium (1985) and the Netherlands (1986). Before and after that, the disease was mainly restricted to the African continent. In 2007 it was, however, introduced in the Caucasus, moving north from there into Russia and entering the EU in 2014. The virus is assumed to be mainly man-driven, through (illegal) transport of infected pigs and swill feeding. As such, infections in wild boar were initially seen as spill-over from infections in domestic pigs. Ever since the virus entered the EU, in 2014, evidence is pointing more and more towards independent circulation of the virus in wild boar populations. Human behaviour (hunters, feeding of wild boar) is still considered a major factor.

Wild boar are a relatively minor species in the Netherlands. Only two nature reserves (Veluwe and Meinweg) are supposed to harbour wild boar. When wild boar populations increased in size, wild boar moved into the "forbidden" areas from the nature reserves or from abroad. In the Netherlands, wild boar have been under active surveillance for CSF ever since the 1990's, while ASF was included in the surveillance in 2014. As long as this surveillance is carried out, no CSF or ASF positive wild boar have been detected.

Poster presentations

Abdominal mass due to *Echinococcus multilocularis* in a two and half years old hard haired dachshund.

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An abdominal mass was detected in a two and half years old male hard haired dachshund. This mass was situated in the left part of the cranial abdomen and seemed evolutive. No other clinical signs were recorded except exhaustion following exertion, and soft faeces. Abdominal echography showed a hepatomegaly with a wide histological modified aspect, multiple hyperechogenic spherical important masses (till 5.15 x 5.7 cm) with fluidic anechogenic central zone and mineralised points. Microscopical examination of smears from hepatic mass and fluid puncture revealed paucicellular samples with protoscolex suspect elements. In front of the importance of the hepatic lesions, euthanasia was realized. Necropsy revealed a severe multilocular chronic active hepatitis with a beginning of right ventricular dilatation. Bacteriological cultures of the mass (Columbia agar plates with 5% sheep blood and specific agar plates for Enterobacteriaceae) were negative. The screening of the intestinal tract for parasites was negative, probably because of previous treatment (milbemycin oxime, praziquantel; Milbemax®). Based on histopathology (numerous multilocular degenerating cysts lined by hyaline membrane, containing eosinophilic debris, mineralized granular material, and one parasitic structure partially fitting with a protoscolex) a diagnosis of cystic hydatidosis due to *Echinococcus multilocularis* was done. The dog was living in the Ardennes region in Belgium (Hastière) known as endemic area of *E. multilocularis*. The high density of foxes (*Vulpes vulpes*) probably explains the occurrence of the disease. Indeed, 1 of the 5 (20%) intestinal scraping of foxes found dead in this area in 2016 was positive for *E. multilocularis*. Fox faeces coprophagia is probably the best explanation for this dog cystic hydatidosis.

The role of birds and bird ticks in transmission of *Borrelia*: a community perspective.

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The principal European vector for *Borrelia burgdorferi s.l.*, the causative agents of Lyme disease, is the host-generalist tick *Ixodes ricinus*. Almost all terrestrial host-specialist ticks have been supposed not to contribute to the terrestrial *Borrelia* transmission cycles. Through experiments with blackbirds and great tits (competent *Borrelia* reservoirs), we tested whether two abundant European bird-specialized ticks, *I. arboricola* and *I. frontalis* act as competent vectors in the *Borrelia* transmission cycles. While the widespread *I. frontalis* shares virtually all terrestrial songbirds with *I. ricinus*, the tree-hole tick (*I. arboricola*) only infests songbirds that breed and roost inside cavities (e.g. great tits).

