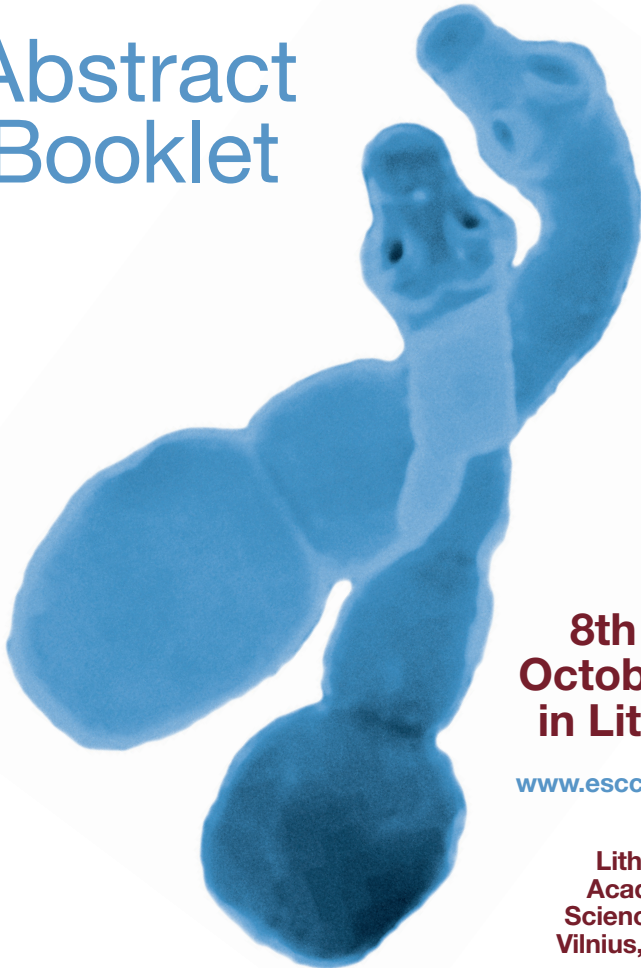




2014
Echinococcus

Abstract
Booklet



**8th – 9th
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in Lithuania**

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**Lithuanian
Academy of
Sciences (LAS),
Vilnius, Lithuania**

Introduction

In Europe, alveolar and cystic echinococcosis (AE, CE) are important parasitic zoonoses transmitted by wild and domestic carnivores. AE, which is caused by larval infections of the fox tapeworm *Echinococcus multilocularis*, represents a serious and steadily increasing threat to large parts of the Central, Eastern and North-Eastern European population. There is clear evidence for rapidly rising infection rates in wildlife in previously endemic areas and for newly emerging foci.

CE, caused by the dog small tapeworm *Echinococcus granulosus*, persists in many European endemic areas in Southern Europe, the Balkans and North-Eastern and Eastern Europe. AE and CE are neglected “malignant” parasitic diseases deserving the same attention as cancer. Well-coordinated veterinary public health actions, including control measures and recommendations for deworming of dogs, have the potential to minimise the infection risk in endemic areas and should be actively encouraged to reduce human diseases.

The ESCCAP *Echinococcus* 2014 meeting will focus on current epidemiological, public health and medical aspects of both zoonoses, AE and CE. Highly-recognised European experts and representatives of many active research groups will provide updates of the scientific field and the two-day, focused scientific meeting will bring together scientists of different areas to discuss common solutions in the frame of a one health concept.

ESCCAP *Echinococcus* 2014 scientific committee
Professors Peter Deplazes, Bruno Gottstein and Thomas Junghanss.

***Echinococcus* 2014 Organising Committee:**

Professor Peter Deplazes, Professor Paul Overgaauw, Ruth Pedder, Valentina Racca and Diane Richards.

***Echinococcus* 2014 Scientific Committee:**

Professor Peter Deplazes (Chair), Professor Bruno Gottstein and Professor Thomas Junghanss.

Acknowledgements

ESCCAP would like to take this opportunity to thank Professors Peter Deplazes, Bruno Gottstein and Thomas Junghanss, members of the *Echinococcus* 2014 scientific committee, for their involvement with the organisation of this important scientific meeting. The directors and members of ESCCAP very much appreciate the contribution they have made.

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Guest Speaker

Clinical epidemiology of human alveolar echinococcosis in Europe

Vuitton, D.A.

On behalf of the FrancEchino Network

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It was expected that alveolar echinococcosis (AE) would disappear from Europe at the end of the 20th century because of 1) major improvements in public health sanitation, individual hygiene and food safety measures; 2) decrease in the European population with occupational risks related to agriculture/animal breeding; 3) changes in behaviour of individuals because of urbanisation and better income. The prediction proved wrong and the number of patients with AE is either increasing or emerging in most of European countries.

AE incidence has doubled within the last 10 years in all countries located in the historical endemic areas (France, Germany and Switzerland). Autochthonous cases of AE are now present in most of central and northern European countries and the situation in the Baltic States is worrisome. In addition, a significant increase in the number of AE cases in patients who received organ transplantation or who were treated for malignant or chronic inflammatory diseases has been observed for the past 15 years; more rapid progression and atypical clinical imaging and serological features may delay AE diagnosis and compromise appropriate treatment in these patients.

Since 2000, potential risk of contamination of humans by *Echinococcus multilocularis* has clearly increased all over Europe, except in a few countries. Urbanisation of the presence of the parasite in foxes and pets, increased numbers of elderly people and of patients with potent immunosuppressive chemo- and/or immunotherapies are concomitant factors for the increase in AE incidence.

Response to the new epidemiological challenges includes the establishment of 1) an accurate European registry of human AE cases, 2) referral centres in highly endemic regions, and 3) a network of health professionals to better manage individual patients, perform prospective studies and deal with the occurrence of the disease in regions where it was previously absent. The development of a vaccine against AE in humans should be reconsidered.

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Guest Speaker

Taxonomy and molecular epidemiology of *Echinococcus multilocularis*: from fundamental knowledge to health ecology

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The taxonomy of the parasite *Echinococcus multilocularis* requires further consideration in order to better define its place in the complex *Echinococcus* group. Its position was studied through morphological and molecular phylogeny investigations, by nuclear and mitochondrial approaches. The recent complete sequencing of the parasite genome and of a few related species certainly offers new possibilities to address ancient taxonomic questions. The construction of a phylogeny on the entire genomic data or the possibility to infer a phylogeny with a large amount of functional genes, (e.g. adaptation genes to the hosts) is now being used to trace back its evolution and adaptive history. Furthermore, based on the current phylogenetic knowledge of the parasite, molecular epidemiology allows us to undertake genotyping studies in order to trace the dynamic activity of the parasite. The need to type strains in animal and patients is fundamental to better understand the contamination events and highlight putative more virulent/pathogen strains for the hosts, or better understand the strategies adopted by the parasite to perpetuate itself.

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Guest Speaker

Host ecology and *Echinococcus canadensis* transmission in the North

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The *Echinococcus granulosus* complex (EG) is the causative agent of cystic echinococcosis (CE). Northern cervid *Echinococcus* has been suggested to be its ancestor. During the last century, EG was regarded to have three (or four) different, but sometimes overlapping, transmission cycles in the circumpolar Arctic and Sub-Arctic: the original wild cervid (reindeer and elk) -wolf cycle; the synanthropic cycle involving semi-domesticated reindeer and herding dogs; and the semi-synanthropic cycle involving wild cervids and sled and hunting dogs. Human infections mainly derived from the latter two cycles, which during the last 50 years have mostly been eliminated due to changes in reindeer husbandry and hunting methods; machines making both herding and sled dogs largely redundant. Typical to human CE in the North has been its relatively benign nature compared with *E. granulosus sensu stricto*. The metacestodes in humans and in the natural cervid hosts predominantly appear in the lungs. The causative agents have recently been identified as *E. granulosus* genotypes G8 and G10 now generally known as *Echinococcus canadensis*, with close relationship to G6 (camel), G7 (pig) and G9 genotypes. The geographical distribution of both G8 and G10 appears to be circumpolar, with G10 currently apparently more common both in the Palearctic and Nearctic. In the future, *E. canadensis* is probably again highly dependent on the wolf, as it was before domestication of the dog.

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Guest Speaker

Actions taken and future considerations due to findings of *Echinococcus multilocularis* in two Scandinavian countries

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When *Echinococcus multilocularis* was first detected in mainland Scandinavia in Denmark in 2000, surveillance was initiated/intensified in Sweden, mainland Norway and Finland. After 10 years of surveillance these countries all fulfilled the requirements of freedom from *E. multilocularis* as defined by EU, i.e. a prevalence in final hosts < 1% with 95% confidence level. However, in 2011 *E. multilocularis* was detected in Sweden for the first time and surveillance was increased in all four countries.

Finland and mainland Norway are currently considered free from *E. multilocularis*, whereas the prevalence in foxes in Sweden and Denmark is approximately 0.1% and 1.0%, respectively. *E. multilocularis* has been found in foxes from three different areas in Denmark: Copenhagen (2000), Højer (2012-14) and Grindsted (2014). Alveolar echinococcosis (AE) is not yet notifiable in Denmark, and the number of human cases is therefore unknown. In Sweden, *E. multilocularis* has been found in foxes in three areas, Västra Götaland, Södermanland and Dalarna Counties (2011) and in an intermediate host in Södermanland County (2014). Two cases of AE have been reported in humans (2012), both infected abroad. No cases of *E. multilocularis* or AE have been reported in Finland and Norway. Recommendations and future considerations will be further discussed.

