4th Symposium of the Belgian Wildlife Disease Society

Consequences of wildlife introductions

Friday 7 October 2011

Veterinary & Agrochemical Research Centre, Tervuren

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Organising Committee: Paul Tavernier, Stefan Roels, Paul Heyman, Alexandre Dobly, Kristof Baert, Leen Claes

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BELGIAN WILDLIFE DISEASE SOCIETY (BWDS)

"Consequences of Wildlife Introductions"

Fourth Symposium of the Belgian Wildlife Disease Society

7th of October 2011,
CODA-CERVA
Veterinary and Agrochemical Research Centre, Tervuren

Bayer HealthCare Animal Health

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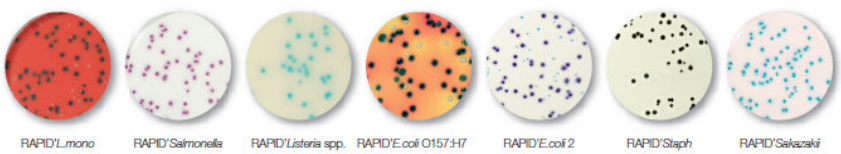

RAPID'*L.mono* RAPID'*Salmonella* RAPID'*Listeria* spp. RAPID'*E.coli* O157:H7 RAPID'*E.coli* 2 RAPID'*Staph* RAPID'*Sakazaki*

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[1]Otranto D, de Caprariis D, Lia RP, et al. Prevention of endemic canine vector-borne diseases using imidacloprid 10% and permethrin 50% in young dogs: a longitudinal field study. *Vet Parasitol.* 2010;172(3-4):323-332.

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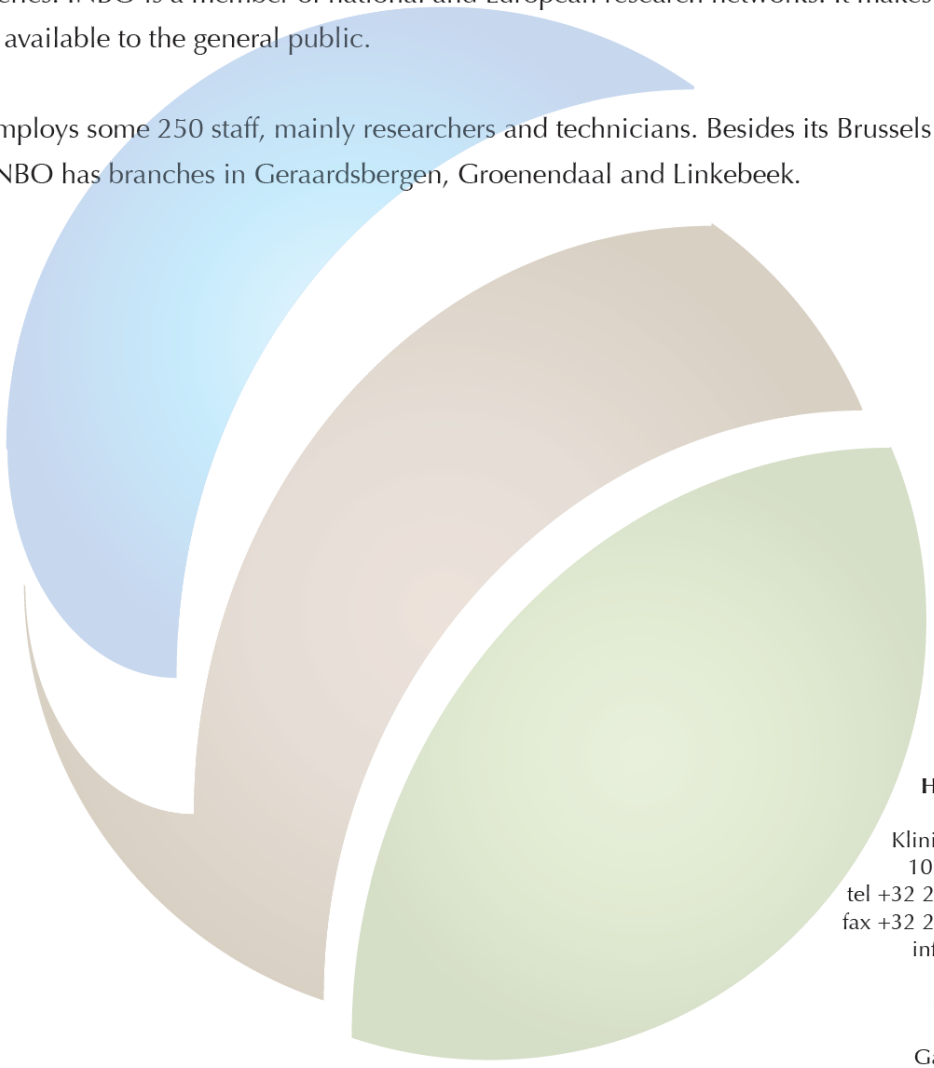

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Program

09:30 Welcome
P. Kerkhofs (General Director VAR - Be)

09:35 Opening
P.P. Pastoret (OIE - Be/Fr)

Session 1: Alien threats
Chairmen: S. Roels & T. Adriaens

09:55 Invasive species
E. Branquart (SP Wallonia / BBPF - Be)

10.25 Exotics and pathogens in the UK
V. Simpson (WVIC - UK)

10:55 – 11:20 Coffee Break

11:20 Exotics and pathogens in France
M. Moinet (J.Barrat) (SAGIR - Fr)

11:50 Risk assessment in reintroduction projects
R.Vaughan (ZSL- UK)

12:20 – 13:45 Lunch with Poster session (on site)

Session 2: Exotic species and endemic pathogens
Chairmen: K. Baert & S. Roelandt

13:45 Borreliosis in chipmunks
M. Marsot (INRA - Fr)

14:15 Echinococcosis in muskrats
J. Stuyck (INBO - Be)

Session 3: Introduced new pathogens
Chairmen: P. Heyman & L. Vanholme

14:45 Chytridiomycosis in frogs
F. Pasmans (UGent - Be)

15:15 – 15:40 Coffee Break

15:40 Aphanomycosis in crayfish
R. Cammaerts (SP Wallonia / ULB - Be)

16:10 Introduced fish pathogens
H. Verreycken (INBO - Be)

16.40 Fascioloides magna in red deer
K.Erdelyi (CVI - Hu)

17:10 Closing remarks & poster awards
L. Claes (ITM - Be)

17:30 End of the day and Coffee

Welcome address: *"Consequences of Wildlife Introductions"*

The fourth Symposium of the Belgian Wildlife Disease Society is focusing on introduced wildlife & pathogens. The BWDS is an independent non-profit working group, uniting scientists and practitioners in order to promote research and to exchange information in the field of wildlife diseases in Belgium. The BWDS is open to veterinarians, biologists, ecologists, bio-engineers, and others actively working in this field. In continuation of the preceding symposia (2005, 2007 & 2009), the fourth edition will explore the ecological and disease risks involved in the introduction of wildlife species

Alien Invasive species issues related to wildlife and domestic animal trade

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The definition of an alien invasive species depends on the viewpoint of the observer, who in some cases may be responsible for introducing the species. History has taught us that humans are the species that has invaded the largest surface area of the planet. It has become increasingly evident that, since the outset, humans have not travelled alone. They have always carried with them their parasites and commensals, their food and ornamental crops and their favourite animals, with the list growing longer and more diverse with each successive millennium.

Gradually certain species came to be labelled universally as “useful” and began to be developed anywhere the environmental conditions were suitable, whereas others became known as “harmful”, irrespective of their biological reality.

The consequences of the invasion by alien species concern many fields including agriculture, animal and public health and biodiversity. It is an issue that affects all regions of the world to a greater or lesser extent. It can have detrimental effects on animal health and biodiversity. For example, the International Union for the Conservation of Nature (IUCN) reported that 625 (51%) of known endangered species are threatened because of invasive (alien) species.

Both wild and domestic animals may be invasive, with detrimental effect on local biodiversity; for instance the introduction of the red fox in Australia for hunting purposes had disastrous effect on some species of autochthonous marsupials. The introduction of goats on the Galapagos Islands had also a negative impact on the native fauna and more recently, the introduction of African sciuridae as new pets in USA imported Monkeypox. All the issues of alien invasive species related to wildlife and domestic animal trade will be discussed.

ORAL PRESENTATIONS

Invasive alien vertebrates in Belgium: risk assessment and pathogen pollution

Branquart E[1], Vanderhoeven S[2]

[1]Invasive Species Unit, General Directorate for Agriculture, Natural Resources and the Environment, Service Public de Wallonie, Belgium; [2]Belgian Biodiversity Platform, General Directorate for Agriculture, Natural Resources and the Environment, Service Public de Wallonie, Belgium etienne.branquart@spw.wallonie.be

Invasive alien species are increasingly observed in Belgium where they are known to cause strong adverse impacts on biodiversity and ecosystem functioning as well as on human and animal health. In order to help land managers and policy makers in the identification of species of most concern for preventive or mitigation actions (namely action plans, legislative tools and voluntary codes of conduct), a list system of non-native organisms established in Belgium has been developed at the initiative of scientists gathered within the Belgian Forum on Invasive Species (1). Lists are built using a standardised assessment protocol, ISEIA (Invasive Species Environmental Impact Assessment), which allows assessing and categorising exotic species from any taxonomic group according to their invasion stage in Belgium and to their impact on native species and ecosystem functions. This protocol is one of the first national standardised risk assessment tools developed for non-native species (2) and it has been used as a model for the development of other comparable initiatives in Europe (3).

The Belgian list system is based on three different categories as recommended in the European strategy on Invasive Alien Species (4). They are defined according to the severity of impacts on the environment: no negative impact (white list), negative impact moderate or suspected (watch or grey list) and negative impact confirmed (black list). The assignment of a non-native species to one of those categories is assessed by four main criteria defined in the ISEIA protocol and matching the last steps of the invasion process: i) the potential for spread, ii) the colonisation of natural habitats and adverse ecological impacts on iii) native species and iv) ecosystems.

