Abstract Book

7th Symposium of the Belgian Wildlife Disease Society

Foodborne Pathogens & Wildlife

Friday 19 October 2018

VAC Herman Teirlinck - Havenlaan 88 - Brussels

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Foodborne Pathogens & Wildlife

7th Symposium of the Belgian Wildlife Disease Society

BELGIAN WILDLIFE
DISEASE SOCIETY
(BWDS)

19 October 2018, VAC Herman Teirlinck, Brussels

Organizing and Scientific Committee:

Paul Tavernier
Stefan Roels
Leen Claes
Kristof Baert

Introduction “Foodborne pathogens & wildlife”

Living creatures need input of energy and essential nutrients to survive. Such a fundamental mechanism for maintaining life is inseparably related to the evolutionary ecology of species in all its diversity. Moreover, often a specific way of feeding provides supplementary ecoservices such as for example the dispersion of plant seeds by birds. Nevertheless the natural rule “to eat and to be eaten” involves consequences, not only for those organisms that are eaten but also for those who eat. Infections from sick or healthy prey animals can be transmitted to their predators, as well as parasites for which these transmissions are often an obligatory part of their natural cycles. In a broader context, the subject “food-borne hazards” goes far beyond the scope of infections through predator/prey relationships. Consuming plants contaminated by survival stages of certain pathogens can harm herbivores, chemical pollutants can cause food-borne intoxications, and environmental conditions can make feeding very risk bearing, think for example about botulismus and cyanotoxins from algal blooms. Furthermore new feeding patterns in the human society carry on new challenges concerning food safety. The promotion of insect consumption offers interesting perspectives for decreasing the ecological footprint of livestock breeding, maybe also for a better balanced way of eating, but who can predict which stages of possibly zoonotic parasites are carried by certain insects ? The risks of eating undercoocked game meat is another example. Will we slowly discover by trial and error, new hazards by changed feeding habits, in the way many other food-borne diseases have been discovered in the past, or are we able to carry out thorough risk analyses in order to avoid possible casualties?

The trade and consumption of bush meat has been increasingly exported to Europe and our country appears to be a hot spot for this illicit trade with a very heavy impact on biodiversity as well as on food safety.

During this one day symposium it is impossible to cover the whole range of food-borne hazards in man and animals. Even if we focus on “pathogens”, we are still spoilt for choice among the diversity of topics fitting within this frame. Human food safety is a societal priority, therefore some of the subjects we selected focus on zoonotic food-borne pathogens originating from wildlife. Next, some current topics about food-borne hazards for other living beings than humans will be discussed, often they include important consequences for biodiversity. Last but not least we will address the most recent facts about the African swine fever outbreak in wild boar in Southern Belgium.

With this 7th BWDS symposium we strongly hope to contribute to increasing the level of “One Health” thinking, by inviting you to approach human and animal food safety not as an isolated subject, but as a part of the whole, more precisely as a consequence, of complex interactions between ecological and human factors.

Paul Tavernier

Programme

09:00 - 09:30 Registration and coffee
09:30 - 09:35 Welcome BWDS (Stefan Roels – BWDS – Belgium)
09:35 - 09:45 Welcome (Maurice Hoffman – INBO – Belgium)
Session 1: moderators Sophie Roelandt & Steven Van Gucht
09:45 - 10:15 EFSA activities on wildlife infectious diseases
(F. Verdonck – European Food Safety Authority – Italy)
10:15 - 10:45 Cases of prion diseases in European wild cervids
(S. Benestad – Norwegian Veterinary Institute – Norway)
10:45 – 11:00 Linking squirrels and hedgehogs with tick-borne diseases in urban settings
(S. Ruyts - Forest & Nature Lab, Ghent University – Belgium)
11:00 - 11:30 Coffee Break
Session 2: moderators Steven Van Gucht & Jef Brandt
11:30 - 12:00 Hepatitis E and other wildlife associated zoonoses
(R. Ulrich – Friedrich-Loeffler-Institute – Germany)
12:00 - 12:30 The effect of neonicotinoids on Bees
(MP. Chauzat – Anses – France)
12:30 - 12:45 Epidemiological study on the impact of in hive pesticide contaminations on bee mortality (N. El Agrebi – UREAR – University of Liege – Belgium)
12:45 - 13:45 Lunch/Poster session (on site)
Session 3: Moderators Jef Brandt & Jean de Borchgrave
13:45 - 14:15 Public Health Risks from Illegally Imported African Bushmeat and Smoked Fish (AL. Chaber – University of Adelaide/Liege – Australia)
14:15 - 14:45 Toxoplasma and wildlife
(P. Dorny – Institute of Tropical Medicine Antwerp – Belgium)
14:45 - 15:15 Coffee Break
Session 4: Moderators Ann Brigitte Cay & Jean de Borchgrave
15:15 - 15:45 Outbreak of African Swine Fever (ASF) in Belgium
(M. Logeot – Federal Agency for the Safety of the Food Chain – Belgium)
15:45 - 16:15 Parasitic zoonoses in wild boar
(I. Vallée – Anses – France)
16:15 - 17:30 Closing remarks & drinks

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Head office

Herman Teirlinckgebouw
Havenlaan 88 bus 73
1000 Brussel
+32 2 430 26 37
info@inbo.be

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.

