

Amsterdam & Utrecht, The Netherlands, 17-18 March 2015

"harmonized Approaches in monitoring wildlife
Population Health, And Ecology and
Abundance"

APHAEA Final Meeting



Programme & Abstract book

Two-days meeting organised by APHAEA
in collaboration with the DWHC (Dutch Wildlife Health Centre)



APHAEA Final Meeting

March 17-18, 2015, The Netherlands



The EMIDA ERA-NET (coordination of European Research on Emerging and Major Infectious Diseases of production Animals) project “**harmonised Approaches in monitoring wildlife Population Health, And Ecology and Abundance**” (APHAEA, 2012-2015) aims at establishing a European wildlife disease surveillance network that is capable of providing reliable estimates of abundance of wildlife species and of pathogen distribution in key wildlife species. In particular, it aims at contributing to the development and implementation of the use of harmonized procedures for wildlife population abundance estimation, sampling and diagnosis, both at national and European levels, in order to improve wildlife health surveillance in general; and at enhancing international collaboration in the frame of but also after the end of the project by developing a European wildlife disease surveillance network that has the potential to monitor pathogen distribution for selected wildlife diseases with an impact on human and livestock health.

The APHAEA project as such will end in the spring 2015 and all partners as well as non-partners are kindly invited to participate in the project final meeting, which will include two parts and take place in the Netherlands:

- (1) The **Satellite Symposium “Geographical coordination of wildlife health surveillance”** held in Amsterdam (NL) on 17 March 2015 during the Third International One Health Congress (IOHC 2015)
- (2) The “**APHAEA Final Consultation Workshop**” held in Utrecht (NL) on 18 March 2015

Programme overview

Sunday 15 March	Monday 16 March	Tuesday 17 March	Wednesday 18 March	Thursday 19 March
	3 rd International One Health Congress			
		APHAEA Final Meeting		
		IOHC Satellite symposium	Consultation workshop	APHAEA Core partners meeting
Amsterdam			Utrecht	

Satellite Symposium

March 17, 2015, Amsterdam

This symposium will aim at providing an overview and comparison of past approaches taken by different networks, providing a blueprint for future initiatives, improving the international coordination of wildlife health surveillance, and promoting integration with similar health surveillance programmes for livestock and public health. Insights from surveillance initiatives in Europe (including the EWDA wildlife health surveillance network and APHAEA), North America, Asia and Oceania will be given by internationally renowned speakers. Having the symposium integrated in the IOHC 2015 will promote the APHAEA project in a broader context and provide opportunities for new collaborations.

Time	Subject	Speaker
12:00	Welcome	Andrea Gröne
12:10	The Canadian Wildlife Health Cooperative: wildlife health surveillance in the world's second biggest country	Craig Stephen
12:30	Monitoring and surveillance for wildlife disease in the United States of America	Tom DeLiberto
12:50	Aphaea: harmonizing estimates of wildlife abundance and wildlife disease diagnosis across Europe	Christian Gortázar
13:10	Setting up an Asian network for wildlife disease surveillance	Tokuma Yanai
13:30	General discussion and closure of formal satellite symposium	Thijs Kuiken (moderator)
14:00	Break-out sessions	Dolores Gavier-Widén (moderator)
15:30	Coffee/tea break	
16:00	Report of break-out groups, general discussion	Dolores Gavier-Widén (moderator)
17:30	Closure	Christian Gortázar
20:00	Optional dinner at restaurant in Utrecht	

Satellite Symposium – Invited speakers & abstracts

March 17, 2015, Amsterdam

THE CANADIAN WILDLIFE HEALTH COOPERATIVE: WILDLIFE HEALTH SURVEILLANCE IN THE WORLD'S SECOND BIGGEST COUNTRY

Craig Stephen, Executive Director CWHC, Saskatoon, Saskatchewan, Canada.

Email: cstephen@cwbc-rscf.ca

Abstract

Surveillance has been defined as close and continuous observation of a population in order to detect signals for action. Meeting that definition can be a challenge in a massive country, with abundant wildlife, large tracts of undeveloped land and a comparatively small population distributed along the southern border. The CWHC addresses this challenge through a network of partners that stretch across the country and penetrate into academia, government and civil society. Our national office serves our network by advocating for resources to support our regional centres (found in each of Canada's veterinary schools), integrating the collective wisdom and information of the network into a single national perspective on wildlife health and identifying strategic priorities for our network to address. The CWHC is a voluntary network that is augmented with some paid staff who enable regional offices to perform surveillance, diagnostic and assessment services. Maintaining this network requires a balance between ensuring the interests of individuals and regions are addressed while at the same time acting as Canada's national wildlife health program that provides a consistent level of surveillance across the country. An additional challenge arises from being outside of government, and thus, lacking legislative authority or dedicated budgets. Maintaining close ties with government partners and making sure our activities meet their needs is crucial to having CWHC outputs affect social change and policy to protect wildlife, public health or economic activities.

Biography

Prof. Dr. Craig Stephen is the Executive Director of the Canadian Wildlife Health Cooperative. He has DVM and PhD (Epidemiology). His academic focus has been on extending population and community health models to fish and wildlife as well as in systems for detecting and coping with environmental surprises. Craig is a Professor (Veterinary Microbiology) at the Western College of Veterinary Medicine, University of Saskatchewan, a Clinical Professor at the School of Population and Public Health, University of British Columbia and adjunct at 4 other Canadian universities.

MONITORING AND SURVEILLANCE FOR WILDLIFE DISEASE IN THE UNITED STATES OF AMERICA

Thomas. J. DeLiberto, United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, Fort Collins, Colorado, U.S.A.

Email: Thomas.J.DeLiberto@aphis.usda.gov

Abstract

The World Organization for Animal Health encourages all countries to develop and maintain wildlife disease surveillance systems, which complement and support human health and agricultural animal disease programs. Most monitoring and surveillance systems (MOSS) implemented for wildlife species have been designed to evaluate the progress of disease control or eradication programs (e.g., bovine tuberculosis, chronic wasting disease, rabies). Recently though, increased awareness by the international community of the important role wildlife can have in the emergence and spread of infectious diseases, has led to recommendations that wildlife resources be included in nationally coordinated animal and human health surveillance programs. However, relatively few nationally coordinated MOSS for wildlife, and fewer international ones, have been implemented, because of the difficulties associated with working with free-ranging animals, quantifying disease in wildlife populations, lack of validated diagnostic and treatment modalities, complicated regulatory authorities of wildlife, and geopolitical conflicts over agricultural trade. I will review some of the main wildlife MOSS implemented in the United States and how these constraints have influenced their implementation.

Biography

Dr. DeLiberto currently serves as an Assistant Director for the NWRC where he coordinates USDA Wildlife Services' surveillance, emergency response, and research projects on diseases, chemistry, genetics, and economics. Dr. DeLiberto also serves on the Interagency Wild Bird HPAI Steering Committee, the White Nose Syndrome Executive and Steering Committees, the National Biosurveillance Working Group, and the Foreign Animal Disease Threats Wildlife Task Force. He represents Wildlife Services on the Board of Directors for the US Animal Health Association and the Wildlife Society's Wildlife Disease Working Group and on the Advisory Council for the American Association of Wildlife Veterinarians. Additionally, Dr. DeLiberto coordinates wildlife disease surveillance and capacity building projects in China, Southeast Asia, Indonesia, Bangladesh, Mexico, Kenya, and Uganda.

