EPIDEMIOLOGY OF ALVEOLAR ECHINOCOCCOSIS IN EUROPE:

Monitoring and Control Perspectives

RECENT DEVELOPMENTS

AND

NEW TRENDS

PROGRAM

ABSTRACTS

POSTERS

Technopôle Agro-Vétérinaire – Domaine de Pixérécourt

NANCY (France) – 8th and 9th of December 2010

GREETINGS:

Dear attenders,

As the President of the French Establishment for studying and fighting against Rabies and Zoonotic diseases (ERZ), let me tell you how glad I am to be in charge of the organization of this European congress on alveolar echinococcosis. Additionally, as a local representative, I am delighted to welcome scientists from 18 European countries to share and centralise information on this particularly concerning topic.

The excellence of the presentations scheduled in the program and the quality of the studies displayed as posters augur a lot to hear and discuss. Undoubtedly this congress will help to homogenize the knowledge about the epidemiology, the control strategies and the human prevention of *Echinococcus multilocularis*.

Since you are going to stay in Lorraine for a few days, we will try to make you appreciate our nice region which is the historical link between eastern and western Europe thanks to Stanislas, King of Poland and father-in-law of Louis the XVth, King of France within the 18th century. This duke of Lorraine would have been glad to personally receive you for the farewell party in the Royal Nancy City Hall.

I wish you all a studious and outstanding stay in Nancy.

Yours faithfully,

Jean-Paul BOLMONT
President of the ERZ

STEERING COMMITTEE:
ANSES (French Food Safety Agency) - National Reference Laboratory for *Echinococcus*
ERZ: French Association for Rabies and Zoonotic Diseases Control.
UFC: University of Franche-Comté - WHO Collaborating Center for prevention and treatment of human echinococcosis.

SCIENTIFIC COMMITTEE:
Dr. A. BERLIOZ-ARTHAUD: Head of the Anses-Nancy laboratory
Dr. F. BOUE: AFSSA-Nancy, Head of the Wildlife Diseases Unit and the French National Reference Laboratory for *Echinococcus*.
Pr. S. BRESSON-HADNI: WHO Collaborating Center, Medical referee in hepatology
Pr. C. CAPDEVILLE-ATKINSON: University of Nancy, Vice President
Mr. B. COMBES: Head of the ERZ
Pr. P. GIRAUDOUX: UFC - WHO Collaborating Center, Professor of ecology
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Pr. G. MANTION: UFC - WHO Collaborating Center, Professor of surgery
Pr. L. MILLON: UFC - WHO Collaborating Center, Professor of parasitology
Dr. F. RAOUL: UFC - WHO Collaborating Center, Associate-professor of ecology
Pr. D.A. VUITTON: UFC - WHO Collaborating Center, Professor emeritus of immunology

A. Animal hosts: current and changing epidemiological/ecological situations in Europe

Keynote speaker	Pr. Thomas Romig	Institute of Parasitology, Hohenheim University, Germany
Chairman	Dr. Joke van der Giessen	National Institute for Public Health and the Environment, The Netherlands
Reporter 1	Dr. Edoardo Pozio	Head of the European Union Reference Laboratory for Parasites - Istituto Superiore di Sanità, Italie
Reporter 2	Pr. Patrick Giraudoux	WHO Collaborating Center Chrono-environnement - UMR 6249 University of Franche-Comté, France

B. Advances in human epidemiology

Keynote speaker	Dr. Bruno Gottstein	Institute of Parasitology, University of Bern, Switzerland
Chairman	Pr. Laurence Millon	WHO Collaborating Center Chrono-environnement - UMR 6249 Department of Parasitology and Mycology CHU Jean Minjoz - Besançon, France
Reporter 1	Dr. Peter Kern	Comprehensive Infectious Diseases Center Div. Infectious Diseases University Hospital and Medical Center Albert-Einstein - Ulm, Germany
Reporter 2	Dr. Solange Bresson-Hadni	Professor of hepatology WHO referee in hepatology CHU Jean MINJOZ - Besancon, France

C. Control strategies

Keynote speaker	Dr. Daniel Hegglin	Institute of Parasitology, University of Zurich, Switzerland
Chairman	Dr. Per Have	European Food Savety Authority (EFSA) AHAW Panel
Reporter 1	Dr. Frank Boué	NRL (National Referency Laboratory) Echinococcoses ANSES Nancy (French Agency for food, environmental and occupational health safety), France
Reporter 2	Dr. Paul Torgeson	Institute of Parasitology, University of Zurich, Switzerland

PROGRAM

Tuesday 7th December 2010: 19h30 Welcome cocktail at the DOUËRA

Wednesday 8th December 2010

TIME		TITLE	SPEAKER	COUNTRY
09:00-09:30		***OFFICIAL OPENNING***		
09:30-10:00	**A**	***ECHINOCOCCUS MULTILOCULARIS* IN ANIMALS: GEOGRAPHICAL DISTRIBUTION AND HOST SPECIES**	**Romig T.**	**Germany**
10:10-10:25	A	*Echinococcus* spp. found in carnivores in Latvia	Bagrade G.	Latvia
10:30-10:45	A	Helminths of red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonides*) in Lithuania	Sarkunas M.	Lithuania
10:50-11:05	A	*Echinococcus multilocularis* in final hosts in Poland - results of ten years survey	Gawor J.	Poland
11:10-11:25	A	Monitoring of the prevalence of *Em* in red foxes in Poland.	Karamon J.	Poland
11:30-11:50		***COFFEE BREAK AND POSTER PRESENTATION****		
11:50-12:05	A	Ten-Year of *Echinococcus multilocularis* occurrence in Slovakia	Miterpakova M.	Slovakia
12:10-12:25	A	Overview of the epidemiological data on the presence of *Echinococcus multilocularis* in northern Belgium.	Vervaeke M.	Belgium
12:30-12:45	A	*Echinococcus multilocularis* in Germany	Schwarz S.	Germany
12:50-13:05	A	Large scale screening of red fox intestines in search of *Echinococcus multilocularis* in France.	Combes B.	France
13:10-13:25	A	Domestic dog situation for *Echinococcus multilocularis* in different endemic areas in France	Umhang G.	France
13:30-14:45		***LUNCH***		
14:45-15:15	**B**	**CURRENT FACTS AND TREND IN HUMAN AE**	**Gottstein B.**	**Switzerland**
15:25-15:40	B	Epidemiological trends of human alveolar echinococosis in Franche-Comté from 1980 to 2010	Giraudoux P.	France
15:45-16:00	B	Human alveolar echinococcosis in France, update 2010	Grenouillet F.	France
16:05-16:20	B	Serological evidence for human alveolar echinococcosis in Slovenia (2006-2010)	Šoba B.	Slovenia
16:25-16:45		***TEA BREAK***		
16:45-17:00	B	*Echinococcus multilocularis* in the Netherlands: what about the human situation?	Kortbeek T.	Netherlands
17:05-17:20	B	Human alveolar echinococcosis in Switzerland 1956-2008	Schweiger A.	Switzerland
17:25-17:40	B	Alveolar echinococcosis emergence in patients with immune deficiency: what is the epidemiological impact	Vuitton D.A.	France
17: 45-18:00	B	In vitro screening for new compounds against *Echinococcus multilocularis* metacestodes identifies anti-echinococcal activity of mefloquine	Hemphill A.	Switzerland
18:05-18:20	AB	Epidemiological human situation and studies on foxes in Austria.	Glawischnig W.	Austria