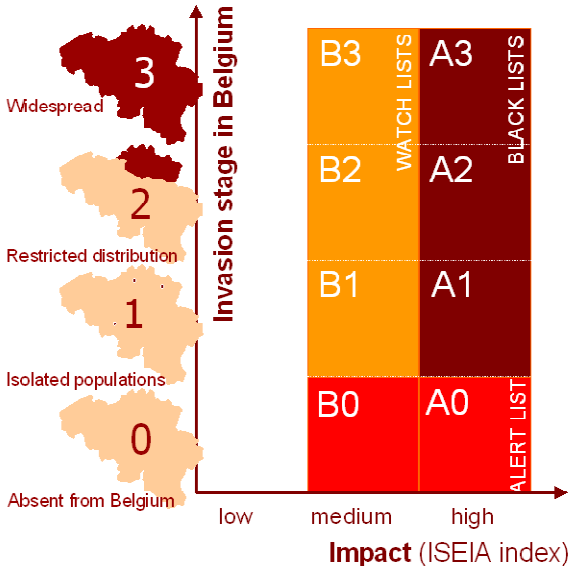

Figure 1 - List system proposed to identify non-native species of most concern for preventive and mitigation actions in Belgium.

Pathogen pollution, i.e. the anthropogenic movement of exotic animals infected by diseases or parasites outside their natural geographic range, is one of the mechanisms that may cause adverse ecological impacts on native species and facilitate their replacement by exotic species (5). Hence, the progressive replacement of the red squirrel by the grey squirrel (introduced in Great Britain from North America) has been favoured by the transmission of a parapoxvirus lethal to the native species (6). Pathogen pollution is known or suspected for most of the 23 vertebrate species included in the Belgian list system of invasive species whatever their taxonomic affiliation (amphibians, birds, fishes and mammals). Specific expertise and research is however needed to properly quantify the threat it may represent to wildlife and human health.

References

1. Branquart E et al. ISEIA, a Belgian non-native species protocol. In : Segers H & Branquart E (Eds), Proceedings of the Science Facing Aliens Conference, Brussels, 2010: 11-18.

2. Verbrugge LNH, et al. Evaluation of international risk assessment protocols for exotic species. Report by Radboub University Wageningen, commissioned by Invasive Alien Species Team, Plant Protection Service, Wageningen, Reports Environmental Science 2010, 352: 54 pp.

3. Parrott D et al. Horizon scanning for new invasive non-native animal species in England. Sheffield, Natural England, 2009: 111 pp.

4. Genovesi P et al. European strategy on invasive alien species. Council of Europe Publishing, Nature and Environment 2004, 137: 70 pp.

5. Daszak P et al. Emerging infectious diseases of wildlife: threats to biodiversity and human health. Science 2000, 287: 443-449; Cunningham AA, Daszak P and Rodrigez JP. Pathogen pollution: defining a parasitological threat to biodiversity conservation. J. Parasitol. 2003, 89: S78-S83.

6. Tompkins D et al. Parapoxvirus causes a deleterious disease of red squirrels associated with UK population declines. Proceedings of the Royal Society of London B, 2002, 269: 529–533.

Exotic species and pathogens in the UK

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Conservationists in the UK are increasingly concerned about the impact that some introduced or invasive species are having on our ecosystem, particularly when they carry pathogens that affect our native wildlife. However, when trying to address the problem of introduced species it is important to remember that many so-called 'native' species were actually introduced, accidentally or otherwise. The British Isles became separated from mainland Europe after the post glacial period, thus preventing natural colonisation. The house mouse (*Mus domesticus*), which originated from India, was introduced during the Iron Age, as was the brown hare (*Lepus europaeus*) which came in via The Netherlands. The Normans introduced rabbits (*Oryctolagus cuniculus*) and fallow deer (*Dama dama*) during the 11C, and the brown rat (*Rattus norvegicus*), originally from Russia, arrived in the 1700s. Later examples include sika deer (*Cervus nipon*) from Japan in 1860, muntjac (*Muntiacus reevesi*) from Asia in the 1890s and grey squirrels (*Sciurus carolinensis*) from North America in 1876. Whilst introductions such as these were deliberate, other species have proliferated after escaping from captivity, either by accident or by default, examples being American mink (*Mustela vison*), Monk parakeets (*Myiopsitta monachus*) and red eared terrapins (*Trachemys scripta elegans*). There is nothing new about exotic or invasive species in the UK; it is just that there are some that we are happy with and others that we see as a problem!

The rabbit was an important human food source in Britain for some 800 years but by the mid-20th century it had become a significant agricultural pest. Therefore, most farmers were delighted when myxoma virus, introduced in 1953, killed 99.8% of the rabbit population. However, the general public were horrified and conservationists were alarmed (1). Much of our natural ecosystem had adapted to this invasive species and the massive population crash caused by myxomatosis had an adverse impact on some predators, such as common buzzard (*Buteo buteo*). It also led, indirectly, to the extinction in Britain of the large blue butterfly (*Phengaris arion*). Over time, the rabbit population slowly recovered but in 1992 they were affected by another exotic disease, Rabbit Haemorrhagic Disease (RHD) (2). The first cases were diagnosed in exhibition rabbits, followed by pet rabbits and

subsequently in the wild population. Although it spread rapidly, mortality was patchy and the overall impact was far less than had been seen when myxomatosis first appeared. The causal agent was a novel calicivirus which had been causing serious losses in farmed rabbits in China. It was thought to have been introduced in to Europe via frozen rabbit meat imported in to Italy.

At about this time farmers were reporting sporadic heavy mortality in brown hares, especially in eastern England (3). What appeared to be the same disease had been spreading through northern Europe and Scandinavia but, as the aetiology was obscure, it had been given the name European Brown Hare Syndrome (4). In due course it was shown to be caused by a calicivirus similar to, but distinct from, that of RHD (5). The origin of the virus has never been determined.

In 1992 a third novel calicivirus appeared in UK wildlife, this time in grey seals (*Halichoerus grypus*). The author had been investigating mortality in seal pups showing severe skin lesions. These proved to be the first documented cases of seal pox in the UK but during the examinations sub lingual lesions were seen and electron microscopy showed that the seals were suffering from a mixed calicivirus and parapox infection (6). The calicivirus was identified as San Miguel Sea Lion Virus (SMSLV), which was of considerable concern to our Ministry of Agriculture as it can cause disease in pigs that closely resembles Foot and Mouth Disease (FMD). This virus is normally restricted to the western seaboard of North America and this was – and I believe remains - the only time it had been recorded in Europe. How the virus arrived in Cornwall is unknown but in the Pacific it is believed to be carried by fish, such as opal-eye perch (*Girella nigricans*). Exotic marine species, such as Chinese mitten crabs (*Eriocheir sinensis*) are thought to have been introduced into our marine environment by ocean-going ships discharging bilge water (7). It is quite possible that SMSLV might have arrived by the same mechanism. Fortunately, it failed to establish itself.

Whilst the introduction of myxoma virus into the UK was probably deliberate, in most cases the introduction of an exotic pathogen owes more to human commercial activities. During the 1980s, farmers in UK were encouraged to diversify and in Cornwall this led to a large number of ponds being dug for commercial coarse fishing. Many of these were stocked with carp - large carp because the bigger the fish the more anglers would pay to catch them. In the summer of 1988 the author investigated heavy mortality in one such carp fishery. Post mortem examinations showed oedema, inflammation and numerous pin point haemorrhages in internal organs, including swim bladder. As the lesions were consistent with Spring Viraemia of Carp (SVC), samples were submitted to the

Weymouth Fish Laboratory where they proved positive. Although SVC had been seen previously in ornamental fish in the UK this was the first outbreak in 'wild' ones. A tracing exercise identified a fish importer's premises as the source of the carp. In the UK, large carp are extremely expensive whereas they are very much cheaper in countries like Czech Republic and Hungary. Although the investigation found no record of large carp being imported, and no offence was proved, it seemed likely that the infected fish came from Eastern Europe - where SVC is common.

There is a huge international trade in fish and other aquatic species but this is an area often ignored by those veterinarians and biologists who study mammals or birds. The danger inherent in this is well illustrated by the appearance in the UK of two diseases, one very serious, and the other apparently less so.

Signal crayfish (*Pacifastacus leniusculus*) from North America were deliberately introduced into the UK in 1976 and bred to supply restaurants. Some escaped from their breeding pools and spread via rivers over most of the country. However, signal crayfish are the principal reservoir of the fungus *Aphanomyces astaci*, the cause of crayfish plague (8). Whilst signal crayfish are not significantly affected, the disease is highly fatal to our native white clawed crayfish (*Austropotamobius pallipes).* Furthermore, the signal crayfish is a voracious omnivore that consumes a wide range of food– detritus, invertebrates, fish eggs and fry, even their own young. It has a high reproduction rate and also makes numerous tunnels into banks, causing them to collapse. Signal crayfish now dominate whole river catchments where they have a pronounced negative impact on the aquatic ecosystem, including its birds and mammals. The introduction of signal crayfish has simply been a disaster.

The second example came to light in 2004 when the author discovered that Eurasian otters (*Lutra lutra*) and American mink in an area of south west England were infected with the bile fluke *Pseudamphistomum truncatum* (9). This is a common parasite of a wide range of carnivores, particularly in Eastern Europe and Russia, but had not previously been recorded in the UK. Carnivores can only become infected by eating infected fish, which act as intermediate hosts. About ten years prior to the infection being diagnosed in otters, two exotic species of fish, sunbleak (*Leucaspius delineatus*) and topmouth gudgeon (*Pseudorasbora parva*), had escaped from an ornamental fish importer and colonised a major water catchment, including the area concerned (10). Both fish species are common in Eastern Europe and Russia and the circumstantial evidence suggests that they were the likely source of the parasite. Early concerns that the parasite might have a negative impact on the

otter population appear unjustified. Post mortem results show that whilst some otters suffer significant liver damage in the early stages of infection, most tolerate the parasite (11). This is also true of American mink and they are probably ideal vectors (10).

American mink which escaped, or were released from, fur farms during the 1960s and 70s bred very successfully and had a major impact on aquatic ecosystems in the UK. The increase in their population coincided with the collapse of that of the otter and as result many people held that mink were responsible, either by competition for resources or as a reservoir of diseases such as Aleutian Disease (AD). However, despite a high prevalence of antibody to AD virus in our mink population (12) there do not appear to be any confirmed deaths due to AD in mink, otters or other small mustelids. We were less fortunate regarding another virus carried by a second species introduced from North America, the grey squirrel.