INBO - The Research Institute for Nature and Forest

Other sites

Gaverstraat 4
9500 Geraardsbergen,
Belgium
+32 54 43 71 11

Dwersbos 28
1630 Linkebeek,
Belgium
+32 2 362 58 10

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.
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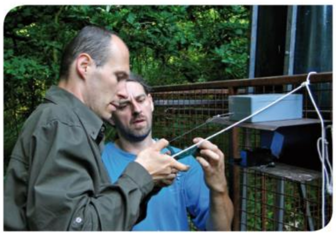

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Oral presentations

EFSA activities on wildlife infectious diseases

F. Baldinelli[1], A. Broglia[1], S. Dhollander[1], C. Fabris[1], A. Gogin[1], A. Ortiz-Pelaez[2], Y. Van der Stede[3], F. Verdonck[1],G. Zancanaro[1]
[1]Animal Health and Welfare team, [2]Biological Hazards team, [3]Biomonitoring team, European Food Safety Authority
frank.verdonck@efsa.europa.eu
The European Food Safety Authority (EFSA) has received several requests from the European Commission in the recent years to provide scientific advice on infectious diseases in wildlife. EFSA is performing risk assessments on the risk of introduction, establishment and spread of transboundary and newly emerged diseases as recently done for *Batrachochytrium salamandrivorans* (Bsal). The situation of Avian Influenza (AI) is monitored using a One Health approach within the Europe and other continents to increase preparedness. For transboundary diseases that occur in more than one Member State, such as African Swine Fever (ASF), regular updates of the epidemiological analyses of outbreaks and risk factor analyses are performed. Literature review, expert opinion and mathematical modelling are used to assess mitigation measures such as fencing, wildlife population management or carcass removal on disease spread. Close collaboration with affected and at-risk Member States is in place to harmonise data collections and exchange practical experiences. This has allowed improving the knowledge on ASF, providing updated scientific advice to risk managers and adjusting the management actions. An animation has been developed and made available on YouTube (with subtitles in many European languages), informing the general public on how to recognise ASF and to prevent spread of the disease. As data on wildlife populations are difficult to obtain, EFSA is funding the ENETWILD project (www.enetwild.com) that initially aims at collecting and harmonizing data on the geographical distribution and abundance of wild boar across Europe. The population data are necessary for outbreak analyses as well as surveillance design and analysis of surveillance results. An online (risk-based estimate of system sensitivity, RiBESS) tool has been developed to substantiate absence of *Echinococcus multilocularis*. Since the confirmation of the first case of CWD in Europe in 2016, in a wild reindeer in Norway, EFSA has been involved in providing support and scientific advice to the EC. Two scientific opinions have been published so far, in 2017 and 2018 respectively, covering the following areas: a proposal of surveillance for a limited number of European countries; the possible public health risks; animal health risk-based measures to prevent the introduction and spread of CWD into/within the EU cervid populations; and the review of the diagnostic methods for CWD in place. Equally EFSA is strengthening the efforts to collate CWD surveillance data from EU and EEA countries and include them in the European Union summary report on surveillance for the presence of transmissible spongiform encephalopathies (TSE), published every year. EFSA is also providing guidance on making surveillance in wildlife more efficient, for instance for AI, by updating the list of target wild bird species and suggesting to focus active sampling at a few priority locations within Europe. This overview shows that many activities related to wildlife disease are ongoing in EFSA. Improving data sharing and providing tools and scientific advice to Member States and data providers needs to be continued to further increase the usefulness and quality of EFSA's scientific outputs.