18:30 DEPARTURE FOR WELCOME EVENING AT LUNEVILLE CASTLE

*Posters will be displayed during the whole congress

Thursday 9th December 2010

TIME		TITLE	SPEAKERS	COUNTRY
09:00-09:35	**A**	**GENETICS AND METHODOLOGY INTRODUCTION** Phylogeny and genotyping studies in Cestodes (*Echinococcus* and *Taenia*) to trace back their molecular evolution history	Knapp J.	France
09:35-09:50	A	Genetic diversity of *Em* in Hungary inferred by multi-locus microsatellite analysis	Casulli A.	Hungary, Italia
09:50-10:00		QUESTIONS		
10:00-10:15	A	Optimization of PCR assays for the specific detection of *E. granulosus* (G1 Genotype), *E. multilocularis*, *E. shiquicus* DNA extracted from tissue and canid faecal samples	Boufana B.	United-Kingdom
10:15-10:30	A	Segmental Sedimentation and Counting Technique (SSCT): an adaptable method for qualitative diagnosis of *Echinococcus multilocularis* in fox intestines	Woronoff N. Boue F.	France
10:30-10:40		QUESTIONS		

10:40-11:00 COFFEE BREAK

11:00-11:30	**C**	**CONTROL OF *ECHINOCOCCUS MULTILOCULARIS*: EPIDEMIOLOGICAL AND STRATEGIC CONSIDERATIONS**	**Hegglin D.**	**Switzerland**
11:40-11:55	C	Scientific Opinion of the AHAW, 18th of January 2007	Have P.	EFSA
12:00-12:15	C	Rural settlements – A habitat for foxes and the fox tapeworm?	Janko C.	Germany
12:20-12:35	C	Fox defecation behaviour in relation to spatial distribution of voles in an urbanized area: An increasing risk of transmission of *Echinococcus multilocularis*	Robardet E.	France
12:40-12:55	C	Capability of *Echinococcus multilocularis* to persist within fox home ranges of increasing sizes depending on vole distribution and fox behaviour	Quintaine T.	France
13:00-13:15	C	Demonstrating freedom from *Echinococcus multilocularis* in Sweden, Norway mainland and Finland	Whalstrom H.	Sweden

13:30-14:45 LUNCH

14:45-15:00	C	Increasing risk of human alveolar echinococcosis in the Netherlands and possible control options	Takumi K.	Netherlands
15:05-15:20	C	Successful long time baiting campaign against the fox tapeworm (*Echinococcus multilocularis*) in Southern Bavaria	Konig A.	Germany
15:25-15:40	C	Urban control of *Echinococcus multilocularis* in France	Comte S. Raton V.	France

15:45-16:15 TEA BREAK

16:20-16:40	**WORKSHOP A CONCLUSIONS**	Pozio E. Giraudoux P.
16:50-17:10	**WORKSHOP B CONCLUSIONS**	Bresson-Hadni S. Kern P.
17:20-17:40	**WORKSHOP C CONCLUSIONS**	Boue F. Torgerson P.

17:50 FAREWELL PARTY IN NANCY CITY HALL

ECHINOCOCCUS MULTILOCULARIS IN ANIMALS: GEOGRAPHICAL DISTRIBUTION AND HOST SPECIES

T. Romig

University of Hohenheim

Abstract

In this introductory talk, the historical development of our knowledge on *E. multilocularis* is briefly reviewed. Until the late 1980s the distribution of this parasite was still largely defined by the occurrence of human AE cases, but in the past 15 years an ever increasing number of studies on *E. multilocularis* in animals, particularly foxes, provided new insights into the range and frequency of this parasite in various parts of Europe. This development was assisted by the European collaborative project EchinoRisk, which gave a particular boost to *Echinococcus* research in the eastern part of central Europe, where ‚new' endemicity regions were identified. Despite these advances, methodical problems remain, e.g. concerning questions of increase or decrease of frequencies, or shift of range, where inadequate surveillance often does not allow valid conclusions. Apart from shortcomings on the descriptive side, our knowledge is even more fragmentary concerning the factors which determine presence, absence, and frequency, of the parasite. The key for this is most likely hidden in the ecology of small mammals, especially rodents, which is surprisingly understudied given the importance of these animals for various zoonotic disease agents.

ECHINOCOCCUS SPP. FOUND IN CARNIVORES IN LATVIA.

G. Bagrade[1,2], M.Kirjusina[3], J. Ozolins[1]

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[2] Natural History Museum of Latvia
[3] Daugavpils University, Institute of Systematic biology

Abstract

The objective of this paper is to present data of *Echinococcus spp.* in carnivores – canids and felids – in Latvia; their intensity and extensity of invasion123 lynxes, 34 wolves, 53 foxes and 23 raccoon dogs were examined according to conventional helminthological methods.
Both *Echinococcus* species known in Europe – *E. multilocularis* and *E. granulosus* – have been found in Latvia. The parasites have been detected only in wild canids – *E. multilocularis* in foxes, wolves and raccoon dogs; *E. granulosus* – in wolf.

The fox is the main definitive host for *E. multilocularis* in wildlife in Latvia with the prevalence of infected animals of 34.0% and the intensity of infection of 1–1438 parasites per animal. For the first time in Latvia *E. multilocularis* was detected in wolves and raccoon dogs. In wolves the parasites are found in 5.9% of cases with an intensity of 62–380 parasites per animal and in raccoon dogs – in 8.7% of cases with an intensity of 1–114 parasites per animal. *E. granulosus* has been found only once – in an adult male wolf. Parasite intensity was very high in the animal – 989 parasites.

The *E. granulosus* and some *E. multilocularis* samples were submitted for genotype determination. Genetic analyses were performed in Parasitological Institute of the Slovak Academy of Sciences in Košice (Slovakia) for some of *Echinococcus* from foxes and in Department of Zoology, Institute of Ecology and Earth Sciences, University of Tartu (Estonia) for *E. multilocularis* and *E. granulosus* from wolves. *E. granulosus* from Latvian wolf belongs to genotype G10. Sequenced isolates from Latvian foxes were allocated to the predominant genetic form in Europe, with partial affinity of one isolate to a genotype that had previously been reported from southern Germany.

The examination of 12 animals was performed in the Scientific Institute of Food Safety, Animal Health and Environment "BIOR". The author is thankful to the Faculty of Veterinary Medicine of the Latvian University of Agriculture for the support of the research.
The results of our study supplement the information available about the *Echinococcus* parasites in the Baltics and in Europe.

HELMINTHS OF RED FOXES (*VULPES VULPES*) AND RACCOON DOGS (*NYCTEREUTES PROCYONOIDES*) IN LITHUANIA.

M. Šarkūnas[1], R. Bružinskaitė[1,2], A. Malakauskas[1], P. Torgerson[3], P. Deplazes[2]

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[3] Section of Epidemiology, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland

Abstract

Red foxes and raccoon dogs are important hosts of a wide range of parasites including important zoonotic helminths. Recent studies have revealed that raccoon dogs are highly susceptible to intestinal *E. multilocularis* infections. However, the contribution of the raccoon dog to parasite transmission has so far not been studied. Between 2001–2006, 310 red foxes and 99 raccoon dogs were collected from 22 districts in various parts of Lithuania. Animals were labeled and sex, age, locality and date of death were recorded. Necropsy revealed that both species are infected with a similar range of helminths. Abdundance and prevalence data were analysed using a generalised mixed modeling approach. Locality of recovery of the animal was used as a random effect. For abundance data an appropriate overdispersed error structure was used during analysis. Both red foxes (58.7 %) and raccoon dogs (8.2 %) were highly infected with *E. multilocularis* although red foxes had a significantly higher abundance and prevalence than raccoon dogs. The abundance of *Mesocestoides* spp*., Taenia* spp, *C. plica*, and *C. aerophila* were also higher in foxes whilst the abundance of *Alaria*, *Uncinaria,* and *C. putorii* was higher in raccoon dogs. The results suggested that age of the fox was significant in determining the mean abundance with *E. multilocularis*, *Alaria*, *Mesocestoides* spp., *Taenia* spp, *Capillaria putorii* and *Cremosoma vulpis*. Likewise in raccoon dogs the age was significant associated with *Mesocestoides* spp. and *C. putorii.* As red foxes were more frequently and more heavily infected with *E. multilocularis* they are likely to play the most important role in transmission of this parasite in Lithuania.

ECHINOCOCCUS MULTILOCULARIS IN FINAL HOSTS IN POLAND - RESULTS OF TEN YEARS SURVEY.