Grey squirrels were originally considered to be a welcome addition to our fauna but by the mid 20 C it was observed that as their populations expanded those of the native red squirrel declined. Initially this was attributed to the grey squirrel being a more dominant competitor. However, in 1980 a red squirrel was found to have died of a previously unknown poxvirus infection (13). Further studies confirmed that pox is a significant cause of red squirrel mortality and that the virus is carried asymptomatically by grey squirrels (14, 15). The last 50 years has seen the relentless expansion of the grey squirrel population and the collapse of the red. Red squirrels are now extinct over most of mainland England and Wales and the Scottish population is infected and in decline (16).

From the foregoing one might expect that we would have learnt lessons about introducing wildlife species from overseas. Unfortunately, this is far from the case. Most animal importers, whether they are dealing in fish, reptiles, birds or mammals, are only interested in those diseases that might affect their investment; rarely do they have any concern that their stock might be carrying infectious agents that could threaten native species. It is all a matter of self-interest. Biologists might be expected to be more vigilant but there is little evidence of this. In recent years some biologists have been enthusiastically promoting the reintroduction of species such as wolves, lynx and beavers. As a result, a significant number beavers have been imported and are now living a semi wild existence. However, as no meaningful health screen is required, either prior to purchase or after importation, it is quite likely that novel diseases will be introduced. Proof of this came when a beaver that died in a ‘wildlife

park' in 2010 was found to be infected with *Echinococcus multilocularis* – a parasite that we do not have in the UK.

In Conclusion, every imported exotic animal should be seen as a potential Trojan horse! Wildlife veterinarians and biologists have a responsibility to try to explain this to the public, the media and government.

References

1. Bartrip PW. Myxomatosis in 1950s Britain. 20 Century British History. 2008, 19 (1): 83-105.

2. Fuller HE, Chasey D, Lucas MH, Gibbens JC. Rabbit haemorrhagic disease in the United Kingdom. Vet. Rec. 1993, 133: 61-13

3. Duff JP, Chasey D, Munro R, and Wooldridge M. European brown hare syndrome in England. Vet. Rec. 1994, 134: 669-673.

4. Gavier-Widen D, Morner T. Epidemiology and diagnosis of the European brown hare syndrome in Scandinavian countries: a review. Rev. Sci. et Tech. OIE 1991, 10: 453-8

5. Chasey D, Lucas M, Westcott D and Williams M. European brown hare syndrome in the UK: a calicivirus related to but distinct from that of viral haemorrhagic disease in rabbits. Arch. Virol. 1992, 124: 363-370

6. Stack MJ, Simpson VR and Scott AC. Mixed poxvirus and calicivirus infections in grey seals (Halichoerus grypus) in Cornwall. Vet. Rec. 1993, 132: 163-165

7. Herborg L, Rushton MSP, Clare AS and Bentley MG. The invasion of the Chinese mitten crab (Eriocheir sinensis) in the United Kingdom and its comparison to continental Eur. Biol. Invas. 2005, 7: 959–968

8. Alderman DJ, Holdich D and Reeve I. Signal crayfish as vectors in crayfish plague in Britain Aquaculture. 1990, 86: (1), 3-6

9. Simpson VR, Gibbons LM, Khalil LF, Williams JLR. Cholecystitis in otters (Lutra lutra) and mink (Mustela vison) caused by the fluke Pseudamphistomum truncatum. Vet. Rec. 2005,157: 49-52

10. Pinder AC and Gozlan RE. Sunbleak and topmouth gudgeon – two new additions to Britain's freshwater fishes. British Wildlife. 2003, 15: 77-83

11. Simpson VR, Tomlinson AJ, Molenaar FM. Prevalence, distribution and pathological significance of the bile fluke Pseudamphistomum truncatum in Eurasian otters (Lutra lutra) in Great Britain. Vet. Rec. 2009, 164: (13) 397-401.

12. Yamaguchi N and Macdonald DW. Detection of Aleutian disease antibodies in feral American mink in southern England. Vet. Rec. 2001, 149: 485-488

13. Scott AC, Keymer IF and Labram J. Parapoxvirus infection of the red squirrel (Sciurus vulgaris). Vet. Rec. 1981, 109: 202

14. Sainsbury AW and Gurnell J. An investigation into the health and welfare of red squirrels, Sciurus vulgaris, involved in reintroduction studies. Vet. Rec. 1995, 137: (15) 367-70.

15. Sainsbury AW, Nettleton P, Gilray J and Gurnell J. Grey squirrels have high prevalence to a parapoxvirus associated with death in red squirrels. Animal Conservation. 2000, 3: 229-233

16. McInnes CJ, Coulter L, Dagleish MP, Fiegna C, Gilray J, Willoughby K, Cole M, Milne E, Meredith A, Everest DJ, Macmaster AM. First cases of squirrelpox in red squirrels (Sciurus vulgaris) in Scotland. Vet. Rec. 2009, 164: (17) 528-31.

Exotics and pathogens in France

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Throughout history, human activities have always been accompanied by intentional or accidental animal and vegetal movements. Some animals were introduced for utilitarian purposes, not only domestic species, but also a number of wild species used in biological pest control or bred for fur. Others were introduced as game or recreational species. Human activities have also been accompanied by unintentional animal movements, such as synanthropic species including rats and other rodents, or farmed animals accidentally released. The presence of an invasive alien species in a territory can have ecological and faunal consequences, but also sanitary consequences both for humans or native/domestic animal species. Alien species can bring new pathogens leading to the establishment of emerging diseases, or participate in the epidemiological cycle of endemic diseases and enhance the disease. We will present some examples of sanitary threats posed by some invasive alien species in France.

Disease risk analysis for conservation translocations, using case examples

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Disease risk analysis (DRA) is an increasingly important tool being used to assess and manage risks of animal movements including translocation from a disease perspective (1). In particular, DRA assesses the risks that animals will carry new diseases into the destination ecosystem that may cause harm to the destination ecosystem and, that the animals being moved will encounter new diseases in the destination ecosystem and, may be harmed by these new diseases. An advanced methodology for undertaking qualitative disease risk analysis for the importation of animals and animal products to protect domestic animal health has been described (2). This methodology can be extrapolated for use in translocations undertaken for biodiversity conservation to identify and analyse risks and, assess the magnitude of the potential consequences, to the ecology and economy of the destination area and, to the overall success of the translocation program (3,4). However for conservation based translocations, in addition we propose that the possible detrimental effects to biodiversity conservation of any disease agent, not only those capable of causing a disease epidemic (5,6) should be considered. Therefore we investigate i) parasite encounters throughout the translocation pathway, ii) changes in the pathogenicity of parasites carried by the translocated animals triggered by stressors and iii) non-infectious hazards at the destination site. We divide our risk assessment into four components (i) release assessment, (ii) exposure assessment, (iii) consequence assessment and (iv) risk estimation as proposed by Leighton (2002) and Murray (2004)(1,2). Then perform disease risk analysis on identified source hazards, infectious transport and carrier hazards, host-immunodeficiency hazards and infectious and non-infectious destination hazards. The results of the DRA are then discussed with principal stakeholders including government authorities and a decision made to proceed if the benefits of translocation are thought to outweigh the risks. If a decision is made to proceed but significant risks have been identified, management options to mitigate disease risk will then be instigated. However we propose that management options should aim to consider ways in which potentially pathogenic and commensal parasites can be conserved through therapeutic regimes and other control measures, in efforts to ensure that native parasites are maintained in the

translocated population. We demonstrate our comprehensive qualitative DRA methodology using examples from avian and invertebrate translocations in doing so providing a framework for DRA for future conservation based translocations.

References

1. Leighton FA. Health risk assessment of the translocation of wild animals. Revised scientific. tech. Office of International Epizootics (OIE) 2002: 21: 187-195.

2. Murray N. Macdiarmid S.C. Wooldridge M. Gummow B. Morley, RS. Weber SE. Giovannini A. & Wilson D. Handbook on Import Risk Analysis for Animals and Animal Products, OIE. 2004.

3. Canadian Co-operative Wildlife Health Centre. Health Risk Analysis in Wild Animal Translocations. 2011. Available online: http://www.ccwhc.ca/wildlife_health_topics/risk_analysis/rskguidintro.php Accessed 1 August 2011.

4. Davidson WR. and VF. Nettles. Relocation of wildlife: identifying and evaluating disease risks. Transcripts of the North American Wildlife Natural Resource Conference: 1992, 57: 466-473.

5. Griffith B. JM Scott. JW Carpenter and C. Reed. Animal Translocations and potential disease transmission. Journal of Zoo and Wildlife Medicine 1993, 24:231-236.

6. Kirkwood JK. and AW. Sainsbury. Diseases and other considerations in wildlife translocations and releases. Proceedings of the World Association of Wildlife Veterinarians. Symposium on Veterinary Involvement with Wildlife Reintroduction and Rehabilitation. 1997. WAWV, Ballygawley, England pp12-16.

Is the introduced Siberian chipmunk (Tamias sibericus barberi) an amplifying host of the Lyme disease risk in a French periurban forest?

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How introduced species may act as a new host for native infectious agents and modify the epidemiology of a local disease has received little attention. In the Sénart forest, near Paris, we studied the consequences of the introduction of a potentially new reservoir host, the Siberian chipmunk (*Tamias sibiricus barberi*), on the ecology of Lyme disease. This disease is caused by pathogenic bacteria of the *Borrelia burgdorferi* sensu lato complex and is transmitted in Europe by *Ixodes ricinus* ticks. We studied the relative importance of chipmunks on the circulation of *B. burgdorferi* sl in comparison to native reservoir hosts, bank voles (*Myodes glareolus*) and wood mice (*Apodemus sylvaticus*).

First, we determined if chipmunks are competent reservoir hosts for *B. burgdorferi* sl. We demonstrated that, in the Sénart forest, chipmunks could carry and maintain *B. burgdorferi* sl. Then, we tested if the presence of the chipmunk modifies the "acarological risk" for human (density of infected questing nymphs) by comparing sites with and without chipmunks at the period of high questing tick density in May-July 2008. Then we calculated the contribution of each reservoir host to the Lyme borreliosis risk for human. A statistical model was developed to estimate the number of infected nymphs produced per host, which take into account the temporal variability of the parameters. If chipmunks are competent reservoir hosts for *B. burgdorferi* sl, they may spillback *B. burgdorferi* sl to native communities and increase the risk of Lyme disease transmission to humans.