Cases of prion diseases in European wild cervids

SL. Benestad[1], K. Madslien[1], T. Vikøren[1], C. Das Neves[1], L Tran[1], Ø. Kolbjørnsen[1], H. Wisløff[1], T. Moldal[1], L Pirisinu[2], G. Vaccari[2], J. Bian[3], JA. Moreno[3], S. Kim[3], T. Spraker[4], O. Andreoletti[5], GC Telling[3],R Nonno[2], J Våge[1]
[1]Norwegian Veterinary Institute, Oslo, Norway
[2]Instituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy
[3]Colorado State University, Fort-Collins, CO, USA
[4]Colorado State University, Prion Research Center and the Department of Microbiology, Immunology & Pathology, Fort Collins, CO USA
[5]INRA, Ecole Vétérinaire, Toulouse, France
sylvie.benestad@vetinst.no

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) in cervids. TSE are prion diseases, like scrapie in small ruminants, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt-Jakob disease in humans.
Detected in USA (Colorado) for the first time in 1967, CWD is now diagnosed in captive and free-ranging cervids (mule deer "*Odocoileus hemionus*", white-tailed deer "*Odocoileus virginianus*", elk "*Cervus Canadensis*" and moose "*Alces alces*") in 24 American states and two Canadian provinces. CWD is still spreading despite considerable efforts to restrain the disease. CWD has also been diagnosed in red deer ("*Cervus elaphus*") and sika deer ("*Cervus nippon*") in South Korea as the result of importing CWD infected elk from North America.
The clinical signs of CWD are subtle and vary from case to case. The first clinical signs are nonspecific and include variable behavioral changes such as listlessness, isolation from the herd, lowering of head and ears, repetitive walking and hyperexcitability, followed by weight loss. Other signs are polydipsia/polyuria, hypersalivation and bruxism. The duration of clinical disease varies among individuals, ranging from weeks to months.
CWD is, together with classical scrapie, the most contagious of the prion diseases, transmitted by direct contact from deer to deer, or in some extend from mother to offspring, or indirectly through contact with environment contaminated by feces, saliva, urine or carcass from infected animals.
In April 2016 CWD was diagnosed for the first time in Europe, in a wild reindeer in the Nordfjella mountain area in Southern Norway (Benestad et al. 2016). An enhanced surveillance program for CWD was then launched in Norway. At the time of writing over 42 000 cervids are tested in the country since the first case, and 23 cervids are identified with the disease: 19 reindeer from the Nordfjella area, and three older moose in two subpopulations approximately 300/500 km apart from Nordfjella and one red deer, in a fourth region.
Prion strains have been characterized in most of the species, with distinct biochemical characteristics of PrPRes, including glycosylation profile, electrophoretic mobility, protease resistance and lesion profile in the brain of affected animals. CWD in the Norwegian reindeer show no evident differences with CWD identified in North America and Korea. The Norwegian Ministry of Agriculture and Food has decided the

reindeer depopulation of the Nordfjella area, and slaughter and testing of the 2 200 local reindeer was achieved in May 2018, identifying 19 CWD positive animals.
Biochemical investigations have shown that the Norwegian moose are affected by a CWD type currently not described in North America. The results of bioassay studies in rodents confirm that it represents a novel strain, designated Nor16CWD (Pirisinu et al. EID in press). Bioassay results show that the Norwegian reindeer have a CWD strain distinct from the Norwegian moose and, more surprisingly, that this reindeer strain is also distinct from the CWD strains described in North America. In addition, the case of CWD in Norwegian red deer appears to have previously undescribed characteristics and is currently undergoing further investigations.

Linking squirrels and hedgehogs with tick-borne diseases in urban settings

S. Ruyts, K. Verheyen
UGent, Belgium
sanneruyts@hotmail.com

Tick-borne diseases are a growing public health concern globally as their incidence is rising. In many forests in Europe, contact with ticks is high. Besides in forests, the favourable habitat of the common *I. ricinus* ticks, hosts for ticks also commonly occur in (sub)urban green spaces in closer proximity to humans. We found many common human pathogens, such as *Lyme borreliosis* causing Borrelia genospecies, but also *Borrelia miyamotoi* and *Anaplasma phagocytophilum*, in squirrels and ticks from hedgehogs collected in urban settings. Hence, humans are likely to encounter ticks infected with one or several pathogens while gardening or recreating in parks, not only in forests.

Hepatitis E and other wildlife associated zoonoses

R. Ryll, E. Heuser, S. Fischer, V. Schulze, S. Drewes, R. Ulrich
Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Greifswald - Insel Riems, Germany and German Center for Infection Research (DZIF), Site Hamburg-Lübeck-Borstel-Insel Riems, Germany
rainer.ulrich@fli.de