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Abstract

Studies done beetwen 2001 and 2008 on the occurrence of *E. multilocularis* in red foxes (*Vulpes vulpes*) in Poland revealed the high prevalence of the tapeworm in many areas across the country. Also racoon dogs (*Nyctereutes procyonoides*) were found to be infected (5,1% among 75 shot in northern Poland). In foxes (more than 3000 examined) frequency of infection exceeding 50% was revealed in lowland areas of northern and central part as well as in southern mountainous region. However, despite more favourable climatic conditions for the transmission intensity of the parasite in the hilly areas (high annual precipitation and low mean temperature of the soil surface, which increase the survival period of eggs) a mean prevalence was lower (14.2%) than in non mountainous parts (26.8%). Landscape patterns not differ much in the regions with presence of permanent grasslands, waste lands and forests. Intermediate host species of *E. multilocularis* were not revealed to date in Poland.

The infection risk of humans in endemic areas is increased, especially due to the tradition of visiting forests for mushroom and berries picking. Since 1992 when the registration of AE started, 76 cases were recorded in Poland.

MONITORING OF THE PREVALENCE OF *ECHINOCOCCUS MULTILOCULARIS* IN RED FOXES IN POLAND – PROJECT REALIZED IN THE FRAME OF THE MULTI - YEAR PROGRAM "PROTECTION OF ANIMAL AND PUBLIC HEALTH" BY NATIONAL VETERINARY RESEARCH INSTITUTE IN PULAWY.

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Abstract

The National Veterinary Research Institute in Pulawy (Poland) coordinates the monitoring multi-year program entitled "Protection of Animal and Public Health, 2009-2013". The main aim of this program is to provide current data from Poland about cases of contamination of animal origin food, prevalence of epizootically significant animal diseases and zoonozes. Monitoring of the prevalence of *Echinococcus* infection in foxes in Poland is one of 35 projects included in this program.

The pilot studies before starting the program (2008) were carried out in two eastern provinces: Lubelskie (242 foxes) and Swietokrzyskie (111 foxes). In the first year of program (year 2009) investigations were carried out in western part of Poland in 3 provinces: West Pomeranian (90 foxes), Lubuskie (107 foxes) and Lower Silesia (53 foxes). This year (2010) samples from southern part of Poland is during examination. Up to now 56 foxes from Malopolskie province and 58 ones from Silesia province were examined. All samples (small intestines of foxes) were examined according to sedimentation and counting technique (SCT).

In pilot study we found 18.2% foxes with *E. multilocularis* in Lubelskie province (significantly higher than results obtained about ten years earlier – then only 1% foxes were positive) and 3.6% in Swietokrzyskie province (it is the first report from this region). Percentages of *E. multilocularis* positive foxes from western Poland were following: West Pomeranian province - 5.6% and Lubuskie province - 4.7% (in this two provinces prevalence was about 4 times higher than data presented about 10 years ago) and no positive ones in Lower Silesian province (one case of *E. multilocularis* infection in fox was reported in this region about 4 years ago). Till now results from 2010 (southern Poland) were following: Malopolskie – 32% and Silesian province – 10%.

Monitoring of the prevalence of *Echinococcus* infection in foxes in Poland will be continued in remaining regions of Poland up to the end of year 2013.

TEN-YEAR HISTORY OF *ECHINOCOCCUS MULTILOCULARIS* OCCURRENCE IN SLOVAKIA.

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Abstract

The first finding of *Echinococcus multilocularis* on the territory of Slovakia was recorded in 1999. In 2000 the first systematic epidemiological survey was initiated.

From 2000 to 2010 the small intestines of 4761 red foxes were investigated for *E. multilocularis* presence. Modified sedimentation and counting technique was used for the parasite detection

E. multilocularis was detected in small intestines of 1441 red foxes (30.3 %). The number of *E. multilocularis* tapeworms found in individual foxes varied from 1 to more than 245 000 with mean worm burden of 1777.

Significant differences were recorded between regions, with the highest prevalence (> 40.0 %) and infection intensity (> 2000 specimens) in the northern part of the country. In several districts prevalence reached more than 60.0 % in individual years. In contrast, in southern parts of Slovakia, from 11.5 % to 24.8 % of red foxes were infected.

In separate study a total of 325 red foxes originated in protected localities of Tatra National Park situated in northern Slovakia were examined for the *E. multilocularis* presence. The tapeworm was detected in small intestines of 140 foxes (42.7 %).

The first human case of alveolar echinococcosis was diagnosed in 2000. Up to now a total of 16 autochthonous human cases of alveolar echinococcosis were recorded in Slovakia, whereas 14 of the cases were diagnosed in people living in northern regions of the country with the highest prevalence of the parasite in foxes.

The results of long-term monitoring refer to the occurrence of high-endemic areas of *E. multilocularis* situated in northern Slovakia. The high number of infected animals inhabiting recreational areas and their close proximity with tourists represents the high transmission risk of this important parasitic zoonosis.

The study was supported by EU project EchinoRisk and Slovak Grant Agency (VEGA 2/0145/09 and VEGA 2/0213/10).

OVERVIEW OF THE EPIDEMIOLOGICAL DATA ON THE PRESENCE OF *ECHINOCOCCUS MULTILOCULARIS* IN NORTHERN BELGIUM.

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Abstract

The tapeworm *Echinococcus multilocularis* (Cestoda, Taeniidae) was detected in 1999 for the first time in Red foxes (*Vulpes vulpes*) in northern Belgium (*i.e.* Flanders)[1]. Between 1996 and 1999, 237 dead foxes were examined for the presence of this tapeworm using the intestinal scraping technique. Four foxes (1.7%) were found to be infected with *E. multilocularis* and showed medium to very high parastic burdens. Three infected foxes originated from the south of Flanders and the fourth animal came from the north of Flanders near the border with The Netherlands. In the period 2007-2008 131 foxes from Flanders were examined for the presence of *Echinococcus multilocularis*, but none of them tested positive[2]. These findings are discussed in relation to (i) the high endemicity of *E. multilocularis* in southern Belgium in final and intermediate hosts, (ii) a temperospatial analysis of compiled epidemiological data that predicted a north-western spread of the cestode from southern Belgium towards Flanders[3], and to (iii) the increased distribution of the Red fox in northern Belgium during the last three decades.

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

[2] Van Gucht S., Van Den Berge K., Quataert P., Verschelde P., Le Roux I., 2010. No Emergence of *Echinococcus multilocularis* in Foxes in Flanders and Brussels Anno 2007–2008. Zoonoses and Public Health 57(7-8): 65-70.

[3] Vervaeke M., van der Giessen J., Brochier B., Losson B., Jordaens K., Verhagen R., de Lezenne Coulander C., Teunis P., 2006. Spatial spreading of *Echinococcus multilocularis* in Red foxes (*Vulpes vulpes*) across nation borders in Western Europe. Prev. Vet. Med. 76(3-4):137-150.

ECHINOCOCCUS MULTILOCULARIS IN GERMANY.

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C. Schulze[2], L. Hoffmann[3], K. Tackmann[1]

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Abstract

Echinococcosis in animals is a notifiable disease in Germany. While infections of animals with *Echinococcus granulosus* s.l. are rarely reported from Germany, *E. multilocularis* is often found in the red fox (*Vulpes vulpes*), its main definitive host in central Europe, and in some regions, particularly in eastern Germany, also in the raccoon dog (*Nyctereutes procyonoides*). Eleven of the 16 Germany federal states reported data on their monitoring activities in 2007 to the National Reference Laboratory for Echinococcosis. Sample sizes, their geographical distribution and the investigated species varied considerably between some states. State-wide monitoring activities were implemented in Brandenburg, Mecklenburg-Western Pomerania, Rhineland-Palatinate und Thuringia. Data on 26,220 foxes that were hunted or found dead in Thuringia, Germany, between 1990 and 2009 were analyzed using a hierarchical Bayesian space-time model. The distribution of the model parameters and their variability was estimated on the basis of the sample size, the number of cases per spatial unit and time interval, and an adjacency matrix of the municipalities by using a Markov chain Monte Carlo simulation technique to assess the spatial and temporal changes in the distribution of the parasite. In the study area, the prevalence increased from 11.9% (95% confidence interval 9.9-14.0%) to a maximum of 42.0 % (39.1-44.1%) in 2005. While the infection was present in foxes only in the North-western parts of Thuringia in 1990, it had spread over the entire state by 2004. These results demand increased vigilance for human alveolar echinococcosis in Thuringia.