APHAEA: HARMONIZING ESTIMATES OF WILDLIFE ABUNDANCE AND WILDLIFE DISEASE ACROSS EUROPE

Christian Gortazar, Head, SaBio (Sanidad y Biotecnología) Research Group, National Wildlife Research Institute IREC (CSIC-Universidad de Castilla – La Mancha), Ciudad Real, Spain.
Email: Christian.Gortazar@uclm.es

Abstract

Harmonizing wildlife population monitoring and wildlife disease surveillance across the about 50 countries that constitute Europe is not an easy task. The EU (EMIDA ERA-NET) funded APHAEA consortium (www.aphaea.eu) has contributed towards the above goal through three actions: (1) producing written recommendations for wildlife abundance monitoring and for wildlife disease surveillance; (2) testing these recommendations on selected host-pathogen binomia; and (3) strengthening the European Wildlife Disease Network. In collaboration with many European and even some non-European experts, APHAEA has written and published species cards describing the tools available for abundance estimation for about 10 key wildlife host species or groups, as well as disease cards describing the key diagnostic tools available for about 20 relevant wildlife diseases. Both kinds of cards include an “APHAEA-recommended protocol”: the one proposed for use across countries. These protocols have been experimentally applied to Aujeszky’s disease virus and Eurasian wild boar (*Sus scrofa*), *Francisella tularensis* and rodents, and *Echinococcus multilocularis* and red fox (*Vulpes vulpes*). These case-studies provided important insights into the practical difficulties to overcome when working across countries and based on volunteers and informal collaboration rather than government agencies. Finally, APHAEA has contributed to push the ongoing European Wildlife Disease Network forward, facilitating a forum for information exchange among the many European research groups interested in wildlife populations and their diseases.

Biography

Prof. Dr. Christian Gortazar got a Degree in Veterinary Sciences at Universidad de Zaragoza in 1990 and a PhD at the same University in 1997. Since 1999, he is Professor at IREC, a multidisciplinary research institute dealing with conservation and management of wildlife and their habitats (www.SaBio-IREC.com). His lecturing on wildlife diseases is part of IRECs MSc and PhD programs, where he has mentored 15 successful international PhD students. He has acted as principal researcher in numerous grants and contracts on wildlife epidemiology and disease control. Research interests include viral, bacterial and parasitic diseases of wildlife, with emphasis on the epidemiology and control of relevant diseases shared with livestock and humans, such as tuberculosis.

SETTING UP AN ASIAN NETWORK FOR WILDLIFE DISEASE SURVEILLANCE

Tokuma Yanai, Senior Board Member, and Junpei Kimura, Secretary General, the Asian Society of Conservation Medicine (ASCM).

Email: yanai@gifu-u.ac.jp

Abstract

Since its first meeting at Kasetsart University in 2005, the Asian Society of Conservation Medicine (ASCM) (before Asian Society of Zoo and Wildlife Medicine, ASZWM) has convened annually, with the goal of “One Health in Asia Pacific”: establishment of an Asian network for conservation medicine and wildlife disease surveillance. These annual meetings have been hosted by various veterinary colleges such as Chulalongkorn (2006), NTU (2007), BAU (2008), SNU (2009), UPM (2010), Tribhuvan (2011), Mahidol (2012) and VNUA (2014), occasionally in collaboration with local veterinary associations. One of our goals is to support “One Health” in Asia by establishing networks for wildlife disease surveillance. Currently, ASCM’s *Mycobacterium* surveillance network is working on surveillance and molecular diagnosis. We are also trying to establish surveillance networks in Asia for equine herpesvirus, rabies, and HPAI. Additionally, we are discussing “How to collaborate with the WDA to join the global wildlife disease surveillance network,” and we started to make arrangements for further collaboration. As a first step, ASCM started to arrange an ASCM-WDA joint session for “Wildlife Disease Surveillance in Asia” from the 2014 annual meeting in Vietnam. The next 8th meeting will be held in Yangon in Myanmar on October 15-19 in 2015. Please join us in Myanmar, a mysterious country.

Biography

Prof. Dr. Tokuma Yanai, DVM, PhD, JCVP, ACCM, is a Professor of Gifu University and Senior Board Member of the Asian Society of Conservation Medicine (ASCM). Prof. Yanai graduated from Miyazaki University, Veterinary Course in 1978, and got his Master's degree (1980) and PhD degree (1992) from the University of Tokyo. From 1980 to 1992, he was a toxicologic pathologist working at a pharmaceutical company, and moved to Gifu University, Department of Veterinary Pathology in 1992. From 1997 to 1999, he worked at Harvard Medical School as a visiting associate professor. In 2005, he became a major organizer of the Asian network for conservation medicine. His interests are pathological aspects of zoo and wild animals, especially zoonoses and other infectious diseases. His group discovered Equine Herpesvirus 9 (EHV-9), which caused an outbreak in a Japanese zoo, and clarified its pathogenicity. Currently his group focuses on nontuberculous mycobacteria in animals.

Satellite Symposium – Access map

March 17, 2015, Amsterdam

The symposium will take place at the Amsterdam RAI Convention Centre (Europaplein 24), located in historic Amsterdam. See <http://www.iohc2015.com/venue/travel> for more detailed information



APHAEA Consultation Workshop

March 18, 2015, Utrecht

The workshop will aim at presenting the final result obtained by project partners on estimates of abundance of selected key wildlife host species and on the distribution of selected pathogen in their wild hosts in Europe. The proposed harmonized methods will be discussed before spreading them to a wider public. The future development of the European wildlife disease surveillance network will also be addressed.

Time	Subject	Speaker
08:00	Registration open	
09:00	Welcome	Christian Gortázar
	Host-pathogen pairs	
09:05	Overview	Christoph Staubach
09:15	Wild boar/Aujeszky's disease	Fran Ruiz-Fons
10:00	Coffee/tea/posters	
11:00	Red fox/Echinococcosis	Franz Conraths
11:30	Rodents/Tularaemia	Rainer Ulrich
12:00	Lunch/posters	
	Harmonization of monitoring wildlife abundance and diagnosing wildlife disease	
13:15	Demonstration of animal cards and disease cards	Ezio Ferroglio
13:45	Wildlife disease surveillance network	Marie-Pierre Ryser
14:15	View of EFSA	Andrea Gervelmeyer
14:45	View of OIE working group on wildlife diseases	Billy Karesh
15:15	Coffee/tea/posters	
15:45	General discussion on future of European wildlife disease surveillance network including use of harmonized protocols	Thijs Kuiken (moderator)
17:15	Conclusions	Christian Gortázar
17:30	Apéritif	
20:00	Optional dinner at restaurant in Utrecht	

APHAEA Consultation Workshop – Invited speakers

March 18, 2015, Utrecht

Dr. Andrea Gervelmeyer, Senior Scientific Officer, Animal Health and Welfare Team, ALPHA Unit, European Food Safety Authority, Italy

Dr. Andrea Gervelmeyer is a veterinary doctor from Germany. She worked for several years with the German Agency for Technical Cooperation (GTZ) and the Food and Agriculture Organization of the United Nations (FAO) on diagnosis and control of epizootics and zoonoses in Sub-Saharan Africa, before shifting her focus to investigation, surveillance and reporting of foodborne diseases of humans, both at the national level in Germany as well as the international level for WHO EURO and the EC. In 2009, she moved to the European Food Safety Authority (EFSA) in Parma, Italy, where she leads the Animal Health and Welfare Team since January 2014. Andrea holds a Ph.D in poultry disease diagnosis, a diploma in tropical animal health management and is an alumnus of the European Programme for Intervention Epidemiology Training (EPIET).