Hepatitis E, hantavirus disease and leptospirosis are wildlife-associated notifiable zoonotic diseases in Germany. During last years the number of registered hepatitis E cases has steadily been increasing in Germany. Wild boar and domestic pig have been found to be the main reservoirs of zoonotic hepatitis E virus (HEV), but HEV was also detected in other wildlife, including deer and rabbit. HEV strains in rabbits are closely related to swine-derived genotype 3 strains, but formed a separate phylogenetic cluster. Rabbit-associated HEV has recently been detected in an urban area of western Germany. Another HEV genotype (ratHEV) has been identified in rats of different species with a global distribution. The detection of ratHEV reactive antibodies in forestry workers raises questions on its zoonotic potential. The rat-associated HEV and rabbit HEV may represent the basis for the development of novel animal models for evaluation of antiviral therapies.
The majority of hantavirus disease cases in Germany are caused by Puumala orthohantavirus (PUUV) with the bank vole (*Myodes glareolus*) as reservoir. The bank vole is widely distributed in Central and Western Europe. PUUV outbreaks mainly affect western and southern Germany, but also parts of Belgium, France and the Netherlands. In contrast, low numbers of human cases have been recorded in northern and eastern parts of Germany. Serological and molecular analyses demonstrated a usually medium to high PUUV prevalence in endemic areas, but an absence of PUUV in bank voles from northern and eastern parts of Germany. An explanation for this heterogeneous distribution of PUUV is its association with a specific evolutionary lineage of the bank vole and the postglacial recolonization of central and Western Europe from a western refuge. Additional cases of human disease are caused by Dobrava-Belgrade orthohantavirus. Its reservoir, the striped field mouse (*Apodemus agrarius*), is only distributed in the eastern part of Germany, resulting in the absence of the virus in the western part. Additional hantaviruses have been detected in shrews, moles and bats from Central Europe, but with unknown zoonotic potential.
Leptospirosis symptoms and reservoir association to rodents are similar to those of hantavirus disease, but the transmission is associated with water bodies. The number of notified cases in Germany is much lower than that for hantavirus disease, but increased numbers were documented in Lower Saxony and North Rhine Westphalia due to clusters of disease cases in strawberry harvesters in 2007 and 2014. A monitoring study in small mammals indicated the highest Leptospira DNA prevalence in common and field voles (*Microtus arvalis*, *M. agrestis*) and the exclusive occurrence of the genomospecies *Leptospira kirschneri*. Norway rats from Europe were found to be exclusively infected by the genomospecies *L. interrogans*. In contrast, in bank voles

and yellow necked mice three different genomospecies were detected, i.e., *L. kirschneri*, *L. interrogans* and *L. borgpetersenii*.
Lethal encephalitis cases in three humans were recently identified to be caused by a novel zoonotic bornavirus, Variegated squirrel bornavirus 1 (VSBV-1). The virus was initially detected in Variegated squirrels (*Sciurus variegatoides*) from private holdings in Germany, but later also in Prevost´s squirrels (*Callosciurus prevostii*) in private holdings and zoological gardens. Initial investigations of Eurasian red squirrels (*Sciurus vulgaris*) did not detect any infected animals. The detection of closely related VSBV-1 strains in squirrels originating from central America and south-east Asia raises the question on the species and geographic origin of this zoonotic virus.
In conclusion, continuous wildlife surveillance is necessary to monitor reservoir populations and to identify reasons for increased numbers of human cases and disease outbreaks. This surveillance should follow a holistic One Health approach targeting animal, human and environmental health.

The effects of neonicotinoids on bees

MP Chauzat
French Agency for Food, Environmental and Occupational Health & Safety
marie-pierre.chauzat@anses.fr

Pollinator health is receiving increased attention as both managed pollinators and native pollinator populations decline worldwide. Pollinators provide the key ecosystem service of pollination to agricultural crops and wild plants. Pollination comprises an integrated system of interactions that links earth's vegetation, wildlife and human welfare. Although bee research mostly focuses on the domesticated *Apis mellifera*, over 25,000 different bee species have been identified. Bees play a key role in the maintenance of biodiversity and in food and fibre production. Over the past decades, a global trend of increasing honeybee disorders and colony losses has emerged. Several causal mechanisms (including viral pathogens, parasitic mites and pesticides) have been proposed and investigated as contributing causes. Pesticide exposure has received significant attention. Of the many compounds detected, the neonicotinoid group has arguably received the most attention. Neonicotinoids act as agonists on nicotinic acetylcholine receptors, causing persistent excitation of these receptors and eventually death. Neonicotinoids are authorised for a wide range of agricultural and horticultural plants that flower at different times of the year. The systemic properties of neonicotinoids imply translocation to pollen, nectar, and guttation droplets. Wild plants and trees surrounding the treated crops have the potential to be contaminated. This means that pollinating insects are likely to be exposed for much of the year to multiple sources of multiple neonicotinoids in their foraging area, but often at very low doses. Neonicotinoids are highly toxic to honeybees, both when administered orally and by contact. Only a few studies assessed the toxicity to other wild pollinators. Little is known about the real exposure to contaminated food for different categories of honeybees in a colony. For wild bees very few data exist on exposure in the field. The amount that wild bees actually consume in the field has not been measured. As mentioned by EFSA in a scientific opinion on the science behind the development of a risk assessment of plant protection products on bees, the overview of the available studies on sub-lethal doses and long-term effects of pesticides on bees highlighted gaps in knowledge and research needs.