LARGE SCALE SCREENING OF RED FOX INTESTINES IN SEARCH OF *ECHINOCOCCUS MULTILOCULARIS* IN FRANCE.

B. Combes[1], S. Comte[1], F. Raoul[2], F. Boue[3], V. Raton[1], P. Giraudoux[2]

[1] Entente Rage et Zoonoses (ERZ)
[2] Chrono-environment – University of Franche-Comté, UMR CNRS 6249 usc INRA
[3] French agency for food, environmental and occupational health safety (ANSES), Nancy laboratory for rabies and wildlife diseases

Abstract

Alveolar echinococcosis is one of today major zoonoses. Its infectious agent, the parasite *E. multilocularis,* is reckoned to be widely present throughout the northern hemisphere. Nonetheless its geographic distribution at a lower scale (continental or national) is hardly described.

In France, data acquired until the early 2000's showed endemic areas confined to the eastern territories and the Auvergne. Hitherto, the landscape described as most suitable for the epidemic cycle of the parasite consisted mainly of grasslands at a medium altitude (400m-800m) where vole's populations, the main intermediate host for *E multilocularis*, frequently reach high densities.

Following the apparition of human cases in territories considered then free of the parasite, a wide screening protocol was implemented over the north-eastern half of France in search of the parasite. Following the gold label SCT technique, adult worms were isolated from intestinal contents of foxes, the main definitive host and major vector of *E. multilocularis*. From 2006 to 2010, 3518 foxes were homogeneously sampled over the whole ERZ territory (around 250,000km²) by night shooting and trapping. Average prevalence in each Department shows great disparities going from 0% to 52%.

The discovery of the parasite in western and southern Departments as well as the increased prevalence in historic endemic areas indicate an under evaluated situation in France. Yet, it is too early to affirm whether our results show a geographic extension of the parasite or depict the increased effort of screening. Active monitoring is now the key to better understand the epidemiology of alveolar echinococcosis in France and to reduce the risk for humans.

ECHINOCOCCUS MULTILOCULARIS IN DOGS OF TWO ENDEMIC AREAS IN FRANCE.

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Abstract

The cycle of the zoonotic parasite *Echinococcus multilocularis* is predominantly sylvatic involving red foxes as definitive host infected by predation of rodents as intermediate host. The North-East French departments of Meuse and Haute-Saône are highly endemic with a fox estimated prevalence respectively of 41% and 36%. Most of the parasites biomass occurs in foxes, although domestic dogs can be infected leading to a major risk of human contamination due to closer contacts.

We organised a collect of dog faeces after praziquantel treatment in partnership with veterinary practitioner. Eight hundred and sixty dogs faeces were collected all over the department of Meuse (n=493) and Haute-Saône (n=367). Intestinal helminth eggs were isolated from the faeces using a flotation technique and observed by microscopy. The species identification of positives samples for taenid eggs was done by sequence analysis after PCR amplification. In order to study explanative factors of infestation, each sample was associated with a questionnaire filled by the dog owners.

A large part of the dog population in Haute-Saône is described as rodent consumer by their owner (38%) whatever the type of dog (hunting, farming or pet dogs). These dogs are less wormed with an efficient anthelmintic against *E. multilocularis* than the others. Even if only 0.81% (n=7) of faeces are infected by taenid eggs, all the rodents consumer dogs in these endemics areas can be considered as at risk for transmission of alveolar echinococcosis to human.

CURRENT FACTS AND TRENDS IN HUMAN ALVEOLAR ECHINOCOCCOSIS.

B. Gottstein

Abstract

The geographic distribution of *Echinococcus multilocularis* is restricted to the northern hemisphere. In Europe, relatively frequent reports of AE in humans occur in North-alpine Switzerland, central and eastern France, Switzerland, Western Austria and South Germany. However, within the past decade, the endemic area of Europe now included many more countries such as Belgium, The Netherlands, Italy, and, more impressively, most former Eastern countries as far as up to Estonia. Worldwide there are scant data on the overall prevalence of human alveolar echinococcosis (AE). Some well-documented studies demonstrate a generally low prevalence among affected human populations. The annual mean incidence of new cases in different areas including Switzerland, France, Germany and Japan has therefore been reported to vary between 0.1 and 1.2/100,000 inhabitants. The incidence of human cases correlates with the prevalence in foxes and the fox population density. Recently, a study documented that a four-fold increase of the fox population in Switzerland resulted in a statistically significant increase of the annual incidence of AE cases.

Clinically, AE is one of the most severe helminthic diseases affecting humans. Infection is acquired upon ingestion of *E. multilocularis* eggs. Amazingly, the minimal infection dose that may inflict AE has not been determined yet for humans. As an example in mice, a peroral infection with approximately 2,000 eggs yielded in approximately 20 primary liver lesions. Once intrahepatic infection is established, the metacestode continuously grows as a tumor-like tissue in the liver. At a later stage, metastasis formation in adjacent and peripheral sites may cause detrimental obstruction of the respectively affected organs. Late diagnosis and non-treatment may result in case fatality. Findings of naturally "aborted" (= calcified) hepatic AE lesions have indicated that not all infected human individuals develop chronically progressing disease. Clinical studies on such “resistant” cases, but conversely also on immunologically impaired AE patients, disclosed the relevance of an appropriate immune response. Immunosuppressive status such as in liver transplantation or by AIDS, especially under suppressed cellular/Th1-related immunity, increases disease severity. Associated to AIDS, restoration of immunity by appropriate antiretroviral therapy leads to reinstallation of the control of metacestode development, and therefore also to responsiveness to chemotherapy. Overall, in humans a large variety of clinical presentations of AE may thus be seen. In fact, the implementation of mass screenings in endemic areas has revealed that the number of established infections in humans was far lower than the number of exposures to parasitic eggs. We assumed that a minority of individuals among humans (estimated to maximally 1 out of 10 subjects) allows the development of the *E. multilocularis* metacestode after contracting a respective parasite egg infection.

Early diagnosis of asymptomatic or clinical AE cases is a prerequisite for efficient management of the disease, such as radical surgery. Consequently, screening may be offered to populations at high risk. The currently optimal tools to be used include (species-) specific immunodiagnosis, to be complemented by imaging, conventionally first-hand ultrasonographic examination. This combined approach allows to discriminate different infection status in AE: (i) clinically manifest AE; (ii) subclinical AE with active hepatic lesions in imaging procedures; (iii) seropositive responders without presenting hepatic active lesions, e.g. individuals presenting fully calcified (dead) lesions; (iv) individuals presenting no detectable lesion at all. The latter two variants are referred to persons exposed to infection but developing a “resistant” course of infection. Serological methods have also been assessed for their value in the follow-up surveillance of operated and/or pharmacologically treated patients. A radical surgical removal of the *E. multilocularis* metacestode resulted in a rapid decrease and subsequent negativation of anti-Em2- and anti-recEm18-antibodies in ELISA. Assessing patients who received partial, palliative or no surgery and continuous benzimidazole therapy is serologically more complex. Retrospective analyses documented a rapid decrease of anti -I/3-10 or -recEm18 serum antibody levels, where the presence of respective antibodies reflects the still presence of a viable metacestode. This serological feature has to be coupled to PET investigation, where disappearance of periparasitic inflammatory reactions correlates with an inactive status of the metacestode. Overall, the backbone of AE treatment still remains the continuous medical treatment with albendazole, and if necessary, individualized interventional measures. With this approach, the prognosis can be improved for the majority of patients with AE.