Echinococcus in muskrats

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In accidental cases, humans may get infected by uptake of eggs of the small fox tapeworm, *Echinococcus multilocularis* (EM), probably via contaminated hands after handling infected carnivores, contaminated plants or soil. Human infection can result in alveolar echinococcosis (AE), a rare but serious zoonotic disease, caused by the development of the larval stage (metacestodes) of the tapeworm. The slow growth of the larvae, usually in the liver of an infected person, results in an asymptomatic phase of several years before diagnosis. When left untreated the mortality exceeds 90% within 10 years. Modern treatments have considerably improved survival, but complete cure is still rare. EM is endemic in large parts of Central Europe and there is growing evidence that the parasite may be emerging in Western Europe.

The life cycle of this parasite is mainly based on a sylvatic cycle, with the red fox, *Vulpes vulpes*, as definitive host and several small rodent species as intermediate hosts. The ongoing expansion of the raccoon dog, *Nyctereutes procyonoides*, in Germany and other parts of Europe is however alarming Schwarz et al., 2011; Kauhala & Kowalczyk, 2011). Indeed, raccoon dogs seem to be highly susceptible for EM (Kapel et al., 2006), thrive very well in anthropogenic habitat and live apparently in concordance with foxes. The abundance and prevalence of EM in the natural definitive host are likely to be higher in geographical regions where there is a concomitant high prevalence in alternative definitive hosts. Besides the sylvatic cycle, domestic dogs and cats may be involved in a synantropic cycle. Their infection rates are generally considered to be low. Nevertheless, their importance in human risk assessment can't be neglected.

In several studies, the common vole (*Microtus arvalis*), the water vole (*Arvicola terrestris*) and the muskrat (*Ondatra zibethicus*) are considered as the most important intermediate host species of EM. Their relative importance differs considerably according to the various countries and regions. The

average prevalence of EM in rodents is generally low (<1% to 6%), but in certain foci higher prevalences, up to 40% have been determined. Besides the very important role of the immunological response of an intermediate host in the development of protoscoleces (fertile stage) (Vuitton & Gottstein, 2010; Matsumoto et al., 2010), local features of the environment and high densities of an appropriate intermediate host population seem to be a precondition for hyper endemic spots.

After the first record of EM in the south-eastern part of Belgium in 1991 (Brochier et al, 1992), several studies were devoted to the incidence and abundance of the fox tapeworm in his definitive host, the red fox. These studies revealed a wide distribution of the parasite in Wallonia (Losson et al., 2003; Hanosset et al., 2004; Hanosset et al., 2008; Mathy et al., 2009), while in Flanders only four cases were recorded (Vervaeke et al., 2003).

In a study on 990 foxes, Hanosset et al. (2008) found the highest prevalence of EM in Fagne-Famenne (61.8%), Ardenne (40.8%) and Belgian Lorraine (33.8%), and the lower prevalences Condroz (24.9%) and Hesbaye (10%). These differences between the geological areas of Wallonia matched with their observations on the occurrence of the metacestodes of EM in 1718 muskrats. On the other hand, the study confirmed the very low incidence of EM infection in other microtines. They concluded that EM prevalences decrease from the south-eastern part to the north-western part of the region. Moreover, these data seem to indicate a wider parasite distribution towards the north than previously recorded (Losson et al., 2003). This phenomenon could be related to the migration of infected red foxes to new places potentially suitable to the establishment of the life cycle of EM (Vervaeke et al., 2006). Recent work on the incidence of EM in Flanders' foxes didn't indicate a further increase in distribution towards the north (Van Gucht et al., 2009).

During a long term survey of the impact of management on the metapopulation ecology of muskrats in Flanders, we gathered data on the incidence of EM in muskrats. From 1994 on, muskrats trapped during the regional muskrat control programme were provided by staff members of the Flemish Environment Agency (VMM) on a regular base from all over Flanders. Date and place of capture were noted, together with gender and bodyweight. During necropsy, besides reproduction data, we recorded the occurrence of several metacestode species in the muskrats (Stuyck et al., 1999; Stuyck

et al., 2009). Liver and viscera were examined visually for the presence of cystic lesions. Identification of the parasite was mainly based on the gross appearance of the cysts or the metacestodes. The presence of *Taenia taeniaeformis* was overwhelming, while *T. martis* and *T. crassiceps* were only sporadically found. In June 2008, in spite of having examined 17767 muskrats, no EM metacestodes were detected until then. On 12/08/08 a first case of EM infestation was detected in a muskrat population from Lessine-Ghoy - Wallonia, near the Flemish border (1/182 – 0.55% (95% conf.int. 0.01%-3.02%)). These muskrats were caught during two consecutive trap nights by VMM staff members in the framework of the Interreg IV-C project Cartora (*www.cartora.eu*). The trap area was solely chosen for having a high muskrat density in the region. No environmental or epidemiological features that could have contributed to the finding were deliberately taken into account.

From 2009 on, we recorded at least 96 additional positive muskrats in the western Flemish-Walloon border region, 11 cases are still awaiting confirmation via PCR. Despite the examination of more than 5000 muskrats, no EM infestations were recorded outside this border region, neither in the rest of Flanders, nor in the French-Flemish border zone. Taking into account the differences in muskrat population density and composition, distribution pattern in the landscape (metapopulation structure) as well as differences in sampling, we postulate an expansion of the Walloon EM range into the adjacent Flemish border municipalities. The records on the incidence of EM metacestodes in the muskrat population are a valuable tool to monitor the expansion of the parasite on a local scale and provide additional evidence for risk mapping (Staubach et al., 2011). Whether the continuation of the stringent Flemish muskrat control strategy, resulting in very low muskrat numbers, can halt this EM expansion further northwards, needs to be awaited.

1. Brochier, B., Coppens, P., and Losson, B. (1992) Enquête sur l'infestation du renard roux par *Echinococcus multilocularis* en province de Luxembourg. Annales de Medecine Veterinaire **136**, 497-501.

2. Hanosset,R., Mignon,B. & Losson,B. (2004) Recent data on a current zoonosis: alveolar echinococcus due to *Echinococcus multilocularis*. *Annales de Medecine Veterinaire*, **148**, 153-167.

3. Hanosset,R., Saegerman,C., Adant,S., Massart,L. & Losson,B. (2008) *Echinococcus multilocularis* in Belgium: Prevalence in red foxes (*Vulpes vulpes*) and in different species of potential intermediate hosts. *Veterinary Parasitology*, **151**, 212-217.

4. Kauhala,K. & Kowalczyk,R. (2011) Invasion of the raccoon dog *Nyctereutes procyonoides* in Europe: History of colonisation, features behind its success, and threats to native fauna. *Current Zoology*, **57**, 584-598.

5. Losson,B., Kervyn,T., Detry,J., Pastoret,P.P., Mignon,B. & Brochier,B. (2003) Prevalence of *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in southern Belgium. *Veterinary Parasitology*, **117**, 23-28.

6. Mathy,A., Hanosset,R., Adant,S. & Losson,B. (2009) The carriage of larval *Echinococcus multilocularis* and other cestodes by the muskrat (*Ondatra zibethicus*) along the Ourthe river and its tributaries (Belgium). *Journal of Wildlife Diseases*, **45**, 279-287.

7. Matsumoto,J., Kouguchi,H., Oku,Y. & Yagi,K. (2010) Primary alveolar echinococcosis: Course of larval development and antibody responses in intermediate host rodents with different genetic backgrounds after oral infection with eggs of *Echinococcus multilocularis*. *Parasitology International*, **59**, 435-444.

8. Schwarz,S., Sutor,A., Staubach,C., Mattis,R., Tackmann,K. & Conraths,F.J. (2011) Estimated prevalence of *Echinococcus multilocularis* in raccoon dogs *Nyctereutes procyonoides* in northern Brandenburg, Germany. *Current Zoology*, **57**, 655-661.

9. Staubach,C., Hoffmann,L., Schmid,V.J., Ziller,M., Tackmann,K. & Conraths,F.J. (2011) Bayesian space-time analysis of *Echinococcus multilocularis*-infections in foxes. *Veterinary Parasitology*, **179**, 77-83.

10. Stuyck,J., De Meyer,A. & Tuyttens,F. (1999) Endoparasieten bij de muskusrat. *Jaarlijks rapport over het jaar 1999. Werkgroep voor wetenschappelijk onderzoek inzake landinrichting.Intern rapport.*

11. Stuyck,J., Baert,K. & Pieters,S. (2009) Het voorkomen van de vossenlintworm *Echinicoccus multilocularis bij de muskustrat*. *Jaarverslag 2009 : Onderzoeksgroep Knaagdierbeheer. Intern Rapport INBO.* pp. 29-33. INBO.IR.2009.

12. Van Gucht, S., Van Den Berge, K., Quataert, P., Verschelde, P., and Le Roux, I. (2009) No emergence of *Echinococcus multilocularis* in foxes in Flanders and Brussels anno 2007–2008. *Zoonoses and Public Health*, **57**, 65-70.

13. Vervaeke,M., Dorny,P., Vercammen,F., Geerts,S., Brandt,J., Van Den Berge,K. & Verhagen,R. (2003) *Echinococcus multilocularis* (Cestoda, Taeniidae) in Red foxes (*Vulpes vulpes*) in northern Belgium. *Veterinary Parasitology*, **115**, 257-263.

14. Vervaeke,M., Dorny,P., De Bruyn,L., Vercammen,F., Jordaens,K., Van Den Berge,K. & Verhagen,R. (2005) A survey of intestinal helminths of red foxes (*Vulpes vulpes*) in northern Belgium. *Acta Parasitologica*, **50**, 221-227.

15. Vervaeke,M., van der Giessen,J., Brochier,B., Losson,B., Jordaens,K., Verhagen,R., Coulander,C.D. & Teunis,P. (2006) Spatial spreading of Echinococcus multilocularis in Red foxes (Vulpes vulpes) across nation borders in Western Europe. *Preventive Veterinary Medicine*, **76**, 137-150.

16. Vuitton,D.A. & Gottstein,B. (2010) Echinococcus multilocularis and Its Intermediate Host: A Model of Parasite-Host Interplay. *Journal of Biomedicine and Biotechnology*, Article ID 923193, 14 pages. Doi :10.1155/2010/923193

Chytridiomycosis in amphibians in Belgium and the Netherlands: a doom scenario?