Dr. William "Billy" Karesh, Executive Vice President for Health and Policy at EcoHealth Alliance, President of the World Animal Health Organization (OIE) Working Group on Wildlife Diseases

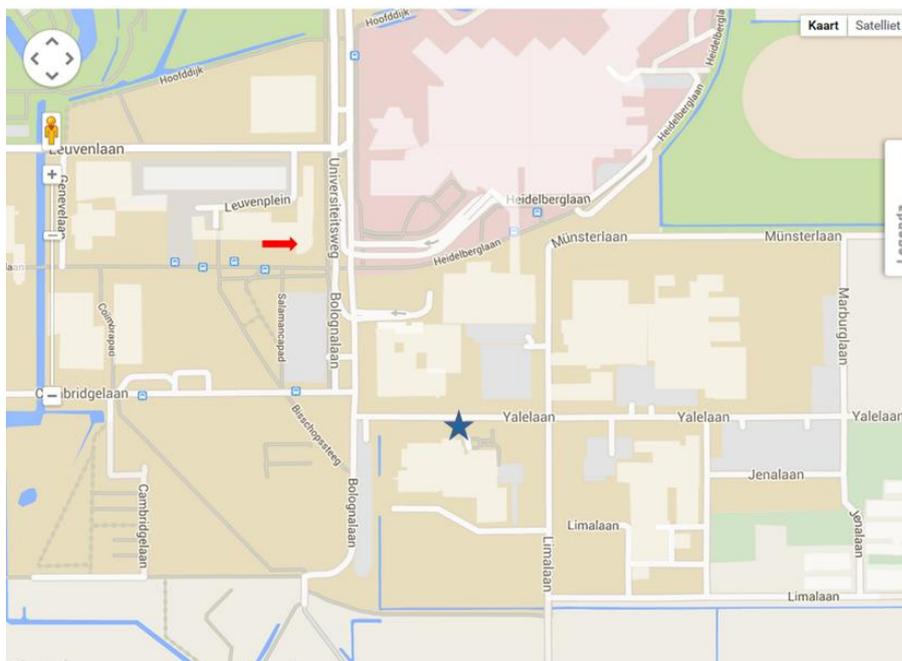
Dr. William Karesh is the Executive Vice President for Health and Policy for EcoHealth Alliance. He is also the President of the World Organisation for Animal Health (OIE) Working Group on Wildlife Diseases and chairs the International Union for the Conservation of Nature (IUCN) Species Survival Commission's Wildlife Health Specialist Group, a network of hundreds of wildlife and health experts around the world. Since 2009, he has served as the Technical Director for the US\$75M USAID Emerging Pandemic Threats PREDICT program.

Dr. Karesh has pioneered initiatives focusing attention and resources on solving problems created by the interactions among wildlife, people, and their animals. He coined the term "One Health" and has created numerous initiatives to encourage linkages among public health, agriculture and environmental health agencies and organizations around the world. He has lead programs and projects in over 60 countries, covering terrain from Argentina to Zambia. In addition to his work in the private sector, Dr. Karesh has also worked for the USDA, DOD, DOI and the Food and Agriculture Organization of the U.N. Dr. Karesh is internationally recognized as an authority on the subject of animal and human health linkages and wildlife. He has published over one hundred and sixty scientific papers and numerous book chapters, and written for broader audience publications such as *Foreign Affairs* and *The Huffington Post*.

APHAEA Consultation Workshop – Access map

March 18, 2015, Utrecht

The workshop will take place at the Faculty of Veterinary Medicine (Heideberglaan 8) in Utrecht..



→ Workshop, from Central Station bus # 28 or 12, exit Heideberglaan Van Lier en Egginkzaal, Heideberglaan 8

★ Apotheek, Yalelaan 1, Androclusgebouw

APHAEA Consultation Workshop – Poster abstracts

March 18, 2015, Utrecht

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SURVEILLANCE FOR BLUETONGUE VIRUS DETECTION IN WILD CERVID SAMPLES IN GREECE DURING THE CURRENT OUTBREAK: RESULTS OF AN ONGOING STUDY

Valiakos George¹; Giannakopoulos, Alexios¹; Birtsas, Periklis²; Chatzopoulos, Dimitrios¹; Sokos, Christos³; Vasileiou, Natalia¹; Papaspyropoulos, Konstantinos³; Tsokana, Constantina¹; Spyrou, Vassiliki²; Fthenakis, George C.¹; Billinis, Charalambos¹

¹ Faculty of Veterinary Medicine, University of Thessaly, 43100 Karditsa, Greece; ² Technological Institute of Thessaly, Larissa, Greece; ³ Research Division, Hunting Federation of Macedonia and Thrace, Thessaloniki, Greece

Key words: Blue Tongue virus, Greece, roe deer, wildlife

BACKGROUND: Since May 2014, a Blue Tongue Virus (BTV) disease outbreak takes place in Greece, spread all over the country; a total number of 32134 sheep, 637 goat and 28 cattle cases have been reported so far (OIE: Event Summary Blue Tongue, Greece, November 2014). The outbreak strain is considered to be a reassortant BTV-4 strain, similar to isolates that have been circulating in the Western Mediterranean and North African countries in recent years (Batten C - ProMED Mail, 2014).

METHODS: Our study objective was to determine the presence of BTV in wild cervids in Greece. Spleen and whole blood samples have been collected from hunter-harvested animals during hunting season, in various controlled hunting areas and in cooperation with regional Hunting Federations. So far, 23 roe deer, 2 red deer and 1 fallow deer samples (6 of which derived from animals found dead) have been collected in order to be tested for the presence of BTV RNA. GIS analysis is being performed regarding the areas where positive samples have been found.

RESULTS: Three samples have been found positive so far, and the presence of BTV nucleic acid was confirmed by sequencing. According to GIS analysis, there are 545 permanent livestock farms near the hunting areas where positive samples were detected, out of which 478 are farms with sheep and goats numbering 44215 animals (range 8-415 ± 88,41 SD). Cattle population reaches 5849 animals. These numbers are even higher, considering the presence of ruminant herds of semi-nomadic state moving through the areas.

CONCLUSIONS: Preliminary findings suggest BTV infects wild cervid populations in Greece and support the potential of wild cervids as bluetongue maintenance and/or spill-over hosts, considering the co-existence of domestic and wild ruminants, as shown by the GIS data presented. However, the possibility that wild cervids are BTV reservoirs in Greece warrants further and larger-scale investigation.