EPIDEMIOLOGICAL TRENDS OF HUMAN ALVEOLAR ECHINOCOCOSIS IN FRANCHE-COMTÉ FROM 1980 TO 2010.

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Abstract

Case records of the FranceEchino register were investigated in order to assess spatial and temporal trends of alveolar echinococcosis (AE) in the Region of Franche-Comté, France, from 1980 to 2010. No changes in the average number of new cases could be detected on the regional scale over the period. However, the number of human cases significantly decreased in the Doubs department and coincidently increased in the neighbouring Haute-Saône. Kulldorff's statistics indicate a large and significant cluster of AE prevalence in the Doubs department (mostly on the Jura plateau) in the period 1980-89. It split into two clusters of smaller size in 1990-99. Then clusters faded in 2000-2010 and areas of higher prevalence maintained on the Doubs plateau and extended to the eastern part of the Haute-Saône and the Jura departments. Moreover, the occupational profile of patients changed over the 30 years of the present study with a large decrease of the 'farmer' category. Those results solidly support the idea that important changes in human AE distribution in Franche-Comté have occurred since the 80s and may still be ongoing.

HUMAN ALVEOLAR ECHINOCOCCOSIS IN FRANCE, UPDATE 2010.

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Abstract

A prospective survey of AE is conducted in France by the FrancEchino network. Analysis of cases recorded from 1982 to 2009 by this network was conducted. Four hundred seventeen AE cases were diagnosed in France during the 1982-2009 period, their median age was 60 years (ranges: 12-89). 73% of patients were symptomatic at diagnosis. Management included surgery in 56% of cases and chemotherapy (parasitocidal compounds, mainly albendazole) for 89% of cases.

Mean annual incidence rate of AE was 0.26 case per 1,000, 000 habitants (ranges: 0.16 à 0.56). At diagnosis, patients were living in the following administrative regions: Franche Comté (40%), Lorraine (19%), Auvergne (8%) and Champagne-Ardenne (8%).

AE remains a rare zoonosis, with a stable incidence in France. Endemic zone showed progressive enlargement during last decades from Eastern France and Alpes to Massif Central and Western regions.

SEROLOGICAL EVIDENCE FOR HUMAN ALVEOLAR ECHINOCOCCOSIS IN SLOVENIA (2006-2010).

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Abstract

Human alveolar echinococcosis (AE) is caused by the larval stage of the tapeworm *Echinococcus multilocularis*. *E. multilocularis* occurs in the northern hemisphere, including central and northern parts of Europe, Asia, and North America. Recent studies have shown that the endemic area of *E. multilocularis* is larger than previously known. Moreover, the parasite is expanding from rural to urban areas. The diagnosis of AE relies mainly on results from imaging studies supported by positive serology. The aim of the present study was to examine serologically whether patients in Slovenia suspected of having echinococcosis in 2006-2010 had been infected by the larvae of *E. multilocularis.*

Between January 2006 and the end of September 2010, 1358 patients suspected of having echinococcosis were examined serologically by indirect haemagglutination assay (IHA). IHA positive patients' sera were retested by Western blot (WB) for confirmation and differentiation between cystic and alveolar echinococcosis. Out of 45 patients confirmed positive for echinococcosis, serum samples from 1 patient showed WB pattern specific for *E. multilocularis* and serum samples from 7 patients showed one of the two WB patterns which cannot distinguish between *E. granulosus* and *E. multilocularis.* These serum samples were therefore retested by enzyme-linked immunosorbent assay (ELISA) for the diagnosis of human AE which confirmed AE in 1/7 patients. Altogether, 2 patients were confirmed serologically of having AE. The serological results of these patients corresponded to clinical and/or imaging findings. The incidence of AE in Slovenia in 2006-2010 is estimated at $0{,}1/10^5$ inhabitants which is slightly lower than incidence in 2001-2005 ($0{,}45/10^5$ inhabitants). However, AE is present in Slovenia and therefore health authorities should give greater attention to the infection.

ECHINOCOCCUS MULTILOCULARIS IN THE NETHERLANDS: WHAT ABOUT THE HUMAN SITUATION?

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Abstract

Introduction: In 2008 the first autochthonous case of alveolar echinococcosis was seen in the Netherlands. The patient lived in an area in the South of the Netherlands where the prevalence of infection in foxes was determined to be more than 12 %. The methods used for diagnosis include *Em* specific PCR and serology. *E.multilocularis* is not a reportable disease in the Netherlands and it is not known if the diagnosis in patients is missed.

Aim: To determine the seroprevalence of *Echinococcus multilocularis* antibodies in areas at risk in the Netherlands

Methods: 1581 human serum samples of 6 municipalities in areas at risk and 5 control municipalities were tested. Antibodies against *Echinococcus spp.* were detected using a commercial available Em2plus ELISA (Bordier) and an in house *E. granulosus* IgG ELISA. All positive samples were tested in an in house Immunoblot *E. granulosus* IgG1 to confirm the reactivity.

Results: 169 out of 1581 sera tested positive in the ELISA, 6 were positive in both ELISAs. The reactivity of the ELISA positive samples (*Eg* or *Em* or both) could not be confirmed by westernblot**.** An unexplained high reactivity was seen in children of 1-4 years and 5-9 years: 4.8 % - 23.5 % were positive, depending on the cut off level. This reactivity was strongest in the *E. multilocularis* serology.

Conclusion: We have found no evidence for specific antibodies in this selection of the dutch population. The seroprevalence is still low (<1:1581). To determine if *E. multilocularis* is a threat, we advise not to study the population but to investigate people at risk that live in regions with infected foxes.

HUMAN ALVEOLAR ECHINOCOCCOSIS IN SWITZERLAND FROM 1956 TO 2008.

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Abstract

Switzerland belongs to the core countries in Central Europe endemic for *Echinococcus multilocularis*. Several case-finding studies have been conducted between1956-2005. The current follow-up study addresses the newest developments in alveolar echinococcosis (AE) from 2006-2008. Thus our dataset covers 53 years of AE in Switzerland.

Only recently we have witnessed a significant increase in the annual incidence of AE in the years 2001-2005 (0.28 cases in 100'000 per year) compared to the years 1993-2000 (0.11 cases in 100'000 per year). From 2006-2008 the average incidence was 0.23 cases in 100'000 per year adding up to approximately 17 newly diagnosed cases annually in the whole country. Thus, the incidence currently appears to stabilize on a higher level. Average age at time of diagnosis in all studies ranged from 52 to 57 years without any significant difference. Nevertheless, the age specific incidence yields a significant increase with every 20 years of life except for persons aged > 80 years (0-19 years: 0.01 (95% CI: 0.00-0.02); 20-39 years: 0.12 (0.08-0.15); 40-59 years: 0.22 (0.17-0.26), 60-79 years: 0.35 (0.29-0.41), > 80 years: 0.31 (0.15-0.47). Thus age appears to be an important factor in the development of clinically relevant AE. In the same time period the proportion of female cases increased significantly to 55% in the years 1984-2008 compared to earlier years (46%). Interestingly, 56% of all AE cases in Switzerland from 1984-2008 have been diagnosed in patients living in urban areas. However, the incidence in rural areas is still significantly higher (0.22 per 100'000 per year from 1984-2008, and 0.14 in urban areas, respectively; $p < 0.001$).

We will also present the first geographical analysis of AE cases in Switzerland.
In conclusion we communicate the changing trends in epidemiology of human AE in Switzerland over the last 53 years.

ALVEOLAR ECHINOCOCCOSIS EMERGENCE IN PATIENTS WITH IMMUNE DEFICIENCY: WHAT IS THE EPIDEMIOLOGICAL IMPACT?

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Abstract

Background: Organ transplantation and AIDS which are associated with more severe course of AE are rare and may not affect the epidemiology of the disease. Within the past decade, patients with malignant and auto-immune diseases have been more and more often treated with Immunosuppressant drugs and biological agents. Actual influence of such situations on AE epidemiology and clinical characteristics was never systematically studied.