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The fungus *Batrachochytrium dendrobatidis* is a major driver of global amphibian declines. Catastrophic, chytridiomycosis related amphibian declines have been reported mainly from North, Central and South America and Australia and, to a lesser extent, from southern Europe. This presentation summarizes our current knowledge concerning the occurrence and the impact of *B. dendrobatidis* infections in amphibians in Belgium and the Netherlands. We studied three categories of animals: captive, native wild and exotic invasive wild amphibians.

In captive amphibians, chytridiomycosis is one of the most important causes of disease and mortality. Disease outbreaks are most often diagnosed in poison dart frogs (Dendrobatidae) and occasionally in other species, notably lungless salamanders (*Bolitoglossa* sp., Pasmans et al., 2005; Van Rooij et al., 2011). However, we recently showed that the majority of chytrid infections in captive amphibians are subclinical (Spitzen – van der Sluijs et al., 2011). Therefore, captive amphibians should be considered a source of infection for native wildlife and we propose stringent measures such as entry control to avoid spread from captive to native amphibians.

With regard to exotic invasive species, we found a high prevalence of chytrid infections in North American bullfrogs (*Lithobates catesbeianus*) but not in Italian crested newts (*Triturus carnifex*). Apparently, bullfrog populations appear not to suffer from clinical chytridiomycosis, confirming their role as important reservoirs from which the fungus may spread to native amphibian assemblages (Spitzen – van der Sluijs et al., in prep.). Besides chytrid infections, we recently showed that bullfrogs are carriers of ranaviruses, the second most important infectious cause of amphibian declines (Sharifian-Fard et al., 2011). Therefore, the significant risk of pathogen pollution this frog species poses, justifies the current efforts to eliminate *L. catesbeianus* populations.

In native, wild amphibians, chytrid infections are widely distributed over Belgium and the Netherlands, infecting most native species (Spitzen – van der Sluijs et al., in prep.). The first *B. dendrobatidis*

infection in museum animals was found in a natterjack toad (*Pseudepidalea calamita*), collected in 1999. The highest prevalences were noticed in yellow bellied toads (*Bombina variegata*) and in midwife toads (*Alytes obstetricans*). Both species are highly threatened in both countries. The impact of the infection at population level is not clear at present, despite the worrying case of clinical chytridiomycosis in a midwife toad in Belgium in 2010 (Pasmans et al., 2010). Overall, our findings suggest that *B. dendrobatidis* has been introduced fairly recently in Belgium and the Netherlands and that, under the present conditions, the impact on our native amphibian communities appears to be limited. However, a thorough risk analysis is urgently needed to predict the impact of chytridiomycosis on our amphibian communities to be able to take proper control measures where and when needed.

References

1. Pasmans F, Zwart P, Hyatt AD. Chytridiomycosis in the Central American bolitoglossine salamander (*Bolitoglossa dofleini*). The Veterinary Record 2005?, 154: 153.

2. Van Rooij P, Martel A, Nerz J, Voitel S, Haesebrouck F, Pasmans F. Detection of *Batrachochytrium dendrobatidis* in Mexican bolitoglossine salamanders using an optimal sampling protocol. Ecohealth 2011, in press.

3. Spitzen – van der Sluijs A., Martel A, Hallmann C., Bosman W., Garner TWJ, Van Rooij P, Jooris R, Haesebrouck F, Pasmans F. *Batrachochytrium dendrobatidis* is a widespread pathogen in the Netherlands and Belgium. In prep.

4. Spitzen – van der Sluijs et al., in prep.

5. Sharifian-Fard M, Pasmans F, Adriaensen C, Devisscher S, Adriaens T, Louette G, Martel A Invasive bullfrogs constitute a reservoir of ranavirosis. Emerging Infectious Diseases 2011, in press.

6. Spitzen – van der Sluijs A, Martel A, Wombwell E, Van Rooij P, Zollinger R, Woeltjes T, Rendle M, Haesebrouck F, Pasmans F. Clinically healthy amphibians in captive collections and at pet fairs: a reservoir of Batrachochytrium dendrobatidis. Amphibia Reptilia 2011, in press.

7. Pasmans F, Muijsers M, Maes S, Van Rooij P, Brutyn M, Ducatelle R, Haesebrouck F, Martel A Chytridiomycosis related mortality in a midwife toad (Alytes obstetricans) in Belgium. Vlaams Diergeneeskundig Tijdschrift 2010, 79 (6): 460-462.

Aphanomycosis in crayfish

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Crayfish plague is the most dramatic disease affecting crayfish, causing total extinction of non-American crayfish populations. The responsible agent is *Aphanomyces astaci*, a common North American crayfish Saprolegniaceae (Oomycete) water mould, enlisted as one of the 100 worst invasive alien species by the IUCN. Its virulence is a classic example to what happens to naïve species after exposure to an alien aggressive pathogen.

The disease was unknown until a first outbreak appeared in Italy in 1859. By 1910 the epizootic reached most of the continental countries of Europe, due to involuntary transport by man, crayfish being then a valuable fishery. The plague hit our country in the 1880's, voiding in a few years our rivers of their native noble crayfish (*Astacus astacus*) populations. Restocking attempts took place, but from 1910 to 1960, twenty major extinctions of Belgian crayfish river populations occurred, most probably due to plague. These new plague episodes may have been the consequence of the import in 1890 in Germany of the American spiny-cheek crayfish (*Orconectes limosus*), a species which established itself in our watercourses in the years 1960. In the 1970's new plague outbreaks occurred after the introduction of the Californian signal crayfish, *Pacifastacus leniusculus*. More recently, the Louisiana red swamp crayfish (*Procambarus clarkii*) joined its two alien relatives. Nowadays, American crayfish plague carriers have largely invaded Belgium, making it a "chronic area of pest". Few noble crayfish populations thus remain, shielded from the omnipresent threat of plague thanks to continuous efforts of a few persons committed to keep them out of reach of their American relatives. Noble crayfish mass mortalities, when happening, become thus confidential events.

The disease is transmitted by the biflagellate swimming zoospores of the Oomycete. When they reach the integument of a crayfish, they fix to it, lose their flagellae, germinate and form a mycelium which grows into the cuticle and eventually enters the vascular system, invading other tissues. A luxurious fungus growth follows, with hyphae growing outwards of the integument and producing zoospores which further spread the pathogen in water.

Infected indigenous crayfish have abnormal locomotion, phototaxic and defensive behaviour. At the end of the disease, the mycelium extrudes from the eye and limb membranes. Death normally ensues in one or two weeks. Clinical expression of the disease in fact depends on the severity of the spore infection and on water temperature.

North American crayfish do not really suffer from aphanomycosis and this makes them a good material for the study of crayfish immunological defence mechanisms against the fungus plague. The immune defence system of crayfish against fungi consists of a humoral defence (the prophenoloxidase (PO) activating system), which ultimately produces melanin, a fungistatic substance which sheaths the mycelium, and in a cellular defence in which the host hemocytes encapsulate the hyphae, sealing them from the blood circulation and destroying them by melanization and superoxydation.

The defence system of North American crayfish permanently produces high levels of PO as a reaction towards the fungus, with up to 90% of the blood cells continuously acting against the plague parasite. The growth of the fungus is therefore slowed and restricted to some integument melanized spots, the crayfish thus bearing the plague fungi in its cuticle as a benign and chronic aphanomycosis which may persist for years. Parasite and host are locked in a standstill: the host cannot produce enough toxic substance to overrun the parasite and the parasite is unable to overtake the host. Nevertheless, the parasite remains alive in its melanin sheath and produces spores, which makes American crayfish extremely contagious for our indigenous crayfish. However, if a further infection occurs or if the crayfish is weakened, its immune system can no longer master this benign aphanomycosis. This explains the sometimes sudden occurrence of plague outbursts in American crayfish, but mortality is rarely total.

Our indigenous crayfish has the same defence reactions, but the balance between host defence and parasite is never established, melanization being considerably less and slower. Thus, in *Astacus astacus*, the fungus always invades the entire crayfish internal cavity before any efficient defence takes place. Affected populations develop total mortality (100%).

Sanitary prophylaxis of crayfish aphanomycosis is impracticable in the wild. Once a crayfish plague happens, we can only prevent it from spreading to further places. Theoretically, the spores of *A. astaci* can stay infectious in water, for up to 2 months. The transport of spores from one location to another by fishing equipment and gear can be prevented by thorough drying (Oomycetes do not resist to

desiccation for more than 2 days) or by disinfection (e.g. with a 15% sodium hypochlorite solution). Fish having eaten infected crayfish cuticle may transmit plague because the fungus remains viable after its passage through the gastrointestinal tract. However, it dies after 12 h at 37°C and is thus unlikely to survive the passage of the digestive tract of mammals or birds. Once a plague episode has passed, restocking with plague-free crayfish is possible if the site can be prevented from further harbouring the plague fungus. This is easy in winter after the drying up of an isolated pond, spores being inactivated when exposed at -5°C for at least 3 days.

Waters connected to river system cannot be shielded from crayfish plague because American crayfish plague carriers now exist almost everywhere. Only few physical barriers like high waterfalls may prevent downstream plague carrying crayfish populations from contaminating upstream susceptible crayfish populations. Quarry lakes, unconnected to rivers and enclosed by high fences may be the best places where to maintain our populations of noble crayfish.

Aquaculture in closed basins enables the use of fungicides to control plague fungus. One of the most efficient decontamination agents for crayfish, fish and water is malachite green oxalate at 1 ppm during 30 minutes at 20°C. RS-1-aminopropylphosphonic acid (Ampropylfos) inhibits the release of swimming spores of *A. astaci* at a concentration of only 0.3 mg ml^{-1}. Water with high magnesium, but low calcium concentration also inhibits sporulation.

In the past there were no laboratories in our country with a practical experience of plague fungus. Secondary contamination by moulds and bacteria and the fact that there is no simple diagnostic feature of *A. astaci* explains the uncertainty about most past mass mortalities. A classical diagnostic method for plague fungus combines culture of infected cuticle in agar (in order to characterise morphological features of the fungus) with experimental contamination of healthy noble crayfish by animals suspected to be infected (in order to show the pattern of mortality). This method takes at least 3 weeks and commonly fails since the *A. astaci* culture is easily overgrown by other moulds. The advent of DNA-polymerase chain reaction method (PCR) and other molecular methods provide rapid tools for the identification of *A. astaci* and its strains, but cross-reactivity with some other moulds is still a problem. One of the aims of a European Community funded crayfish plague consortium established in 2009 is to develop a standardized diagnostic method for crayfish plague. In the meantime it recommends the use of some already operational molecular identity tests (Finnish Food Safety Authority EVIRA: http://www.docstoc.com/docs/35806300/Activities-in2009-Crayfish-plague). See also

the 2010 OIE Manual of Diagnostic Tests for Aquatic Animals and the list of OIE Reference Laboratories (www.oie.int).