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Guest Speaker

***Echinococcus multilocularis*: how much monitoring do we need?**

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Alveolar echinococcosis, caused by infections with the larval (metacestode) stage of *Echinococcus multilocularis*, is considered one of the most dangerous autochthonous parasitic zoonoses in central Europe. The red fox (*Vulpes vulpes*) represents the main definitive host of *E. multilocularis* in Europe, but the raccoon dog also plays a role in the wildlife cycle. Dogs and to a much lesser extent cats are also final hosts of this parasite. The natural intermediate hosts of *E. multilocularis* are rodents, but the spectrum of intermediate and accidental hosts is broad and includes pigs and dogs. Humans get infected by oral uptake of infective *E. multilocularis* eggs. The estimated median number of cases in western, central and eastern Europe is in the range of 170 per year with the highest numbers in Germany, France, Switzerland and Lithuania. Yet, human alveolar echinococcosis is a very rare disease in Europe. However, the infection is widely distributed in foxes with high prevalences of up to 70% in some areas. Moreover, infected foxes in urban areas may represent a particular risk for the potential transmission of the parasite to humans. As a consequence, there is concern that the risk for human infection may generally rise due to the suspected spread of the infection in its definitive hosts and the high prevalences in some regions. Monitoring and surveillance activities have therefore been initiated in several European countries. Several diagnostic strategies have been developed and validated in recent years applying the classical worm detection by microscopy, immunological and molecular tests. However, there is an urgent need for defining minimal standards and harmonised approaches for these activities to allow for a reliable assessment of the epidemiological situation in Europe. Elements for a harmonisation of the monitoring and surveillance activities are presented and control options discussed.

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Guest Speaker

Taxonomy and molecular epidemiology of *Echinococcus granulosus sensu lato*

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Echinococcus granulosus, formerly regarded as a single species with a high geno- and phenotypic diversity, is now increasingly recognised as an assemblage of cryptic species which differ considerably in morphology, development, host specificity (including infectivity/pathogenicity for humans) and other aspects. This diversity is reflected in the mitochondrial and nuclear genomes and has led to the construction of phylogenetic trees and hypotheses on the origin and geographic dispersal of various taxa. Based on phenotypic characters and gene sequences, *E. granulosus* (*sensu lato*) has by now been subdivided into *E. granulosus sensu stricto* (including the formerly identified genotypic variants G1-3), *E. felidis* (the former 'lion strain'), *E. equinus* (the 'horse strain' G4), *E. ortleppi* (the 'cattle strain' G5) and *E. canadensis*. The latter species shows the highest diversity and is composed of the 'camel strain' G6, the 'pig strain' G7 and two 'cervid strains', G8 and G10. There is debate whether the closely related G6 and G7 should be placed in a separate species, but more morphological and biological data are needed to support or reject this view. In this new classification, the application of rules for zoological nomenclature led to the resurrection of old species names, which had long been synonymized with *E. granulosus*. This nomenclatorial subdivision of the agents of cystic echinococcosis (CE) may appear inconvenient for practical applications, especially because molecular tools are needed for identification of the cyst stage, and because retrospective data on '*E. granulosus*' are now difficult to interpret without examination of voucher specimens. However, the increased awareness for the diversity of CE agents – now emphasised by species names rather than genotype numbers – has led to a large number of recent studies on this issue and a rapid increase of knowledge on geographical spread, host range and impact on human health of the various species. *E. granulosus* s.s., often transmitted by sheep, is now clearly identified as the principal CE agent affecting humans. Contrary to previous assumptions, *E. canadensis* G6/7 readily infects humans, although CE incidences are rather low where *E. canadensis* predominates. Sub-Saharan Africa seems to be the region with the highest diversity of *Echinococcus*, and wild carnivores may play a more important role in the lifecycles of various species than previously assumed. Still, a number of issues remain unclear, e.g. possibly diverging parameters of diagnostic tests among the species, different responses to vaccines and, importantly, possibly required modifications of clinical management due to differences in pathogenicity.

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Guest Speaker

Trophic ecology, behaviour and host population dynamics in *Echinococcus multilocularis* transmission

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The life cycle of the cestode *Echinococcus multilocularis* primarily involves canids and small mammals (rodents, lagomorphs) as definitive and intermediate hosts, respectively. Several surveys have identified marked temporal and geographical variations, at different scales, in the parasite's prevalence in both types of hosts, suggesting variations in the biological and ecological factors controlling transmission processes. The parasite transmission from intermediate to definitive hosts is determined by the predator-prey relationship, which theoretically depends on prey population dynamics and the complex dietary response of predators to varying densities of prey species and other food items. The parasite eggs are transmitted to intermediate hosts *via* carnivore faeces the distribution of which in the environment is driven by the defecating behaviour of final hosts. The aim of this article is to review field-based studies that address issues related to trophic ecology and behaviour of definitive hosts, interactions between definitive and intermediate hosts, and *E. multilocularis* transmission both in wildlife and domestic animals in rural and urban environments. Knowledge gaps in this research area will be highlighted.

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Guest Speaker

Susceptibility to, versus resistance against alveolar echinococcosis (larval infection with *Echinococcus multilocularis*)

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Epidemiological studies have demonstrated that the majority of human individuals exposed to infection with *Echinococcus* spp. eggs exhibit resistance to disease as shown by either seroconversion to parasite-specific antigens, and/or the presence of 'dying out' or 'aborted' metacestodes. For those individuals where infection leads to disease, the developing parasite is partially controlled by host immunity: in the case of immunocompetence, a slowly growing metacestode is observed, referring to that form of AE where first clinical signs appear years after infection. In the case of impaired immunity, caused e.g. by AIDS, other immunodeficiencies or immunosuppressing immunotherapy, an uncontrolled exuberant proliferation of the metacestode is observed, leading to a very rapidly progressing disease status.

In infected humans, the kind of immune response developed by the host accounts for the subsequent trichotomy concerning the parasite development: (i) seroconversion proving infection, but the lack of any developing lesion indicating the failure of the parasite to establish and further develop within the hepatic parenchyma; and resistance as shown by the presence of 'dying out' or 'aborted' metacestodes; (ii) controlled susceptibility as shown by a slowly growing metacestode tissue – this group refers to the normal AE patients who first experience clinical signs and symptoms 5–15 years after infection, and (iii) uncontrolled hyperproliferation of the metacestode due to an impaired immune response (AIDS or other immunodeficiencies, e.g. following orthotopic liver transplantation). The host immune mechanisms modulating the course of infection include primarily T cell interactions. Immunomodulation seems to be triggered by parasite metabolites, which directly or indirectly inflict immunosuppressive and/or immunoregulatory processes that are assumed to correlate with parasite survival and proliferation dynamics. TGFβ-driven regulatory T cells have been shown to play a crucial role in the parasite-modulated progressive course of AE. A novel CD4+CD25+ Treg effector molecule FGL2 gave new insight into the tolerance process in *E. multilocularis* infection. This non-cytokine factor was shown to contribute to the outcome of *E. multilocularis* infection by interfering in the maturation of DCs and in promoting Treg cell functions; they also gave evidence for a role of IL-17 in FGL2 regulation.

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Guest Speaker

Recent developments in *Echinococcus* genomics and stem cell research

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Recent years have witnessed significant advancements that are of fundamental importance for understanding *Echinococcus* biology, host-parasite interaction and the development of novel anti-infectives. The genomes of four tapeworms, including *Echinococcus multilocularis* (reference genome) and *Echinococcus granulosus*, have been sequenced and yielded valuable information concerning gene gains/losses associated with the evolution of parasitism, cestode-specific modifications of stem cell regulation, and a plethora of fruitful targets for drug design and development. This is currently combined with extensive transcriptomic analyses of the entire *Echinococcus* life cycle and the characterisation of the transcriptome of the germinative cells, which are of fundamental importance for parasite development and asexual multiplication within the host. Studies on *in vitro* cultivated parasite larvae demonstrated that the germinative cells are the only parasite cells capable of proliferation and that they give rise to all differentiated cells. Germinative cells also display important differences in the expression of genes that belong to the highly conserved multipotency program of metazoan stem cells, which could be involved in the tremendous regenerative capacity (up to immortality) of these worms. Interestingly, drug screening assays indicate that germinative cells are insensitive to benzimidazoles, most probably due to the stem cell-specific expression of a benzimidazole-resistant beta-tubulin, which could explain the high recurrence rates after anti-parasitic chemotherapy. Future efforts should thus concentrate on novel drugs that also target the parasite's stem cell system.

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Guest Speaker

Echinococcus granulosus: epidemiology and state-of-the-art of diagnostics in animals

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Diagnosis and detection of *E. granulosus* (sensu lato) infection in animals is a pre-requisite for epidemiological studies and surveillance of echinococcosis in endemic, re-emergent or emergent transmission zones. Advances in diagnostic approaches for definitive hosts and livestock, however, have not progressed equally over the last 20 years. Development of laboratory-based diagnostics for canids using coproantigen ELISA and also copro-PCR, have made a huge impact for epidemiological studies and more recently for surveillance of hydatid control programmes. In contrast, diagnosis of cystic echinococcosis (CE) in livestock still relies on conventional post-mortem inspection, and current serodiagnostics do not provide a sufficiently specific and sensitive practical pre-mortem alternative. As a result, testing of dog faecal samples by coproantigen ELISA, often combined with mass ultrasound screening programmes for human CE, has been the preferred approach for monitoring and surveillance in resource-poor endemic areas and during control schemes. In this article we review the current options and approaches for diagnosis of *E. granulosus* infection in definitive and animal intermediate hosts (including applications in non-domesticated species) and make conclusions and recommendations for further improvements in diagnosis for use in epidemiological studies and surveillance schemes.