Epidemiological study on the impact of in hive pesticide contaminations on bee mortality

N. El Agrebi, C Saegerman, Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine of Liège, University of Liege, Belgium
nelagrebi@uliege.be

In Europe, colony success depends strongly on the management of the ectoparasitic bee mite, *Varroa destructor*, which historically has been achieved using acaricides that have an impact on honey bee health. Despite honey bees being managed as a domestic pollinator, the impacts of the use of veterinarian pesticides has often been overlooked as a possible factor influencing bee mortality. Honey bees are at risk of being exposed to a broad set of chemicals originating from their environment, inside and outside the hives, from agriculture and from beekeeping practices. In an attempt to better understand the in hive pesticide burden, a pesticide screening (N=186) on the presence of 293 substances was realised in foundation wax by LC–MS/MS and GC–MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE-QuEChERS method and a hazard quotient was calculated. The significant levels of pesticides that are found preserved in wax can be explained by the lipophilic properties of these; the majority of the contaminants are stable and remain unchanged during the process leading to the re-use of the wax by most beekeepers. The pesticide burden in wax was correlated to bee mortality using a logistic regression model. The contamination level found in foundation wax raise our concern. Nevertheless, no correlation was found between pesticide burden in wax and bee mortality. The benefit of pesticides in controlling Varroa infestations should be considered as regard to their toxic effects on bees. Proper diagnosis of Varroa infestation rates should be generalised before using acaricides with parsimony. More efforts are needed in research to characterise the total pesticide burden, which bees and larvae are confronted to in wax, beebread, pollen, nectar, water, honey and propolis.

Public Health Risks from Illegally Imported African Bushmeat and Smoked Fish

AL. Chaber, Y. Lignereux Y, A. Cunningham
University of Adelaide, Australia - University of Liege, Belgium
alchaber@hotmail.com

Concerns have been raised about the illegal import of bushmeat from Africa into Europe, particularly regarding the health risks posed to people and livestock. Bushmeat smuggling was generally assumed to be limited in Europe. We presented the first systematic study of the scale and nature of this international trade and estimate that around five tonnes of bushmeat per week are smuggled in personal baggage through Paris Roissy-Charles de Gaulle airport. We sampled 18 illegal African bushmeat consignments seized at Charles de Gaulle airport, Paris, France and tested for the presence of bacteria. Additionally, five smuggled smoked fish were analysed for polycyclic aromatic hydrocarbons, which are known carcinogens. All bushmeat samples had viable counts of aerobic bacteria above levels considered safe for human consumption. We also identified zoonotic bacterial pathogens in bushmeat and unsafe levels of carcinogens in fish. The illegal importation of meat is a potential risk for the introduction of pathogens. Based on these findings, we suggest ways in which customs, airlines, and airport authorities could reduce imports, focussing on raising awareness of regulations, and improving surveillance and deterrence.

Toxoplasma and wildlife

P. Dorny
Institute of Tropical Medicine, Belgium
pdorny@itg.be

Toxoplasma gondii is among the important food-borne zoonotic parasites globally. Domestic and wild cats are the final hosts; all warm-blooded animals are potential intermediate hosts and may develop tissue cysts in muscles and organs that are infective upon consumption. Meat-producing livestock species, among which are sheep and goats and to a lesser extent pigs, horses and cattle are a source of human infection but meat from wildlife can also be infected. The changing cooking styles of game meat may increase risks for the consumers. Also, meat from wildlife is being traded within the EU and bushmeat is illegally imported, increasing the risk of importing non-endemic strains. In addition, genetic variability of *T. gondii* in wildlife may be higher than in domestic animals, possibly impacting on the virulence of the parasite.

Belgium is among the EU countries with a high seroprevalence of toxoplasmosis in humans. Few data are available on toxoplasmosis in wildlife in Belgium. Decraeye et al. (2011) found 38 out of 73 roe deer (*Capreolus capreolus*) seropositive for *T. gondii*, but 4 fallow deer (*Dama dama*) and 7 red deer (*Cervus elaphus*) were negative. In the brain of wildlife examined by multiplex real-time PCR, Toxoplasma was found in 1/33 samples from roe deer and red deer, and in 57/304 samples from red fox (*Vulpes vulpes*). Genotyping of 26 isolates from foxes revealed that 25 belonged to Type II and one to Type III genotype. Prevalence figures from Belgian wildlife are rather high compared to other European countries. The high demographic pressure in Belgium with a high level of urbanisation, leading to patchy distribution of forests and pasture may allow frequent contacts at the interface between wildlife and domestic animals. Meat from game can thus be considered as a source of infection of *T. gondii* in humans. Of interest is also the evidence of *T. gondii* infection in marine mammals and sporadic descriptions of clinical toxoplasmosis in zoo animals.