Methods: From the French Registry, we collected and compared incident AE cases observed in France in the 1996-1999 and 2006-2009 periods, regarding associated immune deficiency (ID). We prospectively studied diagnosis and follow-up characteristics of incident patients with ID, a diagnosis of AE between Jan 1, 2006 and Dec 31, 2009, and a follow-up until Sept 2010.

Results: Only 2/45 patients with ID were registered in the first period but 11/70 patients were observed in the second period. All 9 patients with inflammatory/malignant diseases, 4 men, 5 women, lived in the endemic area of the north-east of France. Sex ratio was not different from that observed in the Registry or among the patients without ID. Mean age at diagnosis (59.4 yrs) did not differ from that observed in non-ID patients. AE was diagnosed in patients suffering from solid cancer (3), haematological malignancies (3) and auto-immune diseases (6). Three patients combined malignancies and inflammatory diseases. All patients but one received immunosuppressant drugs. AE presentation was more symptomatic (6/9), with an acute liver abscess-like presentation in 3/9, and difficult diagnosis in 4/9; in 1 case liver AE was associated with lung aspergilloma. Serology was negative in 1 patient and diagnosis was confirmed by PCR. Less than 5 years before diagnosis, a normal liver image was available in 6/9 patients and retrospective serological tests were negative for all specific tests in 3/4 patients with available serum.

Discussion: Within the last decade, AE emerged in patients with ID. ID accelerates the course and modifies the classical features of the disease. AE as an opportunistic infection should be systematically evoked in endemic regions, but the current extension of the endemic area may make diagnosis difficult. If contamination by *E. multilocularis* either occurred within the few years before diagnosis or if AE may be ascribed to previous contacts with the parasite is a still open question. AE cases in ID patients may have contributed to the increase in incident cases observed in the past few years in France and Switzerland.

IN VITRO SCREENING FOR NEW COMPOUNDS AGAINST *ECHINOCOCCUS MULTILOCULARIS* METACESTODES IDENTIFIES ANTI-ECHINOCOCCAL ACTIVITY OF MEFLOQUINE.

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Abstract

At present, the chemotherapy of AE is based on mebendazole- and albendazole-treatment, which have been found to be ineffective in some instances, parasitostatic rather than parasiticidal, and treatment regimes usually involve the lifelong uptake of massive doses of drugs. Thus, new treatment options are urgently needed. Within this study, a recently validated parasite viability assay was applied, based on the release of phosphoglucose isomerase (PGI) by dying parasites. A range of 30 thiazolides, 19 pentamidine- and 12 artemisinin-derivatives, and of mefloquine and its (+) and (-) erythro-enantiomers, were tested for their efficacy against *E. multilocularis* metacestodes *in vitro*. Initial screening of compounds was performed at 40 µM, and those compounds exhibiting considerable antiparasitic activity were assessed also at lower concentrations. Mefloquine was chosen for subsequent studies. *In vitro* mefloquine treatment at 20 µM resulted in rapid and complete detachment of large parts of the germinal layer from the inner surface of the laminated layer within a few hours, and prolonged treatment for a period of 10 days was parasiticidal as determined by bioassay in mice. Interestingly, as determined by the PGI-assay, the (-) erythro enantiomer of mefloquine was more active than the (+) enantiomer or a mixture of both erythro-enantiomers. In vivo studies in mice scondarily infected with *E. multilocularis* metacestodes demonstrated that mefloquine, when applied intraperitoneally at 25 mg/kg twice a week for a period of 8 weeks, had a significant impact on the growth of metacestodes, and mefloquine-treated parasite tissue failed to regrow in vitro.

Affinity chromatography employing epoxy-agarose-coupled mefloquine and *E. multilocularis* extracts identified ferritin form this parasite as a mefloquin-binding proteins. Whether ferritin represents a true target for mefloquin in *E. multilocularis* is currently under study. In conclusion, mefloquine represents an interesting drug candidate, and is currently followed in further appropriate *in vivo* studies on alveolar echinococcosis in the mouse model.

PHYLOGENY AND GENOTYPING STUDIES IN CESTODES (*ECHINOCOCCUS* AND *TAENIA*) TO TRACE BACK THEIR MOLECULAR EVOLUTION HISTORY.

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Abstract

Molecular approaches are currently used to better know the evolutionary history of closely related species and the transmission dynamic of infectious agents in the environment. *Echinococcus* and *Taenia* are the two genera of the family Taeniidae, which obligatory parasitize mammals including humans. We introduce here a large nuclear phylogeny study performed on 5 genes coding for proteins in order to establish the nuclear genetic relationships among *Echinococcus* and *Taenia* species and to assess the divergence time of the two genera. The results suggest that a clade of *Taenia* including human-pathogenic species had diversified primarily in the late Miocene (9.0 Ma), whereas *Echinococcus* had begun to diversify later, in the early Pliocene (4.5 Ma). Close genetic relationships among the members of *Echinococcus* imply that the genus is a young group in which speciation and global radiation had occurred rapidly.

A second nuclear genetic study was focused on the transmission dynamic of *E. multilocularis* to human in Europe, by using the tandemly repeated microsatellite EmsB, which present a rapid evolution in time allowing tracing back the dispersion in the environment of different *E. multilocularis* micro-variants. The genotyping of 50 DNA samples from human patients was compared to about 600 genotyped worms from autopsied foxes. Nine profiles already described were found among human and 20 new profiles were determined. Humans living in the same area could harbour isolates matching to a common EmsB profile, calling up a contamination by the same parasite “strain”. Molecular studies allowed us to better understand parasites in their past and current history.

GENETIC DIVERSITY OF *ECHINOCOCCUS MULTILOCULARIS* IN HUNGARY INFERRED BY MULTI-LOCUS MICROSATELLITE ANALYSIS.

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Abstract

Eight hundred and forty red fox (*Vulpes vulpes*) carcasses were randomly collected from the whole Hungarian territory. Ninety foxes resulted positive to the sedimentation and counting technique (SCT). The genomic DNA was extracted from eighty-one single adult worms, purified and concentrated by Wizard Magnetic DNA Purification System for Food. The genetic diversity of *E. multilocularis* was assessed by fluorescent PCR followed by fragment size analyses with the tandem repeated microsatellite target. The hierarchical clustering analysis was done using the Euclidian distance and the average link clustering method (UPGMA) and the genetic isolation by the geographical distance was investigated by Mantel test.

The multi-locus microsatellite analysis showed the presence of four out of the five main European profiles. The H profile was the most common profile with nine genotypes, followed by the G with two genotypes, E with one genotype and D with two genotypes. The genetic distance was not statistically correlated with the geographical distance of the samples, supporting the hypothesis that the geographical distance is only a minor factor among those involved in the genetic distribution of this parasite in Europe. These data indicate that Hungary should be considered as a peripheral area of a single European focus, where the dispersal movement of foxes resulted in the spreading of the parasite from one county to another within a time period short enough to avoid a substantial genetic drift.

OPTIMIZATION OF PCR ASSAYS FOR THE SPECIFIC DETECTION OF *ECHINOCOCCUS GRANULOSUS* (G1 GENOTYPE), *E. MULTILOCULARIS* AND *E. SHIQUICUS* DNA EXTRACTED FROM TISSUE AND CANID FAECAL SAMPLES.