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Guest Speaker

Clinical management of echinococcosis needs attention as long as control fails

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Alveolar echinococcosis (AE) and cystic echinococcosis (CE) will continue to need attention in healthcare services as long as control fails – even more so with the expansion of AE in Europe and the unmet needs of CE patients in endemic regions and in immigrants to CE-free countries.

The setting and the impact of the interdisciplinary clinical centre for patients with CE and AE at Heidelberg University Hospital is presented where infectious disease/tropical medicine physicians, radiologists, abdominal and thoracic surgeons, gastroenterologists and parasitologists work closely together to stage patients and to tailor currently available treatment options of medical treatment with albendazole (AE and CE), percutaneous cyst-sterilisation techniques (CE only), surgery (AE and CE) and ‘watch and wait’ (CE only) to the needs of the individual patient.

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Guest Speaker

Control of cystic echinococcosis in Europe

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Structured control initiatives for cystic echinococcosis (CE) in Europe date back to the second half of the 19th century and have been implemented over the years in several countries, either in their whole territories (Iceland and Cyprus) or in selected regions (e.g. the Autonomous Community of La Rioja in Spain, the island of Sardinia in Italy, the country of Wales in the United Kingdom).

Classical control tools have included targeting dogs and sheep with veterinary interventions, in tandem with the implementation of public education campaigns to raise awareness of the disease and highlight hygienic measures essential for its control. Veterinary interventions have consisted in dog registration, testing and periodic deworming, dog population control, improved surveillance and hygiene in the slaughtering of livestock and proper destruction of infected offal. Only two initiatives have been documented as successful across Europe (Iceland and Cyprus), and far more have been unsuccessful or have only led to a temporary reduction in animal and human CE prevalence. Few of those control activities are fairly documented, while the majority have never been written up and assessed well. Careful planning, effective coordination of competent sectors, strict compliance with the set of proposed measures, adequate funding and sustained support of interventions over the years are crucial elements in a CE control plan.

The immunisation of sheep (and other intermediate hosts of *Echinococcus granulosus*) with the EG95 vaccine is a promising innovative tool that is likely to significantly contribute to the control of the disease in animals, in particular when coupled with classical veterinary interventions and culling of older sheep. With regard to that, mathematical models can assist when choosing the most cost-effective combination of intervention measures to be implemented, depending on local patterns of transmission of the parasite. The World Health Organization (WHO), through its WHO Informal Working Group on Echinococcosis (WHO-IWGE), has recommended the pilot testing of sheep immunisation in field trials with a view to fully evaluating its effectiveness, cost, feasibility and sustainability.

On the human side, across Europe as elsewhere the actual prevalence of CE remains largely unknown, partly due to the lack of efficient and dedicated reporting systems. With regard to that, the Italian Registry of Cystic Echinococcosis (RIEC) - an initiative launched in October 2012 accessible at <http://www.iss.it/riec/index.php?lang=2> on the website of the Istituto Superiore di Sanità (the Italian National Health Institute) in Rome - aims to provide prospective data on the epidemiology and clinical features of human CE. RIEC is meant to be the template for the European Registry of CE (ERCE), that will be implemented in the next few years within the FP7 HERACLES project (<http://www.heracles-fp7.eu/index.html>). The information gathered through ERCE will allow, inter alia, identification of priority areas and regions in Europe where control measures against CE will need to be strengthened.

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Guest Speaker

Epidemiological and clinical aspects of *Echinococcus* infections in the Baltic region

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Echinococcus multilocularis, the causative agent of alveolar echinococcosis (AE), and *Echinococcus granulosus* (pig strain, G7), causing cystic echinococcosis (CE) in humans, are endemic in the whole Baltic region.

In **Lithuania**, *E. multilocularis* was identified for the first time in 2001 in a muskrat. Average prevalence of *E. multilocularis* indicates a high infection pressure in this area. A total of 179 human AE cases have been registered over a 16 year period (1997–2013), and around 16–23 new cases of AE are recorded annually representing around a tenfold increase of AE-incidence. AE was diagnosed in 78% of the patients in the third–fourth clinical stage (PNM). Fifty-three of 179 AE patients (29.6%) died within a 16 year period (longest survival after diagnosis was 16 years and the patients' mean age was 58).

CE in pigs is widespread and known in Lithuania since the sixties. In recent studies, CE was diagnosed in 13.2% of pigs reared in backyard farms and in 4.1% of those from specialised farms; 3.8% of village dogs in the same areas were infected with *E. granulosus* s.l. Molecular analyses from liver cyst tissue of pigs, humans and cattle - as well as from egg samples of dogs - confirmed the occurrence of genotype G6/7. In Lithuania, the number of diagnosed cases of human CE has increased up to 0.4/100 000 of inhabitants (2002-2006). Of the patients, 59.6% (53/89) owned dogs and 41.6% (37/89) originated from rural areas.

In **Latvia**, new records of *E. multilocularis* were reported in 2008 in red foxes (35.5%), raccoon dogs (21.0%) and wolves (5.6%; and 2.9% with *E. granulosus* s.l.). In a recent report, 15 human CE and 29 AE cases were registered (1996-2010). Radical surgery was performed on 12 (41.3%) AE patients, 11 had asymptomatic or uncomplicated infections. Recurrence appeared in one patient (8.3%). Non-radical or palliative surgery due to AE complications was performed on 15 patients (51.7%). Post-surgery complication occurred in 17% of AE patients (lethality rate up to 3.4%). Among 15 CE patients, 3 underwent liver resection, 8 pericyst ectomy and 4 guided percutaneous treatment (PAIR).

An increase in human echinococcosis has been recorded in Latvia, with the majority of patients living in rural households (71.9%) and owning dogs (56.1%) or livestock (35.1%). The most frequent complaints at the time of admission were abdominal discomfort or tightness (38.5%), abdominal pain (24.0%), malaise (15.4%), jaundice (11.5%) and skin itching (10.6%) with the size of lesion < 5 cm in 40.5% of cases. Radical parasitotomy was performed on 18 patients less than 3 months from diagnosis, PAIR was performed on 19 patients.

For **Estonia**, little information is available on the distribution of *Echinococcus* spp. Human *Echinococcus* cases have been recorded; single cases in 2000, 2003 and 2008, 2 cases in 2007 and 3 cases each in 2012 and 2013, two of which were imported from abroad. In all human cases the *Echinococcus* sp. has not been differentiated. The rare occurrence of *Echinococcus* in humans suggests that AE so far has not emerged compared with the other Baltic countries.

Despite diagnostic and treatment innovations, AE still has a high morbidity and lethality in the Baltic region. For the control of CE, on-farm transmission patterns and the seasonal home-slaughtering tradition represent major risk factors.

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Oral Presentation

Cystic echinococcosis in Bosnia and Herzegovina – an overview

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Cystic echinococcosis is a parasitic zoonosis that affects human and animal health and thus represents a public health and economic problem. The causative agent of cystic echinococcosis (CE) is *Echinococcus granulosus*. This tapeworm lives in the intestines of dogs and other canids, which are the final hosts for this pathogen. The life cycle of this parasite includes dogs as definitive hosts and mainly sheep, but also pigs as intermediate hosts. Occasionally, other herbivores and sometimes people can be infected as well.

The aim of this work was to document the distribution of (CE) in animals and humans in Bosnia and Herzegovina. A retrospective compilation study based on the results of various past studies and on the regular report of notifiable infectious diseases in humans was performed.

According to previous studies carried out in ruminants in Bosnia and Herzegovina, CE is the most frequently reported zoonotic disease. *Echinococcus granulosus* (sensu lato) in ruminants was found in 27.2% of cattle and in 80.3% of sheep. According to more recent findings, among 1,939 examined ruminants, *Echinococcus* was identified in 22.22% of all examined animals, with a prevalence of 21.97% in cattle and of 65.25% in sheep.

The problem of CE in Bosnia and Herzegovina has predominantly been handled by veterinarians, while in human medicine it has rarely been addressed, except in the form of reports from surgeons who have treated this disease as a surgical problem.

CE is also an important disease in humans in the whole area of Bosnia and Herzegovina, which is supported by the data of a seroepidemiological study conducted in 2002. Based on 500 blood samples from people living in the area of Herzegovina, 8.30% were seropositive.

A serological study conducted in humans between 2010-2012 with a total of 173 blood samples showed that 31 (17.91%) were seropositive. Among these seropositive individuals, 20 samples were from men and 11 from women, and the predominant age group was 40-50 years.

CE in ruminants and humans is a frequent zoonosis in Bosnia and Herzegovina. Unfortunately, not enough attention has been paid to this problem and systemic prevention programmes are not yet implemented to control this disease.