Efficient tools against aphanomycosis have to be based on the understanding of natural resistance in some susceptible species, e.g. in the Turkish crayfish, *Astacus leptodactylus*, as well as on a thorough knowledge of the functioning of the crayfish immune system. Immunostimulation in crayfish must urgently be studied. So far, only two little experimental studies have been conducted, which show a heightened resistance to plague fungus in the susceptible noble crayfish after the injection of a sub-lethal amount of living *A. astaci* spores or of an antigen similar to that released by fungus wall.

Genetic transformation may well be the future for plague-susceptible crayfish once the complex genetic control of plague resistance will be fully understand. Nevertheless, we should be aware that even if we could “produce” plague-resistant noble crayfish, it would have to face competition with its North American alien relatives, which are more efficient in terms of reproduction success, growth rate and aggressiveness.

Non-indigenous freshwater fish and their pathogens: overview and possible impacts

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The introduction, spread and invasion of non-native (fish) species is a serious threat to biodiversity because of displacement of native species, modification of trophic structure and alteration of ecosystem function but maybe the least recognized threat is the co-introduction of parasites. Belgium has known at least 50 considered, attempted and/or successful introductions with freshwater fish species since 1200. Today, 20 of these species still occur in Flanders and more are on their way in the near future. We will discuss all the present fish species and their parasites, but we will focus on three recently introduced fish species namely topmouth gudgeon (*Pseudorasbora parva*), fathead minnow (*Pimephales promelas*) and round goby (*Neogobius melanostomus*). For these 3 species, scientific literature suggests that their co-introduced parasitic fauna caused harm to fishes in aquaculture facilities and/or detrimentally affected the indigenous freshwater fauna.

Fascioloides magna in red deer – the story of an early hitch-hiker

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The large American liver fluke (*Fascioloides magna* (Bassi 1875)), originally a parasite of white tailed deer (*Odocoileus virginianus*) and other North American deer species, was first detected and described in Europe far away from its endemic distribution area in the USA and Canada. It was introduced from North America to Northern Italy by wapiti (*Cervus elaphus canadensis*) and to Southern Bohemia by white tailed deer during the second half of the 19th century. The parasite became endemic in these two Old World localities and remained confined to these endemic foci for more than a century.

Following the migratory phase of the infection, resulting in severe liver damage, this large trematode settles in pairs within walnut sized cysts in the liver. The strong connective tissue capsules contract after the flukes die thus producing varying degrees of liver cirrhosis. Red deer may present the whole range of clinical disease from acute cases with severe liver damage, through the development of numerous encapsulated adult fluke pairs, to chronic cases with livers containing many dead, necrotic encapsulated flukes and fibrotic nodules.

Host species of *F. magna* are classified as definitive, dead-end and aberrant hosts, depending on the pathology and the ability of the fluke to produce a patent infection in them. As many other invasive parasite species *F. magna* utilizes locally available intermediate hosts to establish its developmental cycle in new habitats. A wide range of Lymneid snails are suitable for the development of *F. magna*, but eventually it seems that its main European intermediate host is *Galba (Lymnea) truncatula*.

The initial impact of *F. magna* infection on naive red deer populations seems to be most severe for the first several years after introduction and characterised by loss of body condition and even mortality. Later, the infections become milder and reinfections of reconvalescent or chronic cases may occur. The new host population usually adapts to the parasite within a few years and achieves an equilibrium similar to the North American situation.

The spread of *F. magna* in Europe is limited by the availability of suitable habitats with adequate intermediate host (snail) and definitive host (red deer) populations. Since such habitats are usually fairly isolated and relatively far from each other, natural movements of deer populations are rarely

sufficient to ensure the expansion of the parasite. Therefore it is either human activity (translocation) or some other natural phenomenon which enables *F. magna* to expand its range.

In 1988, about one century after its European introduction, *F. magna* first occurred outside the Czech endemic area, when infected deer were detected at the Danube floodplain in Slovakia. In 1994 it was also detected at the neighbouring Hungarian areas, but its presence there could be traced back to 1991. *F. magna* subsequently spread downstream to southern Hungary in 1997 and was also found in Eastern Croatia in 2000.

The 200 km leap of *F. magna* along the Danube river to the next suitable deer habitat in southern Hungary suggested that *F. magna* spreads downstream along natural waterways either by eggs contained in floating faeces or infected snails washed downstream during floods. The expansion of the parasite was therefore probably triggered by a local range expansion into a part of Southern Bohemia which lies within the Danube watershed.

Although far from perfect, the monitoring of the range expansion of *F. magna* and its consequences resulted in one of the better documented invasive parasite stories. The conspicuous lesions in an actively managed and utilised game species helped the relatively timely detection of the parasite and provided additional incentive to explore the potential disease management options. However, the presence of the serious liver lesions and the alleged early impact of the infection usually also triggers an active game management response, which makes it almost impossible to document and objectively evaluate the real effect of *F. magna* on a naïve red deer population. This relatively unimportant parasite provides us with a very good opportunity to study the various modes of action used by invasive Trematodes during their expansion and the acquired knowledge could be applied in the development of complex risk assessment tools. The overall experience with *F. magna* suggests that its expansion in Europe may not be over yet.

An active surveillance scheme targeting one parasite species could result in unexpected collateral discoveries, like the detection of the presence of other parasite species beyond their documented geographic range. Thus, *Parafasciolopsis fasciolaemorpha*, a liver fluke of European elk, was found in Hungarian red deer far outside its regular distribution area. Even more interestingly the Asian Schistosomid blood fluke *Orientobilharzia turkestanica* was also found infecting red deer in southern Hungary. These findings lend us an unexpected insight into deer and fluke ecology and they also present us with new evidence for earlier instances of parasite population movements.

POSTERS

The effect of diesel oil on gill histopathology and behavioral changes of the milkfish (*Chanos chanos*)

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In this study, the 96-h LC50 values of diesel oil and its effects on behavioral changes and gill histopathology of Milkfish (*Chanos chanos*) was determined. Young juvenile milkfish (W: 64±1.2gr, SL: 16.7±0.4Cm) were exposed to diesel oil at concentrations of 3.4, 3.9, 4.4, 5.5, and 7 ml/l. The experiments were performed as three replicates, and all changes in the specimens were determined for each concentration. Water quality parameters of the test seawater were: hardness, 193.4 mg/l as CaCO3; pH, 7.6 to 8.1; dissolved oxygen concentration, 6.7 to 7.8 mg/l; temperature, 23ºC and salinity, 38.3 psu. The data obtained were statistically evaluated by the use of the EPA computer program based on Finney's Probit Analysis Method. The 96-h LC50 value was found to be 5.12 ml/l in a static bioassay test system. The gill lamellae became lifted, while the secondary lamella became fused and showed oedema. Hyperplasia, hyperemia and hypertrophy of lamellar epithelial cells were distinct, with the number of mucus cells increasing. Superficial heamorrhages and abnormal behaviour were observed (e,g hyperactivity, loss of balance, convulsions, attaching to the surface, schooling, abnormal behaviour). There were no behavioral changes and deaths observed in the control group throughout the experiment. The result showed that acute oil toxicity severely affects mortality, normal behavior and gill structure which may be deleterious for milkfish populations.

Prevalence of *Toxoplasma gondii* in Belgian Wildlife

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Toxoplasma gondii, an obligate intracellular protozoan parasite, has a worldwide high prevalence in most warm-blooded animals and humans. Few studies are available on the occurrence of this parasite in wild animals. In this study we investigated the prevalence of *T. gondii* in Belgian wildlife. We tested brain samples from red foxes (*Vulpes vulpes*), European polecats (*Mustela putorius*), European Pine Martens (*Martes martes*), raccoons (*Procyon lotor*), brown rats (*Rattus norvegicus*), muskrats (*Ondatra zibethicus*) and roe deer (*Capreolus capreolus*). The samples were tested by Real Time PCR for the presence of *T. gondii* brain cysts. The amplified DNA target was the 529 bp *T.gondii* "repeat element" (AF146527). To check for inhibition, the cellular r18S gene was used. The prevalence was found to be: red fox: 57/304; European polecat: 2/2; European Pine Marten: 1/2; raccoon: 0/2; brown rat 19/335; muskrat 2/10 and roe deer 1/33. Twenty-six of the *T. gondii* positive DNA samples from foxes were genotyped: 25 were type II and one type III. In addition, 73 roe deer serum samples were tested by SAG1 ELISA for the presence of anti- *T. gondii* antibodies, 38 (52%) were positive.

Chlamydia psittaci in Canada geese in Belgium

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Chlamydia psittaci (*C. psittaci*), an obligate intracellular gram-negative bacterium infecting birds all over the world, is an important zoonotic agent. Symptoms in birds include conjunctivitis, rhinitis, dyspnoea, nasal discharge, diarrhoea, polyuria, anorexia and lethargy. Zoonotic transmission takes place trough inhalation of infected aerosols of pharyngeal or nasal excretions or dried feces.

C. psittaci has a broad host range, infecting 467 bird species comprising 30 bird orders. A German study in Canada geese (*Branta canadensis*) could neither isolate nor detect *C. psittaci* by PCR (Bönner et al., 2004). Zweifel *et al.* (2009) failed to detect *C. psittaci* in a recent Swiss study in songbirds and waterfowl. However, in the past, *C. psittaci* was isolated from Swiss Canada Geese (Wilt et al., 1972). In Belgium, the Canada Goose is an alien species presenting a potential threat for indigenous bird populations.

The current study investigates the presence of *C. psittaci* genotypes in a clinically healthy flock of captured Canada Geese in Belgium. In July 2009, pharyngeal swabs and blood samples of 81 wild Canada Geese were collected for culture and serology, respectively. The Canada Geese were captured in the wild and euthanized on the spot as part of pest management, in order to control the number of this alien species in Belgium. Forty-six of 81 (57%) Canada Geese were culture positive, with a low average culture score of 0.44. Seventy of 81 (86%) Canada Geese was seropositive for *C. psittaci*. Our study confirms the occurrence of *C. psittaci* in Canada Geese. Many geese were seropositive. However, less were culture positive. In the absence of clinical symptoms this could reflect a strong natural protection in Canada Geese or a persistent infection or 'carrier status' (Goellner et al., 2006). In carriers, stress or co-infections can trigger recurrent infection and chlamydial shedding. Canada Geese are fecal polluters of parks and pastures (Josefsson & Andersson, 2001). Aerosolized fecal droppings can transmit *C. psittaci* to humans. Thus, Canada Geese should be considered as a potential threat to public health.