African Swine Fever (ASF) outbreak in Belgium

M. Logeot
Federal Agency for the Safety of the Food Chain, Belgium
myriam.logeot@favv-afsca.be

On the 13th of September 2018, the first African Swine Fever case in wild boar was confirmed in Belgium. Until then, we had a distant memory of this virus since we are free from 1985, when there was a short outbreak in domestic pigs in the province of West-Flanders. The new onset in Europe of this epidemic disease, originally from Africa as its name indicates, started in 2007 in Georgia and eventually reached the EU in 2014. In Belgium, it all started suddenly in the small municipality of Etalle in the very southern part of our country. Such as for the other ASF European countries in the beginning, the virus infected the wild boar population. At this moment, the biggest concern is the further dissemination of the virus within the wild boar population and the possible transmission to our domestic pig population. Despite the fact that the virus is only present in wild boar, the presence of this disease in Belgium has already an huge impact on the national market, the intra-community trade and furthermore the worldwide trade in live pigs and pig products. Meanwhile, the different concerned Belgian authorities and stakeholders are aware of the potential and unwanted spread of the disease and are all fully engaged in the collaboration for the common goal of complete eradication of ASF virus in Belgium.

Zoonotic foodborne parasites in wild boar meat: how Trichinella inspection allows identification of other helminths

I. Vallée, P. Boireau, G. Karadjian, B. Polack
UMR BIPAR Anses, ENVA, INRA, Laboratory for Animal Health, Maisons-Alfort, France
isabelle.vallee@anses.fr

When wildlife exhibits a great biodiversity, the risk for wild boars to be infected by parasites is increased. In the past twenty years, *Trichinella spp* was indeed more often identified in wild boars living in natural parks located in south of France or in free-ranging pigs in Corsica. In this island, which was considered as Trichinella free, the circulation of *T. britovi* was analyzed deeply since the parasite emerged in 2004. Different animal species either wildlife or domestic animals in narrow contact with wildlife were analyzed by direct test (artificial digestion) or by serology to detect Trichinella infection. Moreover, epidemiological investigations with hunters and farmers allowed to better understand the factors favorizing the circulation of *T. britovi* in this area.

Thanks to the inspection of wild boar meat regarding the presence of Trichinella muscle larvae, other parasites have been identified in France. *Toxocara cati* larvae were indeed confirmed in wild boar meat using morphological criteria and/or molecular approach based on 18S and ITS2 gene sequencing. The finding of *T. cati* in wild boars, which is a paratenic host of this zoonotic parasite, raises a sanitary question as the consumption of undercooked or raw wild boar meat could be a source of human contamination. At last, *Alaria alata*, a trematode known to circulate in Europe, emerged in East of France in 2004 in wild boar venison. Although *A. alata* is not clearly recognized as zoonotic, its identification requires a sanitary treatment of carcasses as other species of Alaria genus are described as zoonotic.

As the consumption of raw or undercooked wild boar meat is more and more spread as a new culinary habit, the inspection for Trichinella larvae in wild boar venisons should be strongly recommended with hunters as this could avoid foodborne parasitic disease

Poster presentations

Impact of beekeeping management practices on honey bee mortality in Belgium

N. El Agrebi[1], E. Danneels[2], D. de Graaf[2], C. Saegerman[1]
[1]Research Unit of Epidemiology and Risk Analysis applied to veterinary science (UREAR-ULg), Fundamental and Applied Research for Animal and Health (FARAH) Center, Faculty of Veterinary Medicine of Liège, University of Liege, Belgium
[2]Laboratory of Molecular Entomology and Bee Pathology, Department of Biochemistry and Microbiology, University of Gent, Belgium
nelagrebi@uliege.be

Europe and particularly Belgium are strongly impacted by honey bee mortality. Colony success depends also on management of the ectoparasitic bee mite, *Varroa destructor*. Despite honey bees being managed as a domestic pollinator, the impacts of beekeeping management practices has often been overlooked.
In an attempt to understand the impact of bee management practices on bee mortality in Belgium, we correlated the variables obtained from a face to face questionnaire interview, to winter mortality rates. A logistic regression model was built in Stata SE 14.1® using colony losses rate as dependent variable (threshold value of 10% of mortality rate, which is considered acceptable) and questionnaire answers as explanatory variables. A univariate and a multivariate analysis was conducted (odds ratio's with 95% confidence intervals (CI 95%)) completed by a Classification and Regression Tree (CART) Analysis. The sample of Belgian beekeepers was representative, randomized (n=200) and stratified.
In this study, we present the first evidence of a relationship between beekeeping management practices and bee mortality. The results show that the main factors protecting honey bee colonies are the resilience of the beekeepers, the hive type, the equipment use, wintering in proper condition that includes the use of partition, the colony strength, winter check and last but not least, an appropriate integrated pest management.