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Oral Presentation

The high prevalence of *Echinococcus multilocularis* in red foxes in the tourism region of southern Poland

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The study aim was to assess the prevalence of *Echinococcus multilocularis* in red foxes in the tourism region of the sub-Carpathian area during 2011-2012. With the use of the sedimentation and counting technique on 20% of the intestinal sediment we examined 314 samples from foxes shot by hunters in an area of 4,943 km² (four neighbouring counties). The mean prevalence of *E. multilocularis* was 35.0%, with 9.6%, 26.6%, 53.6% and 58.0% in each county. The analysis of data on human alveolar echinococcosis (AE) in the last two decades in Poland showed that AE is emerging in this country. Based on the agglomeration of AE cases (n=85) in north-eastern Poland in the highest risk areas, a national programme was implemented for AE screening and an effort made to increase public awareness and primary care physicians' training to improve early detection of the disease. Current results indicate, that such action should also be applied in other areas with recognised high *E. multilocularis* incidence in foxes, in particular in tourist regions frequently visited by guests including families with children. Of the 121 AE cases recognised to date in Poland, four were detected in children aged 6-11 years which indicates very early exposure. Efforts to increase awareness in local communities, starting with children, are crucial for AE prevention.

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Oral Presentation

Increase of *Echinococcus multilocularis* prevalence in red foxes, but no positive dogs found in South Limburg, the Netherlands

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The zoonotic parasite *Echinococcus multilocularis*, causative agent of alveolar echinococcosis (AE), was first detected in the Netherlands in 1996 in the provinces of Limburg and Groningen. In Limburg, *E. multilocularis* is spreading northwards and the average worm burden of infected foxes is increasing. A risk map model predicted an increase in the number of human AE cases.

To determine the current prevalence of *E. multilocularis* in the eastern suburban areas of Maastricht (South Limburg), 37 foxes in this area were hunted in the winter of 2012-2013. They were examined by the Intestinal Scraping Technique and by a nested PCR on colon contents. Additionally, 142 faecal samples of dogs from Maastricht were analysed for *E. multilocularis* by QPCR.

A prevalence of 59% (95% CI 43-74%) was found, which is a significant increase compared to a study in 2005-2006, when 11% (95% CI 7-18%) of the foxes were infected. Average worm burden increased to 37 worms per fox. However, updated prediction on the number of human AE cases indicates that the risk to local residents in the near future is within the previous prediction. No positive dogs were found, but questionnaires from owners showed that deworming practices need to be improved.

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Oral Presentation

Endemic focus of alveolar echinococcosis in Slovakia

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During the last decades, the known range of *Echinococcus multilocularis* in Europe has extended. In Slovakia, the tapeworm was detected for the first time in red foxes in 1999 and since then intensive epidemiological studies have been carried out.

A modified sedimentation and counting method was applied for *E. multilocularis* detection in the main definitive host, the red fox. Human cases of alveolar echinococcosis were confirmed by a combination of serological methods, PCR, imaging techniques and histological findings in cooperation with medical doctors and hospitals from different Slovak regions.

Actual overall prevalence of *E. multilocularis* in red foxes exceeds 30.0%. The monitoring revealed the existence of highly endemic localities in several northern districts of Slovakia with prevalences reaching as much as 60.0%. The first human case of alveolar echinococcosis in Slovakia was confirmed in 2000 and to date the total number has reached 33 cases. Of these, 26 live in the highly endemic Žilina and Prešov Regions. Mean age of patients is 51.4 years, but what is remarkable is the occurrence of the disease in four (12.1%) people below 20 years of age. The study presents a comprehensive picture of epidemiological situation of *E. multilocularis* in Slovakia.

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Oral Presentation

Cystic echinococcosis in Latvia

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Although Latvia is geographically relatively closely located to known endemic regions of cystic echinococcosis (CE), there are no recent large-scale data on the incidence, diagnosis and treatment of this disease in our country.

Thus we retrospectively analysed data of 93 patients with CE diagnosed in the period between 2002 and 2012.

Geographically, we identified two regions in Latvia where CE is more common. The typical echinococcosis patient is female and aged between 56 and 65 years. The majority of patients live in a rural household (71.9%) and own dogs (56.1%) or livestock (35.1%). The most frequent complaints at the moment of admission were abdominal discomfort or tightness (38.5%), abdominal pain (24.0%), malaise (15.4%), jaundice (11.5%) and skin itching (10.6%). Ultrasound analyses showed that echinococcosis manifested most frequently as a solitary lesion (64.9%) in the right hepatic lobe (62.2%) in an otherwise unchanged liver (65.8%), the size of lesion was < 5cm in 40.5% of cases. *Echinococcus* seropositivity was detected in 41.4% of cases. 88.5% of the patients received chemotherapy. Radical parasitectomy was performed in 18 patients less than 3 months after diagnosis, PAIR was performed on 19 patients.

As echinococcosis is relatively common in Latvia, the diagnostic and treatment algorithm should be improved in order to increase effectiveness of available resources and thus to improve respective control strategies.

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Oral Presentation

Fox culling against *Echinococcus multilocularis*, reverse consequences

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From 2008 to 2012, a fox culling protocol was tested around the city of Nancy to evaluate its potential effects against the parasite *Echinococcus multilocularis*. The study area was divided longitudinally with the northern half devoted to fox culling and the southern half being kept as a control area. For each fox collected, biometric data were recorded (sex, age, weight, placental scars). Population trends were evaluated with spotlight count. From October to April, autopsies were carried out on a batch of foxes in each area. Following the SSCT technique, adult worms were searched for in fox intestines.

Despite the 884 foxes culled, no shift in population dynamics could be assessed. While prevalence remained stable in the control area (42.0%, $p=0.980$), it showed a slight decrease followed by a strong increase in the culled area exceeding the starting level (reaching 55.4%, $p=0.047$). No significant modification in the biometric data could be demonstrated.

Culling efforts were not constant over the whole area. In the most culled foci, the variations of the prevalence seemed to be strengthened. In this particular area, the proportion of immature foxes appeared to be significantly higher than in the control area ($p=0.029$). Young foxes are known to be more sensitive to the parasite with higher worm burdens and higher prevalence.

Our results showed that fox culling over such a territory is highly demanding (time and money) and poorly efficient. Worse; it may favour the presence of the parasite within the fox population, thus increasing the risk to the human population.

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Oral Presentation

Contribution of cats to the lifecycle of *Echinococcus multilocularis* and its zoonotic role in rural areas

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The lifecycle of *Echinococcus multilocularis* is mainly sylvatic between foxes and rodents. Domestic animals such as dogs and cats can also harbour the tapeworm.

Experimental infections conclude that cats play a significant epidemiological role in maintaining the lifecycle of *E. multilocularis* infections but few data of natural infection in cats are available to confirm this. We investigated the presence of *E. multilocularis* in a rural endemic area. 322 cat faeces samples and 30 samples of intestine taken from road-killed cats were analysed by real-time PCR and Segmental Sedimentation Counting Technique, respectively. Immature worms (681 and 7040) were identified in two cats (9%) with only one EmsB microsatellite profile already identified in foxes from the same area. A faecal prevalence of 3% was obtained but no *E. multilocularis* eggs were observed after flotation of positive faeces.

This prevalence in cats is slightly higher than available European data (frequently around 1%) however it confirms the negligible role of environmental contamination. The low zoonotic significance of cats is confirmed by the absence or low excretion of eggs. Nevertheless the infection may occur frequently in endemic rural areas where cats prey daily on rodents. Thus, the zoonotic risk can be higher in preferred defecating areas such as in vegetable plots where human infection can occur.

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Oral Presentation

The effects of seasonal praziquantel dosing of dogs on the prevalence of *Echinococcus granulosus* (G7) in dogs and pigs in Lithuanian villages

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Cystic echinococcosis (CE), caused by *Echinococcus granulosus* (pig strain, G7), is of public health importance in the Baltic region and Eastern Europe. The aim of this study was to examine the efficacy of four praziquantel treatments of dogs during the main slaughtering season on the incidence of CE in pigs in endemic villages in Lithuania.

Twelve villages were selected in southwest Lithuania. In six villages, every dog (n=687) was treated with praziquantel baits (5 mg/kg) in October, December, February and April for four years. Dogs in the other six villages were untreated controls (n=860). Individual faecal samples were collected from the representative number of treated and control dogs in December of each year. Taeniid eggs were isolated by the flotation and sieving method and processed with multiplex PCR (Trachsel *et al.*, 2007). Pig carcasses originating from treatment and control villages were examined morphologically.

The prevalence of *E. granulosus* was 2.76–5.28% during the study in the control dogs; *E. multilocularis* was detected in 0.28–1.11% of the samples. After three years of praziquantel treatment the prevalence of *E. granulosus* infection in dogs was reduced from 5.9% (CI95% 3.6–9.1%) to 0% (CI95% 0.0–0.9%). The prevalence of CE in pigs was 10.7–29.7% in control villages, whereas it was reduced from 39.5 to 1.9% in treated villages during the four year study. The praziquantel treatment of dogs over four years during the slaughtering season strongly reduced the small-scale, on-farm transmission of *E. granulosus* pig strain in a rural environment.