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Abstract

Cystic and alveolar echinococcoses are co-endemic on the Qinghai-Tibetan plateau of western China (Qiu *et al*., 1995; Li *et al*., 2007; 2009). The causative organisms, *Echinococcus granulosus* (*sensu stricto*) and *E. multilocularis* occur sympatrically with *E. shiquicus.* The infectivity of this latter species to humans is unknown (Xiao *et a*l., 2005; Li *et a*l., 2008). The species-specific optimization of PCR assays (van der Giessen *et al*., 1999; Abbasi *et al*., 2003; Štefanić *et al*., 2004; Dinkel *et a*l., 2004) for the detection of *Echinococcus* sp. from tissue and faecal samples pre-dates the description of *E. shiquicus* and thus reduces their diagnostic value within the unique region of western China. Also a recent assessment of three of these assays failed to reproduce the species and/or subspecies specificity reported by the original authors (Boufana *et al*., 2008). Primers were designed within the NADH dehydrogenase subunit 1 (ND1) gene signature sequences of each species with the incorporation of polymorphic nucleotides at both the 5' and the 3' end. Optimization was carried out using various concentrations of $MgCl_2$ and PCR buffers. Specificity of the assay was assessed using a panel of closely related DNA from tapeworm tissue and infected canid faeces and was found to be 100% specific. The three sets of primers detected at least 1.22×10^{-3}ng/ul of target DNA. In addition, *E. granulosus* primers were able to detect at least 1 egg from faeces. The *E. granulosus* and *E. multilocularis* primers were validated independently (AFSSA laboratories, France). A new approach was applied for maximizing the detection of low copy numbers of faecal target DNA.

SEGMENTAL SEDIMENTATION AND COUNTING TECHNIQUE (SSCT): AN ADAPTABLE METHOD FOR QUALITATIVE DIAGNOSIS OF *ECHINOCOCCUS MULTILOCULARIS* IN FOX INTESTINES.

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Abstract

In view of public health, due to the significance of alveolar echinococcosis caused by the parasite, large epidemiological studies are needed in order to determine the infection levels in known areas, but especially in new endemic areas. Simple reliable and rapid diagnosis techniques are required to analyse large fox samples and then assess the presence and the prevalence of this parasite.

In the frame of a national surveillance programme involving a large number of foxes to be tested, a working group was constituted upon the aegis of ADILVA (*Association française des Directeurs et cadres des Laboratoires Vétérinaires Publics d'Analyses*), a French association of laboratory veterinarians, to propose an optimization of the initial SCT technique. Based on the previous experience described the “Segmental Sedimentation and Counting Technique” (SSCT), to examine the presence of *E. multilocularis* helminths in segments of the fox (*Vulpes vulpes*) intestine is described and compared to the gold standard SCT.

Intestines of foxes were divided in five segments of equal length and referenced S1 to S5 from anterior to posterior part and were treated separately in order to have an independent status for each segment. On the 358 foxes collected, 117 were *E. multilocularis* positive (32.7%). According to our technical protocol we found a preferential distribution of the worm in the S4 segment, with more than 40 % of the average parasite burden. The results show that the analysis of the segment S4 associated to the segments S1 or S2 gave 98.3 % and almost 100% of sensitivity and specificity respectively. According to the time saved with the SSCT method, this will be a very useful and reliable technique for large epidemiological studies, particularly in low or in unknown endemic prevalence of *E. Multilocularis* in definitive hosts.

CONTROL OF *ECHINOCOCCUS MULTILOCULARIS*: EPIDEMIOLOGICAL AND STRATEGIC CONSIDERATIONS

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Abstract

Following the successful rabies vaccination campaigns, the zoonotic cestode *Echinococcus multilocularis* benefited from the increasing fox populations all over Europe. In many countries, foxes not only increased in numbers but also started to colonize urban environments where a high anthropogenic food supply sustains population densities exceeding by far those commonly observed in rural habitats. In areas where urban environments overlap with suitable habitats for the main intermediate rodent hosts, the vole species *Microtus arvalis* and *Arvicola terrestris*, parasite transmission is boosted by high densities of both final and intermediate hosts. As a consequence, an extraordinarily high contamination with *E. multilocularis* eggs can be found in these transition zones from rural to urban environments which usually are densely populated. Therefore, cost-effective prevention and control measures should prioritize these defined zones, where on a relatively small area the infection pressure could be substantially reduced for a high proportion of the human population.

Prevention focuses on campaigns which aim at raising the awareness of personal hygiene measures. In addition, different control options to reduce the environmental egg contamination are under discussion. The regulation of the main intermediate and final hosts, which are highly reproductive species, is difficult to achieve with regard to environmental and animal welfare standards. A more promising control option is the deworming of foxes by delivery of anthelmintic baits. However, this strategy has neither a direct impact on the parasite prevalence in the intermediate hosts nor on the survival of eggs which can persist for >1 year. Therefore, an intense, regular baiting scheme over several years is mandatory to achieve a substantial reduction or even a local elimination of the parasite.

RURAL SETTLEMENTS - A HABITAT FOR FOXES AND THE FOX TAPEWORM?

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Abstract

In recent years red foxes (*Vulpes vulpes*) have been recorded in villages and small towns more frequently. Detailed information about red fox behaviour and *Echinococcus multilocularis* prevalence of foxes is lacking for this habitat. Radio-tracking of 17 foxes showed that the mean fox home range was 75ha (95%MCP). At night, foxes spend one third of their time within villages/small towns and the remaining time outside urban areas. Preferred habitats are the built-up area, especially gardens and grasslands outside the villages. Foxes choose daytime resting sites within settlements (16%) under garden sheds or in savaged gardens and outside villages (84%). During the day, foxes exhibited preferences for forests and reed-bed areas. The *E. multilocularis* prevalence of village foxes was 43%, without significant differences to rural foxes (39%) (Intestinal scraping technique; χ^2=0.12, df=1, p=0.727). PCR analyses of faeces could also not proof significant differences between habitats (χ^2=0.68, df=1, p=0.411). We assume that foxes get infected outside villages, where sufficient intermediate-host species are abundant, especially in preferred grassland areas. Furthermore, foxes carry the parasite into villages and small towns. Comparing the number of people living here and considering the high fox abundances as well the potential risk of an infection in villages and small towns is higher than in rural areas. The frequent contacts between foxes and people are enforcing the infection risk as well.

FOX DEFECATION BEHAVIOUR IN RELATION TO SPATIAL DISTRIBUTION OF VOLES IN AN URBANIZED AREA: AN INCREASING RISK OF TRANSMISSION OF *ECHINOCOCCUS MULTILOCULARIS*?

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Abstract

Urbanization of alveolar echinococcosis is a new phenomenon that has been highlighted during the last few decades. It has thus become necessary to understand the dynamics of transmission of *Echinococcus multilocularis* in urbanized areas. Spatial heterogeneity of infection by *E. multilocularis* has been explained as the result of a multifactorial dependence of the transmission in which the factors depend on the scale of the investigation. The aim of this study was to assess, in an urbanized area, the effect of such environmental factors as season, habitat type and the level of urbanization, on the availability of two major intermediate hosts (*Microtus* spp*.* and *Arvicola terrestris*), the distribution of red fox faeces and the distribution of *E. multilocularis* as determined by detection of coproantigens in faeces. Results of the study revealed higher densities of *Microtus* spp. in rural than in peri-urban areas. Moreover this species was highly aggregated in urban wasteland. *Arvicola terrestris* densities did not appear to be linked to the level of urbanization or to the type of habitat studied. Distribution of faeces was positively linked to distance walked and to *Microtus* spp. and *A. terrestris* distributions whatever the level of urbanization. Such a distribution pattern could enhance the transmission cycle in urban areas. The Copro-ELISA test results on faeces collected in the field revealed that O.D. were significantly negatively correlated with the abundance of *A. terrestris.* The larger population densities of *Microtus* spp. found in urban wastelands and the well known predominance of *Microtus* spp. in the red fox diet in the region suggest that *Microtus* spp*.* may play a key-role in urban transmission of the parasite in the study area.

CAPABILITY OF *ECHINOCOCCUS MULTILOCULARIS* TO PERSIST WITHIN FOX HOME RANGES OF INCREASING SIZES DEPENDING ON VOLE DISTRIBUTION AND FOX BEHAVIOUR.

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Abstract

In Western Europe, Red Fox Vulpes vulpes is the main definitive host (DH) of *Echinococcus multilocularis* (*Em*) while meadow voles are its dominant intermediate hosts (IH). As literature report some evidences of a correlation between *Em* prevalence and red fox density, fox culling could be proposed as a strategy to control *Em* prevalence. However, because of the complexity of *Em* transmission cycle, modelling can be used to test the efficiency of a such practice.