Proper diagnosis of Varroa infestation rates should be generalised before using acaricides with parsimony. More efforts are needed in beekeeper training programs to promote good beekeeping practices and achieve early identification of clinical signs of disease.

Searching for lagoviruses in Flemish hares

P. Tavernier[1], L. Capucci[2], A. Lavazza[2]
[1]WILDPAD, Polbroek 17, 9520 St-Lievens-Houtem; [2]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (I.Z.S.L.E.R.), Via Bianchi 7/9, 25124 Brescia (Italy)
paul_tavernier@skynet.be

Different lagoviruses are known in rabbits and hares in Europe. Next to EBHSV, also RHDV2 and non pathogenic HaCV have been found in European brown hares (*Lepus europeus*). In Flanders, information about the prevalence of lagoviruses in hares is marginal. We screened livers from healthy hares, hunted in 2004 and 2017.

Liver tissue samples from hares shot at random locations in Flanders were collected *secundum artem* in a game processing plant in 2004 (n=22) and 2017 (n=16), and were frozen at -20°C until analysis. A sandwich typing ELISA based on the use of specific MAbs and capable of distinguishing between EBHSV and RHDV2 with a specifity >98%, was run. One single sample (1/16: 6%) from the 2004 series was EBHSV positive whereas all hares sampled in 2017 tested virologically negative. No other lagoviruses were detected.

Antibodies against lagoviruses were searched on liver homogenates, using IgG-ELISA (cross reactive lagovirus test) and cELISA specific tests respectively for EBHSV and for RHDV2. For the antibody search, the use of liver homogenate instead of serum (when not available) decreases the sensitivity (factor 8-16). This means that samples negative at the first dilution of 1/40 could correspond to a serum titer range between neg-1/320.

The IgG-ELISA on liver homogenates resulted in 50% with titers equal or below the dilution 1/40 for the 2004 series. Conversely, the 2017 series showed 100% positives with medium titers (from 1/80 to 1/1280). By using the more specific cELISA, the 2004 samples showed 32% EBHS antibody positives with titres from 1/10 to 1/80 whereas in 2017 only 12% of sera resulted positive with very low titre (1/10) close to the threshold value.

In hunter shot healthy hares, the absence of ELISA detectable virus was as expected. Nevertheless, the 2004 sample showing a weak positive signal for EBHSV, could reveal a hare having survived acute EBHS at least 4-8 days before shooting. Antibody detection showed a clear difference between the 2004 and the 2017 results: IgG ELISA indicates a general high circulation of lagovirus, possibly a non-pathogenic one such as HaCV, in Flemish hare populations in 2017, but cELISA EBHSV suggests a higher circulation of EBHSV in 2004. Taken together with the EBHSV positive liver, a circulation of EBHSV with a high population susceptibility is probable for the period 2002-2004.

These findings, together with the apparent phylogenetic evolution of lagoviruses in Europe (emergence of RHDV2) underline the need for continued surveillance in order to monitor the population health and immunity of hares and rabbits.

Blood parasites in passeriform birds in Belgium

Paul Tavernier, WILDPAD, paul_tavernier@skynet.be

Information on avian blood parasites in Belgium is scarce. To collect data on their prevalence, 1007 blood smears from passeriform birds were collected from May to November 1996 in a ringing station. After fixation and staining (Haemacolor®, Merck KGaA, Darmstadt, Germany), they were screened by light microscopy at magnification 10x100 using immersion oil. Blood parasites were identified to the species level, using the experience acquired during a training in an avian hematozoa expert centre in South-Africa in 1994. In 2017, due to new insights in the taxonomy of avian hematozoa, we reviewed our early identifications, and converted them to the current taxonomy according to Valkiũnas (2005). Alltogether 9,3 % of the smears contained gametocytes of hematozoa. Among these, the genus most frequently found was *Haemoproteus* (87,2%), followed by *Leucocytozoon* (10,6%), *Trypanosoma* (2,1%) and *Plasmodium* (1,1%). Mixed infections (4,2%) included: two *Haemoproteus* species (1x), *Haemoproteus + Leucocytozoon (*2x), and *Haemoproteus + Trypanosoma* (1x).