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Oral Presentation

An update on *Echinococcus* in wild canids in Alaska

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Extensive research on *Echinococcus* in Alaskan carnivores was conducted in the mid-1900s but research has since dwindled, leaving a dearth of current data. We sought to opportunistically collect samples to update information on the ecology and geospatial distribution of multiple *Echinococcus* species. From 1999 to 2010, the North Slope Borough Department of Wildlife Management and Veterinary Clinic, and Alaska Department of Fish and Game conveniently collected faeces and gastrointestinal tract samples from the carcasses of wolves (*Canis lupus*) (N=81), arctic foxes (*Vulpes lagopus*) (N=72), red foxes (*Vulpes vulpes*) (N=3), and coyotes (*Canis latrans*) (N=1). Faecal flotation and PCR analysis demonstrated a 12.5% prevalence for *Echinococcus multilocularis* in arctic foxes. Probable *Echinococcus canadensis* was found in 37% of wolves and the coyote. *Taenia* spp., but not *Echinococcus* spp., were detected in red foxes. This data is a platform to conduct further research with the goal of examining the role that climate change and other ecological factors have on the distribution, prevalence, and public health consequences of *Echinococcus* tapeworms in Alaska.

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Oral Presentation

Long-term outcome after surgical therapy of alveolar echinococcosis

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Therapy of alveolar echinococcosis (AE) is challenging. Surgical resection, especially when followed by benzimidazole treatment (according to World Health Organisation guidelines), offers the possibility of a cure.

We retrospectively analysed the long-term outcome after surgical liver resection. Between 1992 and 2013, 355 patients suffering from AE were referred to our hospital. Initial diagnosis of AE in these patients was between 1963 and 2013. 162 of these patients underwent surgical therapy, 139 with a curative intention (resection with safety margin) and 23 with a palliative intention.

In 42 patients, initially operated on with a curative intention, only an inadequate resection was carried out. 31 of all patients operated on had additionally extrahepatic manifestations of AE. Despite a curative operation, seven patients suffered from recurrent AE up to 10 years after surgery. The number of patients with recurrent AE was considerably higher after inadequate surgery. We have limited data about the importance of drug therapy with benzimidazoles after surgery. An additional aim was to evaluate the need of the safety margin and prolonged drug therapy after inadequate resection.

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Oral Presentation

Echinococcus multilocularis in Poland – current prevalence in red foxes and first cases in pigs

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Overall, 1650 intestinal samples of red foxes (collected 2009-2013) from Poland were examined by sedimentation and counting techniques (SCT). The mean prevalence of *Echinococcus multilocularis* in Poland was 16.6% (CI 14.9–18.5%) and was found in 15 of the 16 provinces. Distinct differences in prevalence were observed between regions. There was some observable regularity in the distribution of the infection; those areas with a higher prevalence were located in the eastern half of the country while those areas with a lower prevalence were in western half. The highest level of prevalence was noted in north-eastern Poland in Warminsko-Mazurskie Province (50.0%). Very high prevalence was also observed in two neighbouring provinces: Podlaskie (34.0%) and Mazowieckie (30.8%), as well as provinces in southern Poland: Małopolskie (30.0%), and Podkarpackie (48.1%). The percentage of infected foxes in the western, north-western, south-western and partially central provinces was relatively low, for example: Dolnośląskie (2.0%), Wielkopolskie (2.5%), Kujawsko-pomorskie (3.9%) Opolskie (0.0%).

Moreover, 1250 samples of pig tissues (especially livers) with different lesions (often not typical for *Echinococcus*) were examined microscopically by PCR and sequencing. Among 557 samples identified as Taeniidae, 10 were recognised as *E. multilocularis*.

This investigation shows the risk to human health from these parasites in Poland, especially in those regions with a very high prevalence.

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Oral Presentation

The impact of socio-cultural factors on the transmission of *Taenia* spp. and *Echinococcus granulosus* in Kosovo

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Echinococcus granulosus (G1), *Taenia hydatigena* and *Taenia ovis* are common parasites of sheep in Kosovo and are principally transmitted to dogs during home slaughtering. In particular, during the "Eid Celebration – Feast of Sacrifice", many old sheep are slaughtered based on the tradition to distribute meat to the poor and needy. To determine whether this tradition has a significant impact on sheep-transmitted taeniids to the dog population, a total of 1519 dog faecal samples were collected over most parts of Kosovo from January 2012 to March 2013 in three different time periods (Phases I-III) followed each time by treatment with praziquantel. The religious event was celebrated on 25-27 October 2012 (after Phase II). Using a modified flotation-ovassay and flotation-sieving techniques, taeniid eggs were isolated for DNA extraction and PCR species identification. In Phase I (n=519) taeniid eggs were detected in 33 samples (6.36%) (95% confidence interval, 4.48-8.91), 6 were positive for *E. granulosus* - sheep strain "G1", 31 contained DNA of *Taenia* spp., (in 26 *T. hydatigena* and 1 *T. ovis* were determined). Around 4-6 months later, just before the Eid Celebration, the same dogs (n=508) were tested and treated again (Phase II) and 7 samples contained taeniid eggs (1.38%) (0.61-2.95), (5 positive for *T. hydatigena*, 2 for *T. ovis* and 0 *E. granulosus*). In Phase III, around 2-3 months after the celebration, the dogs were tested again (n=492) and in 21 samples taeniid eggs were found (4.27%) (2.73-6.56) (3 *E. granulosus*, 16 *T. hydatigena* and 1 *T. ovis*). The study indicates that transmission of taeniids including *E. granulosus* increased after the Eid Celebration – Feast of Sacrifice. As the dogs were treated with praziquantel before the celebration at Phase II, these infections must have been acquired during the time of the celebration and not later based on the prepatent period of these taeniids of around 6-10 weeks. The results of this study are important for the design of control measures against cystic echinococcosis in this Balkan area.

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Oral Presentation

Contamination of kitchen gardens by *Echinococcus multilocularis*, *Toxoplasma gondii* and *Toxocara* spp. in northeastern France

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Human infection with *Echinococcus multilocularis* (Em), *Toxoplasma gondii* (Tg) or *Toxocara* spp. occurs following accidental ingestion of parasite eggs spread in the environment in the faeces of foxes, dogs or cats. Consumption of raw vegetables from contaminated kitchen gardens thus induces a zoonotic risk for human beings. The present study aims to assess and prevent this risk by characterising kitchen gardens regarding their exposure to carnivores and their contamination with *Em*, *Tg* and *Toxocara* spp. eggs. In February and March 2014, carnivore faeces were sought in 200 kitchen gardens in northeastern France where *Em* is endemic. Molecular tools applied on faecal material allowed the identification of host species. Cats, foxes and dogs represented respectively 66%, 21% and 13% of the 240 faeces found. Occurrence of *Em*, *Tg* and *Toxocara* spp. in these faeces were assessed by real-time PCR. This innovative approach highlights a sizeable number of faeces with parasite eggs in some kitchen gardens. Finally, we characterised the 57/200 (28.5%) kitchen gardens where carnivore faeces were found with the aim of proposing preventive measures to limit fox, dog and cat access to these gardens.

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Oral Presentation

***Echinococcus granulosus* in Tasmania, still there despite 30 years of control!**

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Following 30 years of well-organised and funded hydatid control in Tasmania, “provisional eradication” of *Echinococcus granulosus* was announced in 1996. However, abattoir monitoring has continued, all dogs entering Tasmania have to be dosed with praziquantel prior to travel and dog owners are required to sign a legally binding document declaring they have done so. There have been no reports of new human infections for over 20 years but there continues to be one or two cases diagnosed annually in older people, thought to have contracted the infection prior to 1996. Nevertheless, there have been over 100 abattoir reports of hydatid disease in cattle since 2000, mostly in cattle from mainland Australia, but 31 infections were in animals less than 3 years old that have never left Tasmania. Curiously, there has only been one abattoir report of infection in sheep during the same period. A recent survey of 306 rural Tasmanian dogs from areas where the infected cattle originated revealed 7.8% positive with the coproantigen test and 3 positive with coproPCR. These data indicate that the transmission of *E. granulosus* is still occurring in Tasmania. Transmission is most likely domestic as sylvatic transmission has never been demonstrated. We suspect hunting dogs may be important but currently have insufficient data to confirm this theory. Plans for continuing investigations will be outlined.

The survey of *E. granulosus* in the Tasmanian rural dogs was funded by Novartis Animal Health

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Oral Presentation

Long-time baiting campaign against the fox tapeworm (*Echinococcus multilocularis*) in Southern Bavaria

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A baiting strategy against *Echinococcus multilocularis* (E.M.) in foxes was conducted in the Starnberg district including the city of Starnberg. Air distribution of baits in agricultural and recreational areas was combined with the distribution of baits by hand in the district's towns and villages in order to cover the entire fox population (Baiting area: 213 km²). Within the first year, baits were put down monthly and within the following years at six week intervals. Bait distribution density was 50 pieces/ km². The pre-baiting prevalence was 51% (45-57% CI 95%, n=286). During a one-year period following the first 4 months' of bait distribution, only one positive fox was found (prevalence 1%; 0-7% CI 95%). Prevalence rates declined to 2% (2007), 3% (2008) and 1% (2011) and could be manifested under a 3% level.

After the baiting campaign stopped, we found in 2012 an increase of prevalence at one border of the baiting area where, during the baiting campaign, we sometimes found foxes with E.M. The other parts of the study area remained tapeworm free.

In contrast, no significant change had occurred in the untreated control area.