LONG TERM SURVEILLANCE ON AUJESZKY'S DISEASE IN WILD BOAR OF CENTRAL ITALIAN ALPS

Chiari, Mario¹; Moreno Martin, Ana^{1,3}; Ferrari, Nicola²; Bertoletti, Marco¹; Avisani, Dominga¹; Zaroni, Mariagrazia¹; Cerioli, Monica¹; Alborali, Loris G.^{1,3}; Lanfranchi, Paolo²; Lell, Davidel^{1,3}; Lavazza, Antonio^{1,3}

¹Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna; ² Department of Veterinary Science and Public Health, University of Milan; ³ National Reference Laboratory for Aujeszky disease

Key words: Aujeszky's disease, wild boar, pig farms, risk factors

BACKGROUND: Aujeszky's disease (AD) is one of the most economically important diseases of pigs. Despite wild boar can serve as a persistent reservoir, few data are available on the long term epidemiology in free ranging wild boar living in absence of industrial swine herds, even though these data may be useful to provide information on the natural dynamics of AD infection.

METHODS: 3260 sera samples were collected from 2006 to 2014 from 4007 hunted free-living wild boar in the alpine footstep mountains (6 hunting districts, Brescia, IT) and tested for anti-AD total gB antibodies. Variables regarding pig farms (Total number of farms; Density; Average number of animals) and wild boar (Age; Sex; Abundance) were recorded. Differences in the composition of wild boar populations among areas (districts?) and factors affecting sero-positivity were investigated. Pig farms in the study area had limited size with usually a small number of pigs bred for a limited period of the year.

RESULTS: A total of 162 (4.97%) out of 3260 wild boar sera resulted positive for anti-AD antibodies. Age, Year and Average number of pigs per farm resulted significant while wild boar Abundance and Total number of pig farms as well as interactions among Age, Year and Sex turned out not to be significant. The odds of being positive increased almost three times for adults with respect to young boars. In addition, for each individual pig increase (on average) per farm, the odds of wild boar being positive decreased by 9%. A positive trend of seropositivity in wild boars was observed along the considered interval, with an increase in positivity of 20% each year.

CONCLUSION: The present long term surveillance showed an increase in AD seroprevalence in wild boar with higher probability of being seropositive among older individuals. In addition, AD sero-positivity in wild boar was tested as independent from the area's likelihood of contact with pigs.

A NOVEL REAL TIME PCR METHOD FOR *GONGYLONEMA* SP. DETECTION, THE CAUSATIVE AGENT OF THE NECROTIC OROPHARYNGEAL DISEASE (NOD) OF THE SCOPS OWL (*OTUS SCOPS*)

Esperón, Fernando¹; Lopes, Francisca²; de la Torre, Ana¹; Orejas, Patricia²; Reoyo, M^a Jesús¹; Alonso Raúl²

¹Centro de Investigación en Sanidad Animal (INIA-CISA); ²Brinjal Owl Rescue Centre

Key words: Necrotic oropharyngeal disease, *Gongylonema*, scops owl, real time PCR

BACKGROUND: Necrotic oropharyngeal disease (NOD) is a necrotic, proliferative disease of the oral cavity that affects young scops owls (*Otus scops*) (2-3 weeks old). This disease occurs every year since 1997 in the City of Madrid and it is caused by third-stage larvae of *Gongylonema* genus. *Gongylonema* infection diagnosis could be challenged since only few larvae can cause the NOD. In addition, while scops owls are considered accidental hosts of the nematode, its cycle is nowadays unknown; so, in order to establish control measures of the infection, it's necessary to elucidate it, especially which arthropod host is involved. Therefore, designing new methods of diagnostic for NOD is needed. **METHODS:** We have developed a new real time PCR based on TaqMan probes, for *Gongylonema* detection. We have tested the sensitivity of the technique by analyzing ten-fold dilutions of a cloned sequence. In a second step, we have analyzed field samples from a tissue bank, comparing the obtained results with the novel rtPCR with an universal PCR for nematode detection or cytology of necrotic plaques. **RESULTS:** This technique has detected as low as 8 copies per reaction. Furthermore, while the sum of positive samples including conventional PCR or cytology were as low as 42%, the percentage of positive samples with lesion by the novel rtPCR was 74%. **CONCLUSIONS:** The novel rtPCR is a sensitive tool for NOD diagnosis. This method will be employed to investigate which arthropod host is involved. This work is supported by the Animal Morris Foundation (D14ZO-834)

A NEW CENSUS TECHNIQUE FOR ESTIMATING WILD BOAR ABUNDANCE

Fischer, Claude ; Félix, Joanne

University of Applied Sciences of Western Switzerland, Dept. Nature Management

Key words: *Sus scrofa*, passive marking, camera traps, CMR

BACKGROUND: The management of wildlife species requires a good knowledge of their population densities and of population trends. The abundance of Wild boar populations is however particularly difficult to assess. The methods considered as the most accurate are often too expensive to be used routinely, and no methods are recognized as being applicable in all the habitats used by this species across its distribution range. This renders any comparison between sites difficult.

METHODS: We are developing a method that should not be biased by climate, habitat type, or hunting regime. The method is based on passive marking using Capture-Mark-Recapture models to evaluate abundances, and as it is passive, costs can be maintained low. Wild boars are marked at feeding sites using marking chinks for cattle and the "capture" and "recapture" are performed by camera traps. Mobile feeding sites are disposed according to a grid system.

RESULTS: First step of our project was to develop the feeders that should allow to passively mark the wild boars, and only wild boars. These feeders are now ready and we could test the method during two seasons in the Jura Mountains. The marking of the wild boars is efficient, and lasts for three to five days on the animals. This allows to get pictures the first night (capture) and to get re-sights the following nights (recaptures), allowing to use CMR models to assess population abundances.

CONCLUSIONS: The system gives interesting results in an area where no other counting method was applied up to know (except for hunting-bag statistics), as this area is not readily accessible (mountainous area) and as it is densely forested. It should be applicable in other habitat types and under different climates, as it is not dependent on the openness of the landscape, of management regimes, or on the detectability of indirect signs.

CAN SARCOCYSTIS SPP. INTERFERE IN MOLECULAR DIAGNOSIS OF TOXOPLASMA GONDII IN WILD UNGULATES?

Gaffuri, Alessandra¹; Formenti Nicoletta²; Vicari Nadia ^{1a}; Paterlini, Franco¹; Lanfranchi, Paolo²

¹Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna- Department of Bergamo and Pavia ^{1a}; ²Department of Veterinary Sciences and Public Health, Università degli Studi di Milano

Key words: wild ungulates; health monitoring; *Toxoplasma gondii*; *Sarcocystis* spp.; PCRs

BACKGROUND: The protozoan *Toxoplasma gondii* affects worldwide several mammalian and avian host species raising public health, economic and conservation concerns. As wild ungulates can be source of *T. gondii* human infection and this protozoan could affect their reproductive performances, a reliable diagnostic trial to monitoring the infection should be defined. Following previous positive serological results in Italian Central Alps, we investigated the reliability of different PCR protocols in wild ungulates.