Presence of extended spectrum β-lactamase producing *Escherichia coli* in wild geese

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Extended-spectrum β-lactamase (ESBL) producing bacteria have increasingly been detected in humans and animals. Recently, some surveys have suggested that European wild birds may act as reservoirs of resistant bacteria and might have an epidemiological role in the dissemination of resistance. Therefore, to gain more insight in the role of migratory birds as reservoir, cloacal swabs from 396 wild geese originating from 6 wildlife areas in Belgium were screened for the presence of ceftiofur resistant *Escherichia coli* (*E. coli*). Of the cultured *E. coli*, resistance against the β-lactams was determined using the disc diffusion test and the β-lactamases were characterized by performing polymerase chain reaction to detect genes encoding TEM-, SHV-, CTX-M- and CMY- type enzymes. To establish the clonal relationship between the *E. coli* isolates, multilocus sequence analysis using seven conserved housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*) was performed. From the 396 faecal samples, 2 ceftiofur resistant *E. coli* isolates were obtained. Characterisation and sequencing of the genes encoding the β-lactamases showed that the first *E. coli* isolate carried a bla_{SHV} gene encoding ESBL SHV-12. The second isolate, originating from a wild domestic goose, was found to carry a bla_{TEM} gene encoding ESBL TEM-52.

In conclusion, although the role of wild geese as a reservoir of bacteria carrying ESBL encoding genes seems to be limited at present, the results of this study may indicate that these resistance determinants have disseminated in the natural environment.

Entomopathogenic nematodes as disseminating agent for *Yersinia pseudotuberculosis*

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Today, the existence of biological reservoirs for pathogenic bacteria has been demonstrated. Entomopathogenic nematodes (EPNs) could be one such reservoir. These nematodes are able to kill insects thanks to their species-specific symbiotic bacteria belonging to the family Enterobacteriaceae (*Xenorhabdus* and *Photorhabdus* spp). Upon invasion of an insect larva, the symbiont is excreted from EPNs. Then, it produces insecticidal toxins and degradative enzymes allowing EPNs to feed on the dead prey. The symbiont also releases antibiotic compounds to prevent bacterial competitors. EPNs reproduce and complete their life cycle inside the cadaver. The symbiont goes back to the digestive tract of new EPNs before they emerge from the dead prey. Here we show that *Yersinia pseudotuberculosis* can colonize and maintain inside EPNs for at least 7 successive EPN generations (10 weeks). Moreover, it seems that this ability is specific to *Yersiniae* since other enterobacteria cannot colonize EPNs. In addition, our results show that *Y. pseudotuberculosis* is found at different places within EPNs along the time.

Yersinia pseudotuberculosis is closely related to *Yersinia pestis*, the causative agent of plague. If *Y.pestis* can also colonize EPNs like *Y. pseudotuberculosis*, it could turn into an interesting model to study the long-term persistence of plague in endemic areas. EPNs may play as a powerful dissemination vector for plague since they are highly mobile in soil. In addition, since up to half a million of EPNs emerge from only one insect larva, EPNs can also sustain an exponential multiplication of the pathogen.

Causes of mortality in roe deer (*Capreolus capreolus*) in Southern Belgium: results of the passive surveillance 2010

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Through a passive surveillance program, 69 roe deer (found dead or selectively culled for illness) were collected in 2010 in Wallonia. Necropsy was performed at the Veterinary Faculty of Liège according to a systematic protocol. Targeted microbiological and histopathological analyses were performed on the basis of gross lesions. No massive mortality in roe deer was recorded this year although several hunting districts deplored a decrease in their roe deer population. This statement is not unusual but the decreases are generally localised and temporary; a territory that suffered a crash of its population recovers one or two years later.

Regarding the found dead animals transported at the Faculty (n = 52), the distribution of causes of death was as follows: parasitic 24/52, traumatic 15/52, infectious 3/52, miscellaneous 4/52 and undetermined causes 6/52. Infectious causes included paratuberculosis, enterotoxemia and acute meningitis; miscellaneous causes were rumenal acidosis and tumours. Animals that didn't show any significant gross lesions or cases for which putrefaction didn't allow a post-mortem diagnosis were listed as undetermined causes. Among culled animals (n = 17), various causes of morbidity were diagnosed: heavy parasitism (n = 6), traumatic injuries (n = 4), ocular lesions (n = 2), acute polyarthritis (n = 1), chronic abscess (n = 1), rumenal acidosis (n = 1), and undetermined etiology in two cases. The roe deer showing heavy parasitic loads were mostly female adults (13/30) and juveniles (16/30). All except one showed severe body alteration. Nine roe deer showed diarrhoea. The diagnosis of heavy parasitism was performed with macroscopic observation in airways/gastrointestinal tract and coprological examination (norms fixed upon animals shoot during hunting season).

Overall, after road injuries, parasitic diseases are the major causes of mortality in roe deer in southern Belgium. Whether or not parasitic diseases are secondary to other pathogens is difficult to determine. More attention should be given to undetermined cases and passive surveillance has to be continuously stimulated to maintain a sufficient caseload.

Monitoring *Geomyces destructans* on hibernating bats: first results in Belgium

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White nose-syndrome (WNS) is a devastating disease causing mass mortalities in hibernating bats in North-America. In May 2009, it was estimated that over one million bats had died from the disease which was first documented in February 2006. A visually conspicuous white fungus grows on the face, ears, or wings of stricken bats with hyphae penetrating deep into the connective tissue of glabrous skin and snout and causing severe damage. To date, *G. destructans* is strongly suspected to be the causative agent of WNS mortality.

The wide distribution of *G. destructans* in Europe and the absence of associated mortality supports the hypothesis that *G. destructans* has co-evolved with European bats and only recently arrived in North America where it is causing unprecedented mass mortalities. Alternatively, *G. destructans* could have been present on both continents and a virulent strain could have evolved in North-America. Until the relationships between *G. destructans* populations across continents are clarified, precautions should be taken to minimise the chances of transcontinental movement of viable *G. destructans*.

The first results from the monitoring conducted in Belgium include two suspected cases in 2008, two other suspected cases in 2010 and three confirmed cases. In 2011, six confirmed cases were detected in Wallonia and a soil sample collected near an infected bat also appeared to be contaminated by *G. destructans*.

The use of spotlight counts for big game species to monitor invasive mammals

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Abundance indices derived from spotlight counts are reliable for the monitoring of red deer populations. They are today commonly used in Wallonia to evaluate the population trends and to compute the annual shooting plans for this big game species. These counts also allow detecting any nocturnal mammal species with eyes reflecting the spotlight thanks to their "tapetum lucidium" (e.g. most of our mammals except Suideae). In 2010, we planned to centralise the observations of every forest district of the Wildlife and Forestry Department of Wallonia using this counting methodology. It concerned an important part of the red deer spread area (2660 km² mainly in Ardenne and Famenne) divided into 173 census sectors. The counts were repeated twice in 44 % of the sectors and 3 or more times in 23 %, with no repetition for the other ones. The ranking in mean amount of individuals for each mammal species was : *Cervus elaphus* (5842), *Capreolus capreolus* (3401), *Sus scrofa* (1571), *Vulpes vulpes* (984), *Lepus europeaus* (857), *Ovis ammon* (115), *Meles meles* (108), *Felis sylvestris* (36), *Felis catus* (23), *Oryctolagus cuniculus* (22), *Martes foina* (14), *Dama dama* (13), *Putorius putorius* (11), *Procyon lotor* (7), *Martes martes* (5). The observations of *Procyon lotor* were located in the Elsenborn (far East nearby Germany), Habay and Florenville (South-East nearby Luxemburg and France) districts. In the future the sampled area will be enlarged to most of the forest districts concerned by red deer (Sankt-Vith, Malmédy, Neufchâteau, Eupen). An unique user-friendly observation form will be used to optimise the quality of the data. The night counts are not only useful for game management. They can also bring relevant information about protected (e.g. *Felis sylvestris*, *Martes martes*,…) and invasive species presence and abundance trends (*Procyon lotor* and *Nyctereutes procyonoides* observed in 2011 in the Verviers district) .

Added value of Natural History collection for the study of Wildlife Introductions

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Natural History collections around the world hold billions of specimens from so to say all known and described species. They are a unique and valuable source of information for studies on Wildlife introductions and associated diseases at different levels.

For instance :

- Experts of Natural History Museums can assist in the identification and taxonomy of the related species
- By consulting the information archived with the specimens, the first sampling or observations of introduced species in specific areas can be traced back by several centuries.
- Specimens or strains of disease causing species are kept in certain dedicated collections. Especially the more recent DNA and tissue collections, as well as the micro-organisms culture collections can play an important role.
- The disease causing vectors and species are often indirectly preserved with the infected specimens of the host species, which thus represents valuable study material
- Collections also contain valuable information of host species and the relationship with their parasites
- To identify future risk zones by the predictive modeling of habitats for disease carrying species in regard to global warming.

Secondly, following best practice in constitution of new collections and management of existing collections largely reduces the risk of spreading diseases. At international level SciColl (Scientific Collections) and at European level the CETAF (Consortium for European Taxonomical Facilities) and the EU project SYNTHESYS (for Synthesis of Systematic Resources) work together in issuing best practice guidelines and recommendation in this domain.

These recommendations include:

- Proper collecting, conservation, packaging and shipping methods when collecting new field specimens
- Decontamination and quarantine procedures before incorporating new specimens in the collections
- Continuous environmental and pest control to avoid contamination and damaging of the collections
- Improved DNA extraction from museum specimens can be used for the study of wildlife diseases
- Standard procedures for packaging, shipping and conservation when specimens are going on loan for studies in other institutions and reintegration procedures when the specimens are shipped back to their original institution

By means of selected examples shown on this poster, the authors would like to encourage more collaborations and joint projects between the Natural History Institutions and the sector of wildlife diseases.