To ensure efficient use of resources it is crucial to know where counter-measures are most beneficial. To assist prevention efforts, a model was developed based on prevalence rates in foxes (*Vulpes vulpes*), fox population densities, fox defaecation rates and human population densities. The model calculates the likelihood of people coming into contact with E.M. For example it demonstrates that in 2005, prior to the deworming programme, the likelihood of contact in our study area was 175% of the Bavarian average. Today, after the 5-year worming programme, this likelihood is only 5% of the Bavarian average infection risk demonstrating the effectiveness of preventing humans in this district getting infected with the fox tapeworm.

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Oral Presentation

Alveolar echinococcosis prophylactic information: how to reach high-risk inhabitants

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Information diffusion is a tricky process with many disturbances potentially impeding the good transmission of the message to the target population. There is often a huge gap between financial and/or human means used for a media campaign and its results. The message content may be misunderstood, partially retained or even deformed. Moreover, as a population receives more and more entertaining messages from an increasing number of media, it becomes very difficult to choose the most efficient media for prophylaxis information. Our study focuses on one question: "how can we inform the high-risk population about alveolar echinococcosis prevention?" To answer this question, we conducted a quantitative survey on a representative sample of inhabitants from two northeast French regions highly endemic for *Echinococcus multilocularis*. From January to March 2014, 343 questionnaires were filled in to identify the socio-demographic profile of people with regards to their knowledge of the disease and their media preferences. The population interviewed did not consider public administration as a source of good information. Paradoxically, it is often considered unreliable. These results will help to develop efficient communication tools to prevent human contamination by *E. multilocularis*.

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Oral Presentation

Geographical information systems: a valid tool to study the epidemiology of cystic echinococcosis

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The aim of this work was to study the distribution and epidemiology of cystic echinococcosis (CE) in water buffalo, cattle and sheep in the Campania region (southern Italy) using Geographical Information Systems (GIS), in order to better understand the chain of transmission of *Echinococcus granulosus*. The results of the epidemiological study showed a prevalence of CE in cattle and water buffalo of 10.4% and 10.5%, respectively, whilst in sheep it was 31.2%. The examination of the epidemiological data provided by the GIS showed a close proximity of the bovine and/or water buffalo CE positive farms with the ovine farms present in the study area. This would suggest that free-ranging canids become infected with *E. granulosus* when feeding on sheep carcasses left on pastures, or from offal resulting from home-slaughter on sheep farms and that these dogs then go on to shed infective eggs on neighbouring cattle/buffalo farms. In conclusion, the use of GIS is a novel approach to further understand the epidemiology of CE and thus to introduce appropriate local control measures.

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Oral Presentation

The ecology of *Echinococcus multilocularis* transmission in urban settings in North America

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Recent work on *Echinococcus multilocularis* has shed light on the distribution, host partitioning and its genetic characterisation in North America. This parasite is likely expanding its range in the central region of North America and invasions of European strains may have occurred. In large urban settings in central western Canada, where domestic dogs overlap with wild canid populations (e.g. fox, coyote) in city parks, the parasite is endemic and the risk of transmission to dogs, hence to people, is unknown.

In 2010, we started a research program on the ecology of *E. multilocularis* in Calgary, AB, Canada, to identify the host partitioning, the spatial and temporal patterns of infections in definitive and intermediate hosts, and the ecological processes that determine these patterns. Through coyote and fox carcass citywide collection and sampling of coyote faeces and small mammals in 5 large city parks, we have monitored the parasite distribution in the main definitive and intermediate hosts. Faecal samples have been genotyped to identify individual coyotes and assess their parasitological status. Faecal samples were also submitted to diet analysis and dietary preferences determined comparing the use vs. the availability of rodent species. Rate of encounter with the parasite was estimated calculating the overall number of intermediate hosts consumed per faeces. Prevalence in dogs was estimated collecting dog faeces from a random sample of 218 owners within a broader study on dog parasites. Parasite specimens have been submitted to molecular confirmation and strain-typing using mitochondrial DNA (*nad2*, *cob*) genes.

We found that the distribution of infections was spatially and temporally structured, with prevalence ranging from 10% (summer) to 43% (spring) in coyotes (overall) and from 0.46% to 3.3% in rodents, with an area of hyper-endemicity in coyotes with prevalence as high as almost 90% in spring. Higher prevalence in definitive host was associated to higher proportion of intermediate host species in rodent assemblages and preference for those species in the coyote diet. In the hyper-endemic area, re-infection rate of individual coyotes was estimated to be 57%.

We estimated that coyotes eat more than 300 intermediate hosts per year, with highest encounter rate with *E. multilocularis* in winter estimated to be 2.4 infected hosts predated/season (95% CI: 0.4 - 8). Two dogs were positive to Taeniidae eggs at faecal analysis, but only one could be molecularly confirmed (prevalence=0.46%). The infected dog was walked regularly in the area of hyper-endemicity and was reported to have rodent hunting habits.

All parasite specimens were genetically consistent with the European strain, except one collected in a fox carcass in Calgary and one coyote from Edmonton were consistent with the North American (N2) strain.

Our results emphasise the need to better understand the complexity of host communities and their role in shaping transmission and distribution of the parasite, particularly in urban settings. A recent human case in Alberta suggests that the scientific community may need to reconsider the public health risk and increase surveillance efforts within a one-health framework to monitor for new potential cases of human alveolar echinococcosis

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Poster Presentation

TGF β production in human PBMC stimulated by both *Echinococcus multilocularis* vesicular fluid and TLR agonist

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Previous works on murine models suggested that *Echinococcus multilocularis* (Em) has developed mechanisms able to impair the natural inflammatory host response. The aim of the present study was to investigate the interaction between Em vesicular fluid (VF), human peripheral blood monocytes cells (PBMC) and different Toll-like receptors (TLR) agonists, in order to better understand the mechanisms involved in the development of alveolar echinococcosis (AE) in humans.

PBMC were obtained using blood from healthy donors and Em-VF was kindly provided by Professor Gottstein (Parasitology Institute of Berne, Switzerland). Four TLR agonists were tested: LPS (TLR 2/4), Poly IC (TLR 3), R848 (TLR 7/8) and CpG (TLR9). TGF β secretion was assessed by ELISA.

A significant increase of TGF β was observed only in the case of stimulation with both LPS and Em-VF ($p < 0.05$, Wilcoxon signed-rank test).

This result suggests that Em-VF needs a concomitant stimulation by TLR 2/4 to induce TGF β secretion in human PBMC from healthy donors. This study also supports the fact that TGF β , a major regulator of the immune response, plays an important role in the development of AE in humans.

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*This work was supported by the network Inflammation-Cancer-Biotherapy of the University of Franche-Comté

Poster Presentation

Decrease of CD86 expression in human MoDC exposed to *Echinococcus multilocularis* vesicular fluid

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Previous works on murine models suggested that *Echinococcus multilocularis* (Em) is able to impair the natural inflammatory host response. The aim of this study was to investigate the impact of Em vesicular fluid (VF) on monocyte-derived human dendritic cell (MoDC) maturation to better understand the mechanisms involved in the development of alveolar echinococcosis (AE) in humans.

MoDC were obtained using blood from healthy donors and Em-VF was kindly provided by Professor Gottstein (Parasitology Institute of Berne, Switzerland). MoDC were exposed to Em-VF and subsequent CD80, C83 and CD86 expression was assessed by flow cytometry. Levels of IL-10 and TGFβ were measured in supernatants using immune-assay.

Exposure of MoDC to Em-VF decreased the expression of CD86 ($p < 0.05$, Wilcoxon signed-rank test). No significant differences between IL-10 and TGFβ levels were observed.

Exposure to Em-VF impacted the maturation of MoDC, but only partially, as the other co-stimulatory molecule's expression and levels of cytokines remained stable.

This result may be due to the use of cells from healthy donors as previous studies demonstrating Em-VF impairment of the host response did use dendritic cells from AE infected mice stressed ex-vivo with Em-VF.

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*This work was supported by the network Inflammation-Cancer-Biotherapy of the University of Franche-Comté

Poster Presentation

Clinical staging of cystic echinococcosis of the liver

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A standard set of definitions that describes the severity of cystic echinococcosis (CE) of the liver is critical in clinical decision making and for collaboration between medical specialists. A TN(R)C point-system was elaborated to combine the World Health Organisation -Informal Working Group on Echinococcosis (WHO-IWGE) staging of echinococcal cyst and clinical cyst complications. Four clinical cyst-scoring parameters were determined: existence of the cyst and its topographic localisation (T), its natural history (N), whether the cyst is recurrent (R) and its complications (C). Three clinical stages of CE that reflect the severity of the disease were identified in a range of 0 to 9 pts:

0 pts - no CE or cured cyst;

1 pt - residual cavity/lesion after treatment;

2-3 pts or Grade I - uncomplicated inactive cysts, both recurrent and non-recurrent, uncomplicated transitional cysts (R0) and inactive cysts with slight complications (R0);

4-6 pts or Grade II - grade of cyst reflects grade of complications;

7-9 pts for Grade III;

The results of the treatment of 87 consecutive patients (during the period 2008-2012) with CE of the liver were analysed. According to the size and number of cysts, the patients were assigned to either open or laparoscopic surgery. Intraoperative assessment of severity and stage of the disease was performed by applying the TN(R)C classification system (view online at <http://www.kjossev.free.bg/>).

In the open surgery the radical technique was used in 21 patients and the conservative technique was used in 52 patients. Clear correlation was noted between cyst stage and rate of intraoperative complications, postoperative morbidity, prolonged length of hospital stay and mortality.