METHODS: We sampled heart muscles of 58 wild boar, 104 roe deer, 10 mouflon and brain samples of, 50 chamois and 159 red deer. Then the diagnostic trial was defined using a PCR targeting a 529 bp non-coding region (protocol 1), a single tube nested PCR (protocol 2) and a PCR-RFLP (protocol 3) using primers that identify also *Neospora caninum* and *Sarcocystis* spp..

RESULTS: *T. gondii* DNA was clearly detected in a chamois and in a mouflon by protocol 1. In 15 red deer samples, weak PCR products resulted from the protocol 1 and thus they were submitted to other PCRs. The *T. gondii* negativity was confirmed with protocols 2 and 3, but the 3 detected *Sarcocystis* spp. DNA in a calf. By the same protocol, *Sarcocystis* DNA was identified also in 34 wild boar and in 37 roe deer. Sequencing analysis discriminated *Sarcocystis hjorti*, *Sarcocystis miescheriana* and *S. cruzi* and/ or *S. gracilis*, respectively in red deer, wild boar and roe deer.

CONCLUSIONS: Protocol 1 is useful in chamois and mouflon but can have a lower specificity than two others in red deer. On the contrary protocol 2 is recommended for the direct *T. gondii* diagnosis. Protocol 3 is useful to differentiate the three protozoa infections, but in latent toxoplasmosis and pauciparasitism infections with the simultaneous presence of *Sarcocystis* the detection of *T. gondii* may fail.

MOLECULAR TYPING OF *ECHINOCOCCUS MULTILOCULARIS* ISOLATES FROM GERMANY

Herzig, Mandy¹; Staubach, Christoph¹; Mattis, Roswitha¹; Knapp, Jenny²; Gottstein, Bruno³; Conraths, Franz J.¹

¹Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald-Insel Riems, Germany; ²Department of Chrono-Environment University of Franche-Comté, Besancon, France; ³Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Key words: *Echinococcus multilocularis*, molecular typing

BACKGROUND: Alveolar echinococcosis is a serious zoonosis caused by the tapeworm *Echinococcus multilocularis*. If the disease remains untreated in humans, it may take a lethal course. Infection occurs through ingestion of eggs of *E. multilocularis*. In Europe, the red fox (*Vulpes vulpes*) is the main definitive host of the parasite and rodents serve as intermediate hosts. Other carnivores such as the raccoon dog, domestic dogs and cats also represent definitive hosts of the parasite. *E. multilocularis* occurs in the temperate and arctic region of the northern hemisphere.

METHODS: Since little is known about the genetic diversity of *E. multilocularis*, we set out to study this feature in Brandenburg, Germany, because a large collection of parasite isolates with accompanying information on the infected hosts is available that covers the entire region over a long period of time. Three mitochondrial markers and the microsatellite EmsB are used for genotyping individual adult *E. multilocularis* parasites. The PCR products of the mitochondrial targets are sequenced to detect differences suitable for genotyping. Moreover, length polymorphisms of the EmsB profile are determined. The respective protocols are harmonized with other groups in Europe to ensure that the results are comparable.

RESULTS: So far, we detected one fox with worm sequences, which are different in two mitochondrial markers from those of other worms isolated from the same fox. Two foxes were detected that harbored multiple atp6-types and one fox had parasite with multiple nd1 types. In summary, three different atp6 and two nd1 types were identified and four EmsB profiles detected. The most common one is profile G followed by profile D.

CONCLUSIONS: Genetic variability of *E. multilocularis* isolates from foxes in Brandenburg was limited as determined with the EmsB and mitochondrial markers. Differences in mitochondrial markers do not match with particular EmsB profiles.

EVALUATION OF METHODS FOR ESTIMATING WILD BOAR ABUNDANCE IN SWITZERLAND

Meier, Roman K¹; Fischer, Claude²; Ryser-Degiorgis, Marie-Pierre¹

¹Centre for Fish and Wildlife Health, University of Bern; ²University of Applied Sciences of Western Switzerland, Dept. Nature Management

Key words: Census, hunting statistics, pellet counts, thermal imaging, *Sus scrofa*

BACKGROUND: A large number of direct and indirect methods are applied for estimating wild boar abundance, resulting in a confusing heterogeneity of data. Efforts to harmonize methods among European regions are thus required. The aim of this study was to evaluate three methods for wild boar abundance estimation under Swiss environmental conditions (richly structured habitats with dense forests): hunting statistics, a widespread but controversial method; faeces counts along transects, an established method in Mediterranean regions; and night vision counts, a promising new approach.

METHODS: We pooled data on dead wild boar (shot and found dead) from Switzerland (2004-2013) into three time periods and produced graduated color maps, representing the average number of dead animals/100ha/year. In winter 2014/2015 we applied additional methods in two study sites: Per site we carried out faecal counts on six randomly chosen transects of approx. 1 km length and performed three night vision counts with a FLIR thermal imaging camera on one transect of approx. 18 km.

RESULTS: Data from the hunting statistics revealed large differences at local scale (LAU-1) ranging from 0.0 to 19.1 ind/year/100ha, with a national average of 0.17 ind/year/100ha for the whole period. We observed an increasing number of dead wild boar at national and regional scales and a geographic spread. The number of faeces ranged from 0 to 3/km and night vision counts fluctuated between 0-17 individuals per trial (mean 2.8 ind/10km \pm 3.7). Detectability of wild boar in forests was low.

CONCLUSIONS: Despite the bias inherent in hunting bags, they represent an interesting tool to illustrate spatio-temporal trends of wild boar abundance and occurrence allowing comparisons among regions with similar hunting management and pressure. Detectability of wild boar by night vision and of faecal droppings was low in forests and therefore could be deemed unsuitable under Swiss conditions.

A RETROSPECTIVE STUDY OF CAUSES OF MORTALITY AND MORBIDITY IN ROE DEER IN SWITZERLAND FROM 1975 TO 2013: PRELIMINARY RESULTS

Pewsner, Mirjam¹; Origi, Francesco Carlo¹; Marie-Pierre Ryser-Degiorgis¹

¹Centre for Fish and Wildlife Health, University of Bern

Key words: *Capreolus capreolus capreolus*, disease, pathology

BACKGROUND: The roe deer is the most abundant and widespread wild ungulate in Switzerland, with 40,000 animals hunted and consumed annually. The aim of this study is to gain an overview of the diseases diagnosed in roe deer in Switzerland over the past decades and to assess the limits and advantages of such a dataset.

METHODS: We analyzed the necropsy reports of 888 roe deer (carcasses or selected organs of 386 males, 431 females and 71 deer of unknown sex) examined between 1975 and 2013. All submitted cases were examined macroscopically. Additional examinations included mostly histology (55.4%), bacteriology (46.3%) and parasitology (47.5%). Animal data, case history, results and pathologist responsible were entered into a database. Diagnoses were classified as infectious, noninfectious or unclear. In case of unclear etiology, classification was based on syndromes.