Detection of avian influenza virus from infected Pekin ducks (*Anas platyrhynchos*) by two different swabbing methods

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The role of wild birds in the epidemiology and ecology of influenza A viruses has long been recognized. As a result of the emergence of H5N1 highly pathogenic avian influenza (HPAI) and the role of wild birds in its spread across Asia, Europe and Africa, avian influenza (AI) wild bird surveillance has been implemented in many countries. This has included, since February 2006, a mandatory programme in the European Union. We investigated the detection of virus excreted from Pekin ducks (*Anas platyrhynchos*) infected experimentally with 10^6 EID_{50} of A/mallard/England/2126/07(H3N6) over a fourteen day period post-infection using cloacal and oropharyngeal swabs of two different types: Dryswab™ ENT (dry) swabs and Virocult® (wet) swabs with viral transport medium (VTM) (Medical Wire & Equipment, England). The swabs, which were collected from each duck in alternating order (wet-dry), were stored at +4°C for 2-4 days to mimic transport conditions and then tested by RRT-PCR for the detection of influenza A virus Matrix gene RNA. Data will be presented regarding the detection of avian influenza virus using the two different types of swab. Results will be interpreted with reference to EU-mandated guidelines for avian influenza surveillance in wild birds.

Transmissible Cancer within the Tasmanian Devil Population

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The Tasmanian Devil, the largest carnivorous marsupial, is threatened with extinction by a contagious neoplasm. The cancer, known as devil facial tumour disease (DFTD), has first been reported in 1996. Today the disease has spread across the island and in some regions the population of devils has declined to 10% of its former size. The fatal tumour, growing primarily around the mouth, is an allograft and transmitted between devils by biting. Death usually follows within 6 months of infection. Biting is a normal behaviour of Tasmanian devils, associated with feeding but mostly with mating. This makes the transmission likely to be frequency dependent and thus disease induced extinction possible.

This clonally transmitted tumour is most likely of Schwann cell origin. It is even more unusual because it does not invoke an immune response, although the devil has a competent immune system. The lack of MHC diversity in the Tasmanian devil population could be the cause of its inability to recognise the foreign tumour cells. Without human intervention in the form of active disease control or the development of a vaccine, the Tasmanian devil might face extinction.

Improving tools and strategies for the prevention and control of classical swine fever (CSF) in European wild boar

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Classical swine fever (CSF) is a highly contagious disease of domestic pigs and wild boar and outbreaks can generate immense socio-economic losses. Although CSF has been eradicated from the European domestic pig population, CSFV is still present in some wild boar populations in the EU. Questions remain about the epidemiology of CSF in wild boar and about their potential role as a reservoir for infection of domestic pigs. Currently, a CSF non-vaccination policy exists within the EU. However, derogations from this policy have been granted, where necessary, usually for vaccination of wild boar. For this purpose, a modified live C-strain vaccine administered via baits is commonly used. This vaccine is very efficacious but its major disadvantage is that no differentiation can be made between vaccinated and infected animals, causing serious complications to the monitoring and surveillance of CSF in wild boar.

Within the EU FP7 project CSFV_goDIVA, a live CSFV marker vaccine with an accompanying DIVA (Differentiation of Infected from Vaccinated Animals) assay is currently under development for oral administration to wild boar via baits. Preliminary challenge studies in wild boar under experimental conditions showed a high level of efficacy. A field study in wild boar in Italy is currently ongoing. In parallel with the development of the new live marker vaccine, further attempts are made to improve the uptake of baits in young wild boar as even the newly developed smaller spherical baits were insufficiently ingested by piglets. In a second part, the project includes further studies into the epidemiology and persistence of CSF in wild boar and into the benefits of oral mass vaccination of wild boar. For the latter, an already existing CSF spread model was further developed and demonstrated that, in addition to vaccination of the infected area, the inclusion of a buffer zone equal to the annual spread distance of the virus can be recommended. The model also showed that the efficiency of vaccination may vary according to the season. Finally, the current surveillance strategies for CSF in wild boar were reviewed. A preliminary simulation study demonstrated that some modifications could be made to the sampling interval and/or sample size to save resources. An optimal scheme for sample size and sampling interval based on population density and design prevalence is currently being developed.

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Follow-up of a bite incident by a rabid bat infected with the European bat lyssavirus-1

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The classic rabies virus has been eliminated in Western Europe, but related lyssaviruses still circulate in European bats. In August 2010, a Belgian photographer was bitten upon provocation of an *Eptesicus serotinus* bat in Spain. The bat was disorientated, weak, active during the day time and exhibited uncoordinated flight. Its eyes were running. The bat had to be removed by force. The animal succumbed one hour after the bite and the carcass was submitted for rabies diagnosis

The patient applied immediate pressure on the thump to bleed out both puncture wounds, followed by disinfection with polyvidone-iodine (Isobetadine®). The bite victim was immunized preventively and received appropriate post exposure prophylactic care. He received two booster vaccinations five and eight days later (inactivated rabies vaccine Mérieux HDCV, Sanofi Pasteur MSD). He had received preventive immunisation with the same vaccine eight (3 doses) and three years (1 dose) before the bite. No symptoms were reported upon 12 months after the incident.

Despite the moribund state of the bat, there were no significant gross or microscopic lesions in organs other than the brain. A high load of rabies virus could be detected in the brain with the fluorescent antigen test and RT-PCR. Sequence analysis identified the virus as European bat lyssavirus-1 (EBLV-1). The isolate (named AF-2010) proved highly neurovirulent in mice, comparable to the classic rabies virus. Eight days after intracerebral inoculation with the isolate, all mice developed rabies disease signs, followed by death with a median survival time of 14 days.

Available vaccines are based on the classic rabies virus (genotype 1), which is significantly divergent from EBLV-1 (genotype 5). Considering the lethal risk of the infection, the divergence with the vaccine strain and the paucity of cross-protection studies, a follow up of the bite victim's serological protection against the EBLV-1 isolate was performed. We chose an *in vivo* challenge model to examine the vaccine efficacy. A mix of the patient's post-vaccine serum and challenge virus was inoculated directly into the brain of mice. At the 5% concentration (but not at lower concentrations), the patient's post-vaccine serum provided complete neutralisation of the 2010 EBLV-1 isolate. Fortunately, the follow up of the patient's serological immune response demonstrated thus satisfactory neutralisation of the bat virus isolate.

This case highlights the need for preventive rabies vaccination in people who (can) come in contact with bats and to seek medical council after a scratch or bite from a bat. In Europe, the risk of bat rabies is limited for the general public, possibly thanks to the rarity of unprovoked attacks. Besides virological surveillance of rabies in bats, it might be interesting to also record the number and type of bat bites. An increasing trend of unprovoked bites might be an early indicator of an increased risk of transmission to humans or other mammals.

Likelihood of Low pathogenic avian influenza introduction into domestic poultry pens ? Approach for a risk-based surveillance

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Following the devastating avian influenza epidemics in the years 2003-2005, international standards as well as European Union legislation decided to recommend risk-based surveillance in each Member State. If a country needs to establish a surveillance program based on risk, the influence of wild bird's movements and the probability of introduction of low pathogenic influenza in domestic poultry pens cannot be ignored in this context (Alexander, 2008; Gilbert et al., 2006). The aim of the present study was to better characterize the contact patterns of wild waterfowl and domestic poultry pens in order to better apprehend the role of these risk factors and enable a more targeted surveillance taking into account those risk factors.

Two experimental open range poultry pens were selected for this study: one in an urban area and another in a more rural area. Within each poultry pen, continuous video recording was performed. A grand total of 21.780 h recording was obtained for the poultry pens under study. The observed variables were the number of incursions of wild waterfowl into the domestic poultry pens, the absence or presence of feed and/or water in the open range poultry pens, whether the poultry pens were close to wetlands or not, and the number of wild waterfowl counted in the surroundings of the poultry pens, as well as the number of domestic birds within the poultry pens. A first step consisted in a descriptive analysis of the incursions over time, following which a time cluster analysis was performed to determine whether the cluster of incursions observed in time was significant or not. Non-parametric and parametric separate univariate analyses were carried out to measure the significance of each risk factor separately before selecting the variables to be incorporated in a generalized linear mixed model. The significance of the relative risk of contacts between wild birds and experimental poultry pens according to season, feed, proximity to wetland, and local abundance of wild waterfowl were estimated.

Results enabled the identification of interesting features, such as seasonal patterns that clearly showed time periods with a significantly increased probability of introduction. Outdoor feeding seemed to play a crucial role in the number of incursions of wild waterfowl. Wetland surfaces and higher migratory bird concentrations were not significantly associated with an increased risk of waterfowl contact with outdoor raised poultry. Despite the limited number of poultry pens under observation, the high number of observations per poultry pen brought interesting results to light. These results still need further investigation. However, these results, together with the variability and uncertainty around them, could be used as input parameters to better quantify the efficiency of risk-based surveillance such as described by Welby et al. (2010).

***Dermacentor reticulatus* as vector of *Anaplama phagocytophilum* ?**

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As the role of wildlife is recognized as major in emerging diseases in humans as well as in domestic animals, tick borne diseases are in this context a subject of growing concern. In Belgium, the most health implicated tick is *Ixodes ricinus.* However, *Dermacentor reticulatus* seems to extend its geographical distribution more than previously thought, like in other parts of northern Europe. *D. reticulatus* can potentially or successfully transmit viruses, bacteria or piroplasms such as *Rickettsia sp, Francisella tularensis, Babesia canis, Coxiella burnetii* or TBEV, but was never related with *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis. Indeed, in Europe, only the sheep tick (*I. ricinus*) was recognized to carry these bacteria. Here we deal with a new potential vector species.

In the field of the disease monitoring activities of the WILDSCREEN network, we found on an adult red deer 35 ticks that were morphologically and genetically identified as *D. reticulatus*. We tested them for *Anaplasma phagocytophilum* by PCR and sequencing a part of the Msp2 gene and some of them were positive as presented previously. One of them was enhanced by sequencing a part of the 16S RNA gene. To extend our knowledge about the variants that may be found in *D. reticulatus,* 108 additional specimens were collected by flagging and some of them were tested positive for *A. phagocytophilum* by Real Time PCR on the Msp2 gene. The positive ticks will be sequenced regarding a part of the 16S RNA gene to compare them with *A. phagocytophilum* isolated from *I. ricinus* ticks, humans, red deer and roe deer.

This approach will enable us to learn more about the variants isolated from *D. reticulatus* and if they display a genotype similar to variants isolated from other sources.