The present study suggests that the surgical procedures should be planned by correct assessment of cyst severity using the TN(R)C point system. This clinical staging system may have prognostic and therapeutic implications. In our view, such a tool is potentially important for epidemiological studies, confirmation of the infection status and for treatment and monitoring in control programmes.

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Poster Presentation

Strain specification of liver hydatid cysts obtained from paraffin-embedded tissues in Southern Iran – a 12 year investigation

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Different strains of *Echinococcus granulosus* are the cause of cystic echinococcosis (CE) in humans. The present study aimed to determine the genotypes of *Echinococcus granulosus* utilizing NAD1 mitochondrial genes and sequencing. A total of 114 paraffin blocks and pathology slides of CE patients admitted to affiliated hospitals at Shiraz University of Medical Sciences during the years of 2000 to 2011 were selected. The specimens were de-paraffinized, total DNA was extracted and amplified by PCR on 91 samples and finally 21 samples were sequenced. The sequences were blasted, aligned and compared using GenBank data and finally a phylogenetic tree was drawn using the sequences. Out of 21 samples sequenced, 17 cases were diagnosed as G1 and 4 cases as G6. The mean age of the patients was 30.35 in patients infected with G1 and 41 years in patients infected with G6 strains). The distribution of patients according to sex 16 (76.19%) were female (13 and 3 cases infected with G1 and G6 strains respectively) and 5 (23.80%) were male (4 and 1 cases infected with G1 and one case by G6 respectively). The mean diameter of the cysts was 6.76 and 11.5 cm in G1 and G6 strains; showing G1 as more common strain in human in this region.

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Poster Presentation

Echinococcus granulosus in pigs in central Poland – the pilot study

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Cystic echinococcosis (CE), an important zoonotic infection, is considered an emerging disease in Eastern Europe and Asia. Recently in Poland the number of reported CE human cases was 29-36 per year (2010-2012, data of National Institute of Public Health). According to data from regional Veterinary Inspectorates, hundreds of morphologically unidentified lesions have been detected in pig livers during post-slaughter inspection. Since many of the lesions have been identified and reported as hydatid cysts based only on visual identification, this pilot study aimed to assess the prevalence of metacestodes of *E. granulosus* in pigs. The livers with lesions were collected in slaughterhouses subjected to four County Veterinary Inspectorates in central Poland. Data of pigs' origin were gathered with the aim for a further investigation of dog faecal samples from areas where *E. granulosus* positive pigs originated. To date we have collected over 170 samples of pig livers with unidentified nodular foci or/and cysts. Morphological examination (histopathology) to investigate the presence of fertile/non fertile cysts and the genetic identification of *Echinococcus* species are currently being performed.

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Poster Presentation

Bone manifestation of alveolar echinococcosis in a child (case report)

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Bone involvement is a very rare manifestation of alveolar echinococcosis especially in childhood. The authors present a case of a 9 year old boy with a bone manifestation of the infection. The diagnosis was established at the age of 7. The boy suffered from pain and limited mobility in the right leg. He underwent surgery twice – excochleation of bone cystae of the femur. Diagnosis was confirmed histologically, serologically and by means of PCR. Treatment with albendazole was started but it had to be interrupted several times based on adverse reactions (severe leukopenia, anaemia, alopecia). The child's parents repeatedly refused radical surgery, so treatment with albendazole continued, monitoring the levels of haemoglobin and leukocytes regularly, and therapy interrupted if necessary. PET/CT scan was performed twice within a one-year interval and improvement revealed.

Herein we report the first case of bone echinococcosis in Slovakia. This is an unusual localisation in an early childhood patient with complicated treatment due to adverse reactions to long-term therapy with albendazole. Refusal of the recommended radical surgery and no alternative treatment regimen epitomise the possibility of further severe complications.

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Oral Presentation

Identification of the lifecycle of *Echinococcus canadensis* in Corsica

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The lifecycles of *Echinococcus granulosus* sensu lato are mainly domestic between dogs and livestock but wildlife species can also be partially or totally involved. Corsica is the most important focus of human cystic echinococcosis in France with an annual incidence of 1.3 cases for every 100 000. In recent slaughterhouse surveys, only pigs were observed infected but with a high prevalence of 5% and at the same time a similar prevalence of wild boars harvested during the hunting season was established. The mixed genotype G6/7 of *Echinococcus canadensis* was systematically identified.

259 samples of faeces from hunting dogs were collected and analysed by a new multiplex real-time PCR for *E. granulosus* leading to a prevalence of 1.2% of *E. canadensis* G6/7.

The dog is confirmed as the definitive host of the *E. canadensis* lifecycle in Corsica. It is the first molecular diagnosis of *E. granulosus* s.l. in dogs in France. The analysis of the owner questionnaire revealed hazardous practices concerning deworming frequency and the availability of raw viscera for dogs after hunting or worse by direct feeding. This first survey targeting hunting dogs needs to be completed by others and also by the investigation of the potential role of the fox as a wild definitive host since foxes are widely present in the island.

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Poster Presentation

Genotype identification of *Echinococcus granulosus* isolates from human cystic echinococcosis patients in Kerman, Iran

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Cystic echinococcosis (CE) is endemic in most provinces of Iran. This study aimed to identify the genotypes of *Echinococcus granulosus* sensu lato in CE patients in Kerman, southeastern Iran.

Individuals who had undergone hydatid cyst surgery in Afzalipour Medical Centre from April 2011 to September 2013 were included in the present study. Patient information, including demographical and clinical data, was collected. Hydatid cyst samples were collected from operating rooms. Protoscoleces/germinal layers from each individual isolate were washed three times in normal saline and each isolate was kept at -20 °C until use. After total genomic DNA extraction, genotype of each *E. granulosus* isolate was determined using PCR-sequencing of two mitochondrial *cox1* and *nad1* genes.

Forty-two patients (23 females, 19 males) participated in the study; the mean age was 43 ± 31.9 years. Pulmonary hydatid disease was observed in 34 patients (81.0%), 11 patients (26%) had a history of previous hydatid surgery. Eighteen (42.9%) and 24 (57.1%) patients were infected by *E. granulosus* sensu stricto (G1-G3) and *E. canadensis* G6 genotype, respectively.

Both sheep (G1) and camel (G6) strains of *E. granulosus* occur in Kerman Province. G6 genotype was significantly more frequent than G1-G3 in the southern regions of the province.

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Poster Presentation

Long-term trends in prevalence and ecology of *Echinococcus* in wolves (*Canis lupus*) in Isle Royale National Park, Michigan, USA

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Echinococcus multilocularis is present in wild canid populations in central North America. However, Isle Royale National Park (ISRO) wolves were not previously studied for the presence of any species of *Echinococcus*. From 1999 to 2014, over 300 faecal samples and complete demographic, pack and genetic data were collected from wolves in ISRO. Initial analysis of 35 faecal samples collected from 23 wolves from 2010 to 2014 (15 samples from 15 individual wolves; 8 samples from 4 wolves collected over 2 different years; and 12 samples from 4 wolves collected over 3 different years) (2010: N=8; 2011: N=13; 2012: N=8; 2013: N=5; 2014: N=1) found taeniid eggs in 8 (34.8%)

individual wolves. Two of these individuals were infected over two consecutive years. Multiplex PCR analysis indicated an *Echinococcus granulosus* sensu lato genotype. *Echinococcus canadensis* is expected upon sequencing. These initial samples will be examined in combination with the rest of the faecal samples to study the prevalence and ecological trends of *Echinococcus* spp. in ISRO wolves over the last 15 years.

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Poster Presentation

Morphological analysis of hepatic hydatid disease in an extensive single-centre study of surgically resected human tissue samples

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Hepatic echinococcosis is a parasitic zoonosis caused by the tapeworm genus *Echinococcus*. Two species are recognised as being able to cause disease in Latvia: *Echinococcus granulosus* causing cystic echinococcosis (= hydatid disease) and *Echinococcus multilocularis*, causing alveolar echinococcosis. So far, we do not know which species is most common in human patients.

The purpose of this study was to examine microscopic slides of human liver tissue obtained during surgical treatment in a single university hospital (time range 2003-2012), in order to determine the *Echinococcus* species based on morphological features. The parameters to analyse the samples comprised morphological characteristics of the parasite endocyst, ectocyst, adventitia and tissues around the cystic lesions.

In total, 48 echinococcosis cases were examined and 33 were suitable for further examination, as with those patients a definitive diagnosis of hepatic echinococcosis was reliably feasible. In rare cases, we were able to assume also the species of the parasite, as they presented specific morphological structures typical for the two *Echinococcus* species. From this collected data, we can conclude that patients in Latvia can be infected with both *Echinococcus* species. However, a definitive species-specific diagnosis just from the histopathology of the parasite is not always reliable. Therefore we recommend to further confirm the diagnosis e.g. by PCR.

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Poster Presentation

Development of a new sensitive and high throughput MC-PCR assay applied in the surveillance of *Echinococcus multilocularis* in fox scats in Sweden

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We describe a new magnetic capture probe DNA extraction method and real time PCR assay (MC-PCR) for the detection of *Echinococcus multilocularis* in fox scats. Taeniid-specific DNA extraction from 3 grams of faeces using a biotinylated hybridization probe and streptavidine-coated magnetic beads ensures high sensitivity and low inhibition of the assay. The sensitivity was determined by validating the method against the sedimentation and counting technique (SCT) on faecal samples from foxes originating from Switzerland, where *E. multilocularis* is highly endemic. Of 93 fox samples that were positive using the SCT, 82 (88%) were positive in the MC-PCR. The specificity was evaluated on 2158 fox scats collected in Sweden, a low prevalence area. 2156 samples were negative in the MC-PCR implying a diagnostic specificity of at least 99.9%.