RESULTS: Infectious diseases (n=352) were slightly more represented than noninfectious diseases (n=321). Among infections, bacteria represented the most common etiology (n=213, predominately lung and brain), followed by parasites (n=100, mainly lung and gastrointestinal tract) and fungi (n=20, mostly lungs). Viral etiology was rare (n= 5) but likely underrepresented as virology testing was not routinely performed. Noninfectious diseases consisted principally of trauma but also included metabolic disorders, neoplasia and fawn starvation. Four syndromes were regularly mentioned: gastrointestinal disorders, blindness, myopathies and seasonal alopecia. Fluctuations in disease frequency were attributed to specific disease events, changing surveillance strategies and to the senior pathologist responsible.

CONCLUSIONS: The power of retrospective analyses of scanning surveillance data is limited by a number of biases inherent in such datasets. However, we observed a similar disease pattern over 40 years despite methodical and personal changes, which suggests that the general picture is representative. Interestingly, we noted the repeated occurrence of syndromes for which an etiology has not been identified to date.

RISKSUR – ENHANCING ANIMAL HEALTH SURVEILLANCE SYSTEMS

Katja Schulz¹, Franz J. Conraths¹, Christoph Staubach¹, Birgit Schauer¹, Marta Martinez Aviles², Marisa Peyre³, Katharina Stärk⁴, Chirag Doshi⁵, Ann Lindberg⁶ & Dirk Pfeiffer⁷

¹Friedrich-Loeffler-Institut, Institute of Epidemiology, Insel Riems, Germany, ²The University Complutense of Madrid, Spain, ³Le Centre de coopération internationale en recherche agronomique pour le développement, Montpellier, France, ⁴Safoso AG, Liebefeld, Switzerland, ⁵Tracetracker, Oslo, Norway, ⁶SVA National Veterinary institute, Uppsala, Sweden, ⁷Royal Veterinary College, London, United Kingdom

Keywords: RISKSUR, surveillance, animal disease, evaluation

BACKGROUND: The aim of the RISKSUR project (www.fp7-risksur.eu) is to develop and validate conceptual frameworks and tools for designing efficient animal health surveillance systems. The possibility to use risk-based approaches to enhance surveillance effectiveness has specific focus. Three surveillance objectives are targeted: early detection of emerging diseases, declaration of freedom from specified diseases and monitoring of endemic diseases. The consortium consists of 12 European partners representing academic institutions, institutions involved in animal health surveillance, economic institutions and industry.

METHODS: The project is divided into eight work packages (WP). WP 1 develops a conceptual framework for evaluation of risk-based surveillance systems. WP 2-4 construct frameworks for the design of surveillance, for each surveillance objective, and applies these through a series of case studies. WP 5 is responsible for evaluating the economic efficiency of surveillance designs tested in case studies and WP 6 develops tools incorporating the design and evaluation frameworks developed in previous WPs. WP 7 focuses on communication and training and WP 8 is responsible for project management. An extra working group on terminology has also been established.

RESULTS: Project outputs are summarized in 36 documents, some of which are accessible by the public and available online. Currently, WP 2-4 are conducting case studies to support the development of the design framework and evaluation tool, to test their practicability. Some of the epidemiological tools (RSurveillance package in R) developed by WP 6 are already accessible online. In addition, several webinars have been given, and two modules of an online surveillance training series are available on the RISKSUR homepage.

CONCLUSIONS: During the course of the project, the need for a consistent terminology regarding surveillance has been highlighted. The output so far; the web-based decision-support tools, the online training as well as results from the case study applications are promising results.

PROPOSAL TO CREATE A US NATIONAL FISH AND WILDLIFE HEALTH NETWORK

Jonathan Sleeman¹; Colin Gillin²; Teri Rowles³; Frances Gulland⁴; Gabriela Chavarria⁵; Frederick Leighton⁶; Arpita Choudhury⁷; Terra Rentz⁸; Paul Johansen⁹

¹USGS National Wildlife Health Center, Madison, Wisconsin, USA; ²Oregon Department of Fish and Wildlife, Corvallis, Oregon, USA; ³National Oceanographic and Atmospheric Administration, Silver Spring, Maryland, USA; ⁴Marine Mammal Commission, Bethesda, Maryland, USA; ⁵US Fish and Wildlife Service, Oregon, DC, USA; ⁶Canadian Cooperative Wildlife Health Centre, Saskatoon, Saskatchewan, Canada; ⁷Association of Fish and Wildlife Agencies, Washington, DC, USA; ⁸The Wildlife Society, Bethesda, Maryland, USA; ⁹West Virginia Division of Natural Resources, South Charleston, West Virginia, USA

Threats to fish and wildlife from endemic and emerging diseases are a growing concern for natural resource managers. Disease threats to free-ranging fish and wildlife populations transcend state and national borders, cross jurisdictions and have multiple societal and ecological impacts. Cross sector and multidisciplinary partnerships to address these issues of mutual concern is imperative. We propose the creation of a US National Fish and Wildlife Health Network designed to build a collaborative, operational framework by which government agencies, tribes, universities and professional conservation organizations coordinate to assist tribal, state and federal agencies in their responsibilities to manage these diseases. The mission of the Network will be achieved through collaborative partnerships and the collective, voluntary adoption of protocols, procedures and actions to address fish and wildlife health issues. A Coordinating Committee will be established to create, oversee and coordinate implementation of the Network and it shall be a self-directed, voluntary partnership of public and private sectors. The Network will consist of tribal entities, state and federal government agencies, academic institutions and professional organizations charged with implementing the guidelines and plans developed by the Coordinating Committee. The primary users of this Network are those tribal, state and federal government agencies responsible for managing fish and wildlife diseases and the health of free-ranging fish, terrestrial wildlife and marine animal populations. Specific areas of focus for the Network will include the following: 1) wildlife diagnostic laboratory protocols; 2) disease information management and dissemination; 3) coordinated disease surveillance; 4) interagency communication and response plans; 5) species specific health issues. The Network will address deficiencies in fish and wildlife disease monitoring and prevention programs where they exist, and facilitate the work of existing systems. The creation of this Network will be an important step in addressing the critical need to protect fish and wildlife health.

EVALUATION OF THE NEED AND POTENTIAL OF HARMONIZING METHODS FOR COMMON VOLE ABUNDANCE ESTIMATION AND THE DIAGNOSIS OF INFECTIONS WITH *FRANCISELLA TULARENSIS* – A QUESTIONNAIRE SURVEY

Sonnenburg, Jana¹; Staubach, Christoph¹; Ulrich, Rainer, G.¹; Imholt, Christian²; Ferroglio, Ezio³; Ryser, Marie-Pierre⁴; Kuiken, Thijs⁵; Conraths, Franz, J.¹; Gortazar, Christian⁶

¹Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; ²Julius Kühn-Institute, Münster, Germany; ³University of Turin, Turin, Italy; ⁴University of Bern, Bern, Switzerland; ⁵Erasmus Medical Centre, Rotterdam, The Netherlands; ⁶IREC Universidad de Castilla, La Mancha, Spain

Keywords: APHAEA, common vole, *Francisella tularensis*, questionnaire

BACKGROUND: The APHAEA (harmonized Approaches in monitoring wildlife Population Health, and Ecology and Abundance, www.aphaea.org) project aims to establish a European wildlife disease surveillance network capable of providing reliable estimates on abundance of wildlife species and occurrence and distribution of pathogens in key wildlife species. For this purpose, the host-pathogen combination Common Vole (*Microtus arvalis*) and *Francisella tularensis* was selected. **METHODS:** A questionnaire was designed to collect information on host abundance and pathogen occurrence from historical records, current studies or data which will be potentially accessible in the future. The questionnaire was circulated among the core project partners and voluntary external partners.