In the first phase of the experiments, we obtained *Borrelia*-infected ornithophilic nymphs by exposing larvae to birds that had previously been exposed to *Borrelia* from the wild. For all bird and tick species, we had records of successful host-to-vector transmission, and larva-to-nymph transmission over moulting (i.e. transstadial transmission). In the second phase, *Borrelia*-naïve birds were exposed to the infected ornithophilic nymphs. We showed successful transmission of *B. turdi* by *I. frontalis*. Neither *B. garinii*, nor *B. valaisiana* was vectored by any of the bird-specialized ticks. In contrast, *I. ricinus* successfully transmitted all avian *Borrelia* genospecies mentioned above. European enzootic cycles of *Borrelia* between songbirds and their ornithophilic ticks do exist, with *I. ricinus* breaking the cryptic ornithophilic *Borrelia turdi* cycles, potentially acting as a bridging vector towards mammals, including man.

The role of *Vibrio tapetis* in the development of skin ulcerations in common dab (*Limanda limanda*).

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Introduction: An increasing prevalence of skin ulcers in flatfish in the North Sea is noticed. The cause is hitherto not known. However, we have isolated *Vibrio tapetis* as a pure culture from several lesions. The aim of this study was to elucidate the role of *V. tapetis* in the development of skin ulcers.

Materials and Methods: On the pigmented and non-pigmented skin of 60 individually tagged wild-caught dab three adjacent areas were demarcated: a zone where skin was descaled; a zone where mucus was removed and an intact zone. The order of the three treatments was randomized for all individuals. Immediately thereafter, a group of 36 dab was challenged with *V. tapetis* by immersion (3,28 x 10⁵ colony forming units/ml) during one hour. The other 24 animals were sham treated (controls). Fish were daily monitored for clinical signs and gross lesions during 21 days. In animals that died or have been euthanized, gross lesions were scored. At necropsy, samples were taken for histological, immunohistochemical and bacteriological examination.

Results: In the challenged group, significant more animals died compared to the controls. Lesions were significantly most severe in descaled areas and in challenged dab. Preliminary results of bacteriology and immunohistochemistry confirmed the presence of *V. tapetis* in the lesions.

Conclusions: Our preliminary results point towards *V. tapetis* possibly having an etiological role in the development of skin ulcerations in dab subsequent to prior skin damage. However, this research hypothesis needs to be verified by means of the results of the on-going analyses.

Occurrence of the new pathogenic variant of *rabbit haemorrhagic disease virus (RHDV2)* in wild populations of rabbits in Southern Belgium.

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Abstract: Rabbit haemorrhagic disease (RHD) is a highly infectious and fatal disease of the European rabbit (*Oryctolagus cuniculus*), responsible for important economic losses in the rabbit industry. The aetiological agent of the disease is a RNA virus (RHDV, *Lagovirus*, *Caliciviridae*) first detected in China in 1984. Phylogenetic analyses of RHDV strains have identified 3 distinct groups : the classic RHDV, the variant RHDVa and RHDV2. This latter has been detected in France for the first time in 2010 in domestic and wild rabbits and since then has spread throughout Europe, replacing the circulating RHDV/RHDVa strains in most european countries. RHDV2 has already been detected in Belgium in rabbitries. Here, we report for the first time the presence of RHDV2 in wild rabbits in Southern Belgium. In november 2015, the Surveillance Network of Wildlife Diseases received seven dead wild rabbits for necropsy (Faculty of Veterinary Medicine of the University of Liege) according to a systematic protocol based on gross lesions, histopathological and targeted microbiological analysis. Examinations of the carcasses showed congestion of lungs/kidneys and livers were macroscopically normal. Histopathological examination revealed haemorrhagic lung lesions in one animal while 5 of them presented severe necrotic hepatitis. Samples of livers were sent to Scanelis Laboratory (Toulouse, France) for RHDV RT-qPCR diagnostic. The results were positive for the new variant RHDV2 in 5 out of the 7 rabbit livers. Samples were negative for the classic RHDV. For a retrospective study, we tested a series of livers that had been collected in 2013 and 2014. Among the 25 rabbit livers checked, 12 presented necrotic hepatitis and were sent for analysis. Ten were confirmed positive by RT-qPCR for RHDV2. In conclusion, this is the first report which confirms the presence of RHDV2 in wild populations of rabbits in Southern Belgium. Additional data are needed to strengthen the epidemiological picture of RHDV2.

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Notes

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