This test is well suited to surveillance programs for *E. multilocularis* where sampling of fox scats is done to reduce the costs of sampling and where a test with a high sensitivity and a very high specificity is needed.

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Poster Presentation

Animal and human echinococcosis/hydatidosis: updates on the epidemiological status of the disease in Portugal

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Echinococcus granulosus is the only species described in our country, and the genotypes most associated with ruminant and human infection are G1, G3 or the complex G1-G3. Recent data highlight the finding of G7 genotype in wolves and in cattle.

The incidence of human hydatidosis increases from north to south, Alentejo being the province with highest record, while animal hydatidosis follows an opposite trend, increasing from south to north. Both medical and veterinary state officers consider that the disease is somewhat underestimated due to the lack of report. Following a rise up until the 1980s, it seems the human disease is declining, probably due to widespread public information regarding basic hygiene measures, a greater role in primary prevention, as exemplified in the regular deworming of dogs, as well as to the establishment of multidisciplinary groups dedicated to the control of hydatidosis, such as occurs in some areas of the Alentejo province. However, this province is still considered an endemic one by the official Public Health National Laboratory, especially the Évora district. In 2008-2013, 35% of new positive cases and 71% of positive humans following hydatid surgery came from that area. In the north province of Trás-os-Montes, *E. granulosus* showed 8-11%, 30% and 7-12% prevalence, in swine, small ruminants and dogs, respectively. Feeding 80% of dogs with viscera/raw meat and possession of dogs by 92% of elementary school children can be troublesome regarding its dissemination and control in this region. The recent findings of *E. granulosus* in wolves (G7) and hydatid cysts in wild boar highlight the importance of wild hosts and a possible wild cycle or the intersection of domestic and wild ones.

In conclusion, echinococcosis/hydatidosis is slowing down, but its endemic status in some southern and northern locations, together with the new findings in wildlife and low report level, stress the importance of establishing an active surveillance of this disease, allowing timely public-health intervention directed at target populations.

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Poster Presentation

Ring-tailed lemur (*Lemur catta*), another intermediate host for *Echinococcus granulosus*

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Cystic echinococcosis (CE) caused by *Echinococcus granulosus* is an important zoonosis, endemic in the Mediterranean region. This tapeworm has a wide host-wild species range but it never was observed, to our knowledge, in lemurs. The only reported cases refer to *Echinococcus multilocularis* in Swiss (1989) and French (2013) zoos. The lemur is an omnivorous primate, endemic only in Madagascar and present in many zoos. A 13 year old male lemur, host of a colony in a zoo of the province of Ravenna (Northern Italy) died and at the necropsy a lot of transparent cysts were observed in the lungs and abdominal cavity. The cysts were of different sizes from 4 cm to a few mm, many were free floating in the peritoneal cavity; no protoscolex was microscopically observed. Molecular identification revealed that the specimen found was *E. granulosus sensu stricto*. Histologically a multifocal severe parasitic granulomatous pneumonia was noted. In this case, suitable definitive hosts (canidae) for *Echinococcus* are not present and the region is not endemic for CE, but the lemur originated from a southern Italian zoo in an area highly endemic for CE.

The authors believe that this report represents the first case of CE in *L. catta*.

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Poster Presentation

***Echinococcus multilocularis* in rodents (EMIRO): field investigation to assess the transmission pathways**

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EMIRO (www.emiro.org) is multinational EU funded project (EMIDA Era-Net) designed to improve our understanding of the role of different wild rodent communities in the lifecycle of *Echinococcus multilocularis*. The overall aim is to provide fundamental knowledge on the role of rodent intermediate hosts to identify key factors that may limit the parasite transmission in low and high endemic areas of its distribution (i.e. in Sweden, Lithuania and Switzerland). This is done by prevalence studies and by investigating predation rates of foxes on different rodent species.

Additionally, the susceptibility of different intermediate hosts is investigated in Denmark by experimental infections in a selection of rodent models (see separate poster). Four field study sites to collect rodents and fox scat samples have been outlined in each country. Several records of infected intermediate hosts (i.e. in *Arvicola terrestris* and *Microtus arvalis*) have been made both in Lithuania and Switzerland. The first positive record of an infected intermediate host (*Arvicola amphibius*) has been made in central Sweden. Several PCR-confirmed findings of *E. multilocularis* eggs have been made in fox scats collected in the different study sites. Status after the first year of data collection is as follows: Sweden (5 positives, n=131), Lithuania (n=45, PCR has not been conducted), Switzerland (6 positives, n=60). It is planned to investigate the predation rate of foxes on the different infected rodent species by faecal analyses. This data in combination with the prevalence rates of different intermediate hosts should allow the assessment of the relative importance of the different species for the transmission of *E. multilocularis*. This knowledge should contribute to forecast the risk for *E. multilocularis* infections and spread in different European countries.

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Poster Presentation

Establishment, immune response and corticosterone level dynamics in the intermediate host *Microtus agrestis* following experimental infection with defined doses of *Echinococcus multilocularis* eggs

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Various defined doses (100, 500 and 1000) of sodium hypochlorite resistant *Echinococcus multilocularis* eggs were administered via primary infection to the field vole (*Microtus agrestis*), a known intermediate host of the parasite. Primary infection most accurately mimics natural transmission of the helminth and is therefore most relevant to elucidating its ecological dynamics. Four C57BL/6J mice (inoculated with 1000 eggs) were used as positive controls with 3 of 4 becoming infected. Morphological (lesion establishment), immunological (lymphocyte proliferation and TNF production in response to *E. multilocularis* metacystode crude antigen, EmAg) and endocrinologic (corticosterone) responses were analysed in response to the infections. Lesion counts were not significantly different across doses however lesion establishment as a percentage of egg dose was suggesting an apparent upper limit of oncosphere establishment in the liver of this species. The remaining variables were significantly correlated with lesion establishment however no variable could be associated with the higher percentage establishment in the 100-dose group.

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Poster Presentation

Ongoing research project on the importance of rodent communities for the transmission of *Echinococcus multilocularis* in Lithuania

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The high prevalence of *Echinococcus multilocularis* in foxes and raccoon dogs demonstrates that *E. multilocularis* is of emerging concern in Lithuania. However, so far there is no information on the role of different intermediate host species in the transmission of *E. multilocularis* in Lithuania.

In the framework of a European research project (EMIDA-EMIRO), the significance of different rodent species for the transmission of *E. multilocularis* will be investigated in European countries with different epidemiological settings. In Lithuania, high endemic areas with prevalence rates in foxes ranging from 62,3% - 83,3% and areas which previously were not examined were selected for our investigations. In these areas we collected different rodent species and faeces of foxes and raccoon dogs. Rodents were trapped in places with high fox activity. Snap traps were placed in small quadrats (Myllymäki, 1971). Top Cat traps were set up in places of high activity of *Arvicola* sp.. Fox faecal samples were collected using transects. Samples were collected in spring and autumn, 2013 and 2014.

During the study 342 rodent samples, 544 fox and 15 raccoon dog faecal samples were collected. *Microtus* sp. (n=167), *Myodes glareolus* (n=68), *Apodemus agrarius* (n=56), *Apodemus flavicollis* (n=39), *Apodemus sylvaticus* (n=11) and *Arvicola* sp. (n=1) were trapped in the investigated areas. First analyses revealed that *A. agrarius* (p=0.0052) was more frequently recorded in known endemic areas while *Microtus* sp. were more frequent in previously unexamined areas (p=0.0096). DNA from liver lesions and Taeniidae eggs isolated from faeces will be tested using multiplex PCR protocol (Trachsel *et al.*, 2007).

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Poster Presentation

Molecular characterisation of *Echinococcus* spp. from human FFPE tissue samples from Latvia

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Formalin-fixed, paraffin-embedded (FFPE) tissue samples are widely used for the preservation of clinical samples, which are a reliable source of DNA. *Echinococcus* spp. in humans cause echinococcoses, which are widespread around the world. There are little facts known about the epidemiology and the genotypes of *Echinococcus* spp. in Latvia. FFPE samples are available at Riga Stradiņš University for molecular analyses, and here we present the first report about molecular characterisation of such sample types in Latvia. Cyst walls from different tissues (liver, gallbladder, diaphragm and lungs) from 50 patients, collected in the years 2001-2012 were used for a more thorough investigation. During this study, we have optimised a protocol for DNA extraction from FFPE tissue samples using the QIAamp DNA FFPE tissue kit protocol (Qiagen, Germany). Volumes of lysis buffer were increased to 200 µl, proteinase K to 25 µl with 2 h incubation (+56 °C) for liver tissue, and 1 h for other tissues. DNA quality and quantity were analysed by spectrophotometry. DNA concentration varied from 1.82 to 34.78 ng/µl. A genus-specific real-time PCR was used for the diagnosis of *Echinococcus*. In 84% of the samples was *Echinococcus* spp., detected but sequencing analysis is required for species identification.

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