RESULTS: We received 17 completed questionnaires from 13 participating European countries. There is a substantial heterogeneity concerning the used data sources for estimating common vole abundance. Even if snap trapping was performed as proposed by APHAEA, different snap trapping protocols were used. Furthermore, the questionnaire showed that the population abundance estimation is mostly not accompanied by disease monitoring and surveillance; and that different protocols may be used for both purposes.

CONCLUSIONS: Although heterogeneity could be shown, the intention to harmonize the used methods was clearly demonstrated by the participants. Crucial information regarding the used methods for abundance estimation as compared to the proposed harmonized methods was obtained.

EVALUATION OF THE NEED AND POTENTIAL OF HARMONIZING METHODS FOR RED FOX ABUNDANCE ESTIMATION AND THE DIAGNOSIS OF INFECTIONS WITH *ECHINOCOCCUS MULTILOCULARIS* – A QUESTIONNAIRE SURVEY

Sonnenburg, Jana¹; Staubach, Christoph¹; Ferroglio, Ezio²; Ryser, Marie-Pierre³; Kuiken, Thijs⁴; Conraths, Franz, J.¹; Gortazar, Christian⁵

¹Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; ²University of Turin, Turin, Italy; ³University of Bern, Bern, Switzerland; ⁴Erasmus Medical Centre, Rotterdam, The Netherlands; ⁵IREC Universidad de Castilla, La Mancha, Spain

Keywords: APHAEA, red fox, *Echinococcus multilocularis*, questionnaire

BACKGROUND: The APHAEA (harmonized Approaches in monitoring wildlife Population Health, and Ecology and Abundance, www.aphaea.org) project aims to establish a European wildlife disease surveillance network capable of providing reliable estimates on abundance of wildlife species and occurrence and distribution of pathogens in key wildlife species. For this purpose, the host-pathogen combination red fox (*Vulpes vulpes*) and *Echinococcus multilocularis* (fox tapeworm) was selected.

RESULTS: We received 22 completed questionnaires from 11 participating European countries. There is substantial heterogeneity concerning the methods used for estimating red fox abundance. Information on population densities is available on a detailed local level and mostly on a yearly basis independent of the method used.

CONCLUSIONS: Although the available data are very heterogeneous, participants clearly demonstrated their intention to harmonize their methods in the future. Crucial information regarding the used methods for abundance estimation compared to the proposed harmonized methods was obtained. With regard to the disease-related questions, harmonization is already established at a high level. Sera or tissue sample were exchanged to promote the harmonization idea.

EVALUATION OF THE NEED AND POTENTIAL OF HARMONIZING METHODS FOR WILDBOAR ABUNDANCE ESTIMATION AND THE DIAGNOSIS OF INFECTIONS WITH THE AUJESZKY'S DISEASE VIRUS – A QUESTIONNAIRE SURVEY

Sonnenburg, Jana¹; Staubach, Christoph¹; Ferroglio, Ezio²; Ryser, Marie-Pierre³; Kuiken, Thijs⁴; Conraths, Franz, J.¹; Gortazar, Christian⁵

¹Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany; ²University of Turin, Turin, Italy; ³University of Bern, Bern, Switzerland; ⁴Erasmus Medical Centre, Rotterdam, The Netherlands; ⁵IREC Universidad de Castilla, La Mancha, Spain

Keywords: APHAEA, wild boar, Aujeszky's disease virus, questionnaire

BACKGROUND: The APHAEA (harmonized Approaches in monitoring wildlife Population Health, and Ecology and Abundance, www.aphaea.org) project aims to establish a European wildlife disease surveillance network capable of providing reliable estimates on abundance of wildlife species and occurrence and distribution of pathogens in key wildlife species. For this purpose the host-pathogen combination wild boar (*Sus Scrofa*) and Aujeszky's disease virus was selected. **METHODS:** A questionnaire was designed to collect information on host abundance and pathogen occurrence from historical records, current studies or data which will be potentially accessible in the future. The questionnaire was circulated among the core project partners and voluntary external partners.

RESULTS: We received 31 completed questionnaires from 13 participating European countries. In most cases, data available to estimate wild boar density consisted in hunting bags. The information was available at different regional scales and mainly on an annual basis. The definition of hunting seasons and hunting strategies varied widely. But the questionnaire demonstrated that harmonized protocols already exist for the collection of information for the hunting statistics and investigation protocols (e.g. age classification, gender, type of carcass, date, location, test results).

CONCLUSIONS: Although heterogeneity could be shown, the intention to harmonize methods was clearly demonstrated by the participants. Crucial information regarding the used methods for abundance estimation compared to the proposed harmonized methods was obtained. Given the known limitations of hunting bag data, people were encouraged to also provide data obtained by other methods, such as thermal imaging and distance sampling or camera-trapping at local scale. With regard to the disease-related questions, harmonization is already established at a high level.

GEOGRAPHICAL DISTRIBUTION AND ANALYSIS OF HUMAN LEISHMANIASIS CASES AND LEISHMANIA INFECTED EUROPEAN BROWN HARES AND DOGS IN THE REGION OF THESSALY, GREECE: RESULTS OF AN ONGOING STUDY

Tsokana, Constantina N.¹; Giannakopoulos, Alexios¹; Papaspyropoulos, Konstantinos²; Sokos, Christos¹; Birtsas, Periklis³; Pervanidou, Danae⁴; Triantafyllou, Eleni⁴; Georgakopoulou, Theano⁴; Valiakos, George¹; Spyrou, Vassiliki⁵; Chatzopoulos, Dimitrios C.¹; Athanasiou, Labrini V.⁶; Rodi Burriel, Angeliki¹; Hadjichristodoulou, Christos^{4,7}; Billinis, Charalambos¹

¹Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; ²Research Division, Hunting Federation of Macedonia & Thrace, Thessaloniki, Greece; ³Department of Forestry and Management of Natural Environment, Technological Education Institute of Larissa, Karditsa, Greece; ⁴Hellenic Center for Disease Control and Prevention, Athens, Greece; ⁵Department of Animal Production, Technological Education Institute of Larissa, Larissa, Greece; ⁶Department of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; ⁷Department of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, Larissa, Greece

Key words: *Leishmania*, hares, dogs, humans, GIS analysis

BACKGROUND: Leishmaniasis, a vector-borne mammalian disease caused by a protozoan flagellate of the genus *Leishmania*, transmitted by phlebotomine sandfly species, is endemic in nearly all geographical areas of Greece. Our study aimed to report the geographical distribution and the environmental parameters related to *Leishmania* infection in hares and dogs (data obtained from the research funding program THALES) in conjunction with the human leishmaniasis cases reported to Hellenic Center for Disease Control and Prevention (HCDCP) between 2007-2014 in Thessaly, Greece, a highly endemic region for Leishmaniasis.