Sylvia atricapilla, the bird species with most samples, appeared to be also the most infected one (*H. belopolskyi* 25%), followed by *Sylvia borin* (*H. belopolskyi* 24%, including one mixed infection with *L. majoris), Sylvia curruca (H. belopolskyi 9%*; *L. majoris 9%*), *Fringilla coelebs* (*H. fringillae 10%,* including one mixed infection with *H. magnus; L. fringillinarum 5%*), *Parus major* (*L. majoris 6%*; *P. relictum 2%*), *Turdus merula* (*H. minutus 3%*; *L. dubreuilli* 2%), and *Erythacus rubecula* (*H. fallisi 1%*, *H. neseri 3%*). (Percentages rounded to the unit). No haematozoa were found in *Passer domesticus*, *Prunella modularis*, *Carduelis spinus*, *Cyanistes caeruleus*, *Troglodytes troglodytes*, *Regulus ignicapillis* and *Regulus regulus*. Bird species with less than 10 samples were not included. The sensitivity of light microscopy, if performed properly, is comparable to molecular PCR techniques (Valkiũnas et al., 2008). Moreover, light microscopy allows the identification of avian haematozoa up to the species level. The prevalence of blood parasites depends on a complex mix of factors, including the vector activity (distribution, climate, season, pollution, insecticides), bird age and body size, breeding behaviour (in colony or solitary, nest type and location, duration of stay in the nest), and migration pattern. Migrating birds mix up with local populations, impacting on the prevalence of blood parasites. We found haematozoa in both long- (*Sylvinae*) and short-distance migrators (*Fringilla coelebs*). Bird species in which we found no parasites were non-migrating, except *P. modularis* and *C.spinus* (short-distance i.e. within Europe migration).

Interestingly, *H. neseri*, known only from South Africa, was observed in *E. rubecula*, the morphology matching entirely with the description (average 30 pigment granules/gametocyte, dumbbell shaped gametocytes).

In conclusion, the most frequently found blood parasites in passerine birds in Flanders were *H. belopolskyi* (in *Sylvia spp.)*, *H. fringillae* and *L. fringillarum (*in *F.coelebs)*, and *L.majoris* (in *P. major*). Infections with other blood parasites were marginal (<5%), or inconclusive given the small sample size per species.

Yellow ear: etiology and incidence of pinna injuries in bats in Belgium

Frédéric Forget[1,2], Pierrette Nyssen[1], Nicolas Hougardy[1,2], Paul Tavernier[4], Quentin Smits[1,3]
[1]Natagora, [2]Vivalia, [3]DEMNA, [4]BWDS/WILDPAD
paul_tavernier@skynet.be

The emergence of white nose syndrome has emphasized the need for more knowledge about diseases in bats. During the winter of 2012 we discovered 3 bats with ear lesions. Below, we illustrate our findings. The observed cases prompted us to screen for pinna injuries in bats

Between 29/01/2012 and 13/02/2012, a cold wave hit Belgium. This condition enabled the frost to penetrate in the caves much deeper than usual. Just after the cold wave, in 3 different caves of the area of Rochefort, we observed 3 bats (1 *Myotis bechsteinii* and 2 *Myotis nattereri*) close to the entrances, with external otitis. A thick purulent yellow ear discharge was visible at the tip of the pinnae and was sampled for bacteriological examination. Light microscopy of a Gram-stained smear made from the pus allowed to detect leucocytes and bacteria. The P.A.S. staining method excluded mycosis. The bacteriological culture identified two Gram-negative bacteria, *Serratia liquefaciens* and *Acinetobacter lwoffii*, and a Gram-positive bacterium *Kocuria rosea*.

The bacteria we found are widespread in the environment but are not able to destroy the epidermis. In humans, similar bacteria are found in external otitis but additional etiologic factors are needed for an infection to start. Destruction of the cutaneous barrier, in association with moisture, is a common cause of external otitis. In our case, we assume that a frostbite lesion was secondarily infected by bacteria present in the moist environment of the cave.

Masing (1984) reported that in Estonia, 3.8 % (n=52) of bats hibernating in natural caves had pinnae partially amputated, versus 0.4% (n=260) of bats hibernating in mines. He assumed this difference is due to the fact that mines are deeper than most of the caves, and better protected from the frost.

At the entrance of a cave close to where the described cases were found, capture sessions with mist nets are organized every fall, for biological research purposes. This site is a known swarming site. From 2012 to 2017, the pinnae of the captured bats (n = 1056) were screened for lesions by visual inspection. Overall, 6% of bats showed pinna lesions (males 8%, females 2%). Only *Myotis* species were affected (*M. emarginatus* 11 %, *M. bechsteinii* 10%, *M. daubentonii* 2%, *M. nattereri* 2%).

Pinna injuries in bats are common but are not well described in the literature. Frostbite in combination with bacterial infection is probably a frequent cause of such lesions, but other etiologies are highly probable, particularly for species like *M. emarginatus* which are known to hibernate far from the cave entrance. We have no explanation for the apparent sex ratio difference in the occurrence of these injuries.

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