METHODS: The regions where the human Leishmaniasis cases and the *Leishmania* infected hares and dogs were found, were recorded. The data were statistically analyzed and Geographical Information System (GIS) analysis was also performed using the software ArcGIS Desktop 10.1, three applications (ArcMap, ArcCatalog, ArcToolbox) and Google Earth.

RESULTS: GIS analysis revealed that the *Leishmania* positive hares were found in shrubland with pasture land and in agroforestry formations while the mean distance from villages and towns was 500 m. The majority of *Leishmania* infected dogs was found in urban and rural areas (irrigated and non-irrigated land). Most of the human cases were found in discontinuous urban fabric and permanently irrigated land. The *Leishmania* positive hares and dogs, as well as the human cases were found at low altitude. Importantly, human cases have been recorded in 19 of the 26 Municipalities of Thessaly while the 43.3% of the human cases was recorded in the Regional Unit of Larissa. Interestingly, the preliminary GIS analysis revealed the presence of *Leishmania* positive hares and dogs in the same regions with human leishmaniasis cases.

CONCLUSIONS: Our study indicates the possible role of hares and dogs in the epidemiology of Leishmaniasis in Thessaly, Greece. Moreover, any possible association between the human leishmaniasis cases and the *Leishmania* infection in hares and dogs deserves further elucidation.

PATHOGEN HUNTING IN GERMANY: THE NETWORK “RODENT-BORNE PATHOGENS”

Fischer, Stefan¹; Schmidt, Sabrina¹; Rosenfeld, Ulrike M.¹; Drewes, Stephan¹; Heuser, Elisa¹; Lenk, Matthias²; Hoffmann, Bernd³; Hoffmann, Donata³; Röhrs, Susanne³; Beer, Martin³; Imholt, Christian⁴; Reil, Daniela⁴; Jacob, Jens⁴; Drexler, J. Felix⁵; Eckerle, Isabella⁵; Drosten, Christian⁵; Mayer-Scholl, Anne⁶; Nöckler, Karsten⁶; Eßbauer, Sandra⁷; Ulrich, Rainer G.¹

Friedrich-Loeffler-Institut, ¹Institute for Novel and Emerging Infectious Diseases, ²Department of Experimental Animal Facilities and Biorisk Management, ³Institute of Diagnostic Virology, Greifswald-Insel Riems; ⁴Julius Kühn-Institute, Münster; ⁵Institute of Virology, Universitätsklinikum Bonn; ⁶Federal Institute of Risk Assessment, Berlin; ⁷Bundeswehr Institute of Microbiology, Munich, Germany

Key words: Rodents, zoonosis, viruses, bacteria, reservoir population

Rodents and other small mammals are important reservoirs for a large number of zoonotic viruses, bacteria and endoparasites. In addition, small mammals carry pathogens that might not be zoonotic. Knowledge of the geographical distribution, molecular evolution of rodent-borne pathogens and of reasons for clusters of human infections in Germany is limited. Therefore the network “Rodent-borne pathogens” was initiated for a synergistic collaboration of research groups in rodent biology and molecular epidemiology of rodent-borne pathogens. In the frame of the network, a total of about 18,000 wild and commensal rodents and other small mammals were collected in Germany. Using these animals the host association and geographical distribution of known zoonotic pathogens, as hanta- and orthopox viruses, *Leptospira* spp. and *Rickettsia* spp. were investigated. In addition, novel rodent-associated viruses have been discovered, e.g. rodent hepaciviruses. Moreover, the network aims to study potential influences of alterations in small mammal populations on the emergence, prevalence and molecular evolution of zoonotic pathogens. Besides the field studies the network coordinates the generation of cell lines derived from wild rodents.

SEROLOGICAL SURVEILLANCE OF WILD BIRDS FOR EXPOSURE TO WEST NILE VIRUS IN GREECE, 2009 TO 2014

Valiakos, George^{1,2}; Papaspyropoulos Konstantinos^{1,3}; Giannakopoulos, Alexios¹; Birtsas, Periklis⁴; Tsiodras, Sotirios^{5,6}; Hutchings, Michael R.⁷; Spyrou, Vassiliki⁴; Pervanidou, Danai⁵; Athanasiou, Labrini V.¹; Papadopoulos, Nikolaos⁸; Hadjichristodoulou, Christos⁹; Tsokana, Constantina¹; Baka, Agoritsa⁵; Manolakou, Katerina¹; Chatzopoulos, Dimitrios¹; Artois, Marc¹⁰; Yon, Lisa¹¹; Hannant, Duncan¹¹; Petrovska, Liljana¹²;
Billinis, Charalambos^{1,2}

¹Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece; ²Department of Biomedicine, Institute for Research and Technology of Thessaly, Centre For Research and Technology Hellas, Larissa, Greece; ³Research Division, Hunting Federation of Macedonia and Thrace, Thessaloniki, Greece; ⁴Technological Education Institute of Thessaly, Larissa, Greece; ⁵Hellenic Center for Disease Control and Prevention (HCDCP) Ministry of Health, Athens, Greece; ⁶National and Kapodistrian University of Athens, Faculty of Medicine, Athens, Greece; ⁷Disease Systems, SRUC, Edinburgh, UK; ⁸Department of Agriculture Crop Production and Rural Environment, University of Thessaly, Volos, Greece; ⁹Faculty of Medicine, University of Thessaly, Larissa, Greece; ¹⁰VetAgro Sup, Campus Vétérinaire de Lyon, France; ¹¹School of Veterinary Medicine & Science, University of Nottingham, Nottingham, UK; ¹²Animal Health and Veterinary Laboratories Agency - Weybridge, UK

Keywords: Greece, Serological surveillance, West Nile virus, Wild birds

BACKGROUND: From 2010 until nowadays, one of the largest European West Nile virus (WNV) outbreaks took place in Greece with more than 524 laboratory-confirmed human cases. A lineage 2 strain of WNV was detected in humans, horses, wild birds and mosquito pools.

METHODS: Our research team conducted a study to assess the exposure of various species of wild birds to the virus. In particular, a serological surveillance was performed, in 679 serum samples of wild birds that were hunted, found dead or trapped since 2009 in mainland Greece.

RESULTS: During the study the following interesting results were found and reported: a) Seropositive birds were hunted eight months before the outbreak of human cases in 2010. b) Migratory birds were found to be exposed to WNV prior to their time of arrival in Greece, during autumn migration, suggesting that avian species with similar migration routes may be responsible for the entrance of the virus in Greece. c) Many corvid samples were found to be seropositive, indicating an extended exposure of resident wild birds to the virus and implicating them in the epidemiology of the disease. d) Seropositive birds were found in each region where human cases were reported. e) Distance from stagnant water and altitude were identified using Geographic Information Systems (GIS) and statistical analysis, (two-step cluster analysis) as environmental factors associated with the presence of seropositive birds indicating high-risk areas. These sites were reported and later in many of them, human cases were detected, confirming the previous analysis.

CONCLUSIONS: These findings underscore the importance of surveillance of wild birds for zoonotic diseases such as WNV and that pre-emergence surveillance of wildlife can be a powerful tool as part of an effective warning system to prevent or reduce the impact of emerging zoonoses.