Assuming an increase of the mean home range size in a fox population we used a spatially explicit individual-based model to test the capability of *Em* to persist within fox home ranges of increasing sizes and according to different fox defecation behaviors and predation rates. Model output fit *Em* prevalence observed in fox and in vole populations when fox drops feces randomly within its home range and whith simultaneously high predation rate on voles and low number of infective feces deposited by foxes.

Then, frequency of *Em* extinction was calculated for a range of home-range sizes assuming a range of contact rates between *Em* and its hosts. We found that *Em* transmission endured very few infective feces deposited by foxes as soon as IH consumption by foxes was sufficiently high even in the largest fox home-ranges.

We conclude that fox culling will be efficient in limiting *Em* prevalence only if consumption of intermediate hosts by foxes is weak.

DEMONSTRATING FREEDOM FROM *ECHINOCOCCUS MULTILOCULARIS* IN SWEDEN, NORWAY MAINLAND AND FINLAND.

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Abstract

Background: *Echinococcus multilocularis* (*Em*) is an emerging zoonotic parasite in Central EuropeAt present, five EU member states, including Sweden and Finland, and in addition Norway mainland consider themselves free from *Em* and national requirements for dogs and cats to be treated against *Em* before entering the country are in place. However, the EU Commission has indicated that due to the cost and inconvenience these requirements are considered disproportionate. To be able to keep the present legislation there is a need to document the probability of freedom from *Em*.

Methods: Probability of disease freedom was estimated using a methodology for quantitative analysis of multiple complex data sources. The model was adapted to include surveillance of several different species, thereby requiring definition of separate design prevalences for each species. Survey data from different surveillance systems as regards *Em* in foxes, rodents, out-door pigs, wild boars, dogs and humans from each country (Sweden, Norway and Finland) was collected from 1st January 2000 to 31st December 2009. Because data on the sensitivity of surveillance of the human population was not possible to obtain, the contribution of this surveillance was included in the model as an increased prior probability of freedom in year 2000.

Results and Conclusion: Preliminary results of the model will be presented. Strengths and weaknesses of the present model to document disease freedom on country basis for *Em* will be discussed. In particular the inclusion of different species in the model, which to the authors' knowledge has not been done before, and, the magnitude of the design prevalences that was found important in the sensitivity analysis.

INCREASING RISK OF HUMAN ALVEOLAR ECHINOCOCCOSIS IN THE NETHERLANDS AND POSSIBLE CONTROL OPTIONS.

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Abstract

Background: Alveolar echinococcosis (AE) is one of the most pathogenic parasitic zoonoses in central Europe. Humans are infected when accidentally ingesting the parasite eggs that are shed into the environement by infected foxes. The parasite was first detected in the Netherlands in 1996 and subsequently spread in the local population of foxes. Using the spreading of the parasite as a predictor, we assess the risk of human alveolar echinococcosis in the new endemic regions, and evaluate parasite controls.

Methods: Red foxes were collected from two provinces of Limburg and Groningen and analyzed by mucosal scrapings. Spatial-temporal dynamics of the parasite infection was modelled by a diffusion equation with a local exponential growth of the parasite population. The basic reproduction number (R_0) for the parasite was derived and estimated from the worm burdens of the foxes collected in NL. The speed at which contour line of a constant mean parasite burden is advancing was estimated. The human risk in Limburg was simulated by a Monte Carlo approach based partly on the historical records of AE epidemiology in Switzerland. Effect of reducing the parasite lifespan by the application of anthelmintic treatment on foxes was evaluated using a mathematical model of the parasite transmission.

Results: Estimated reproduction numbers of the parasite were 1.6 in Limburg and 2.0 in Groningen. The infection front is advancing into the Netherlands at the speed of 2.7 km per year from the Belgium border and at the speed of 3.4 km from the German border. In Limburg, up to 30 human cases are predicted by 2030. The duration of the control is a critical factor for a successful parasite control.

Conclusions: The epidemiology of AE in the Netherlands might have changed from the period of zero risk in the past to the period of increasing risk in the coming years.

SUCCESSFUL LONG TIME BAITING CAMPAIGN AGAINST THE FOX TAPEWORM (*ECHINOCOCCUS MULTILOCULARIS*) IN SOUTHERN BAVARIA.

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Abstract

A baiting strategy against E. multilocularis 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 km^2). Within the first year, baits were put down monthly and within the following years in six weeks intervals. Bait distribution density was 50 pieces/km^2. 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 2% (2009)/and could be manifested under a 3% level. In contrast, no significant change had occurred in the untreated control area.

As 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 defecation rates and human population densities. The model calculates the likelihood for people to get in contact with *E. multilocularis*. For example it demonstrates that in 2005, prior to the worming programme, the likelihood of contact in our study area was 175% of the Bavarian average. Today, after the 5-year worming program, this likelihood is only 5 % of the Bavarian average infection risk. Herewith it is shown that it's a great effort of preventing humans in this district of getting infected with the fox tapeworm.

URBAN CONTROL OF *ECHINOCOCCUS MULTILOCULARIS* IN FRANCE.

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Abstract

In Europe, most cities are currently colonized by red foxes (*Vulpes vulpes*) which are considered to be the main vector of the parasite *Echinococcus multilocularis*. The risk of transmission to humans is thus particularly worrying.

The distribution of baits containing a wormer (*praziquantel*) has already shown promising results in rural areas and on small plots in urban areas. The purpose of this study was to assess the feasibility of such a treatment in two medium-sized cities and their outskirts: Pontarlier and Annemasse. After five annual treatments over two and a half years between 2006 and 2009, the spring-time prevalence in foxes, as determined by ELISA tests on samples of faeces collected in the field, decreased from 13.3% to 2.1% in Annemasse. No prevalence differences were detected around Pontarlier (stable prevalence 10.9% - 7.0%). Working with veterinary clinics in the two towns also allowed us to screen the canine population in search of the parasite. More than one thousand samples were collected, among which only two were positive, one in each city. Thus, although bait distribution targeted toward fox populations has decreased *E. multilocularis* prevalence, it failed to prevent (indirectly) dog infection. Costs and benefits of these kinds of actions are discussed. What is at stake is the question of optimizing such actions by focusing treatments on higher risk areas (parks, urban gardens, green corridors, river banks, railways, etc.) with a higher bait distribution frequency (one per month).

In parallel, in 2006 the town of Nancy wanted to reduce the risk of human contamination by financing trappers to cull the fox population. The direct regulation of foxes though is a sensitive subject; in fact some people emphasize it as the final solution whereas it is considered to be cruel and useless by others. In such a context, it is difficult to advice authorities without scientifical facts. Therefore, we decided to implement a protocol of fox culling through night shooting whose aim is to assess the cost/benefits of such a control method. The study area is a 20km radius circle centred on Nancy. The northern half is devoted to fox culling while no change on the hunting pressure is made in the south. Each year of the study, which will last from 2008 until 2011, a batch of fox will be sampled homogeneously on each area from October to April. Intestines will be tested using the scrapping technique to assess the presence of *Echinococcus* worms. During the first session, the fox prevalence was 39% and 45% (north and south respectively) and reached 31% (north) and 40% (south) in the second year. In the same time, fox populations were monitored by night counting showing IKAs of 6.1 and 4.9 foxes per 10km in the north whereas in the south theses indices were 4 and 3.7 for session one and two respectively. Consequently, the culling protocol tested around Nancy seems to be poorly effective both on the fox population and on the fox prevalence. We still have to wait for the results of the third session to have a better comprehension of the real impact of fox culling.

S. COMTE, V. RATON, S. FAVIER, B. COMBES

European Congress On Alveolar Echinococcosis

Nancy, 8th and 9th of December 2010

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Echinococcus surveillance in Finland

Antti Oksanen, Finnish Food Safety Authority Evira[1],
Heikki Henttonen[2], Voitto Haukisalmi[2], Finnish Forest Research Institute (Metla)[2],
Antti Lavikainen, University of Helsinki[3]

2b 2a 1 3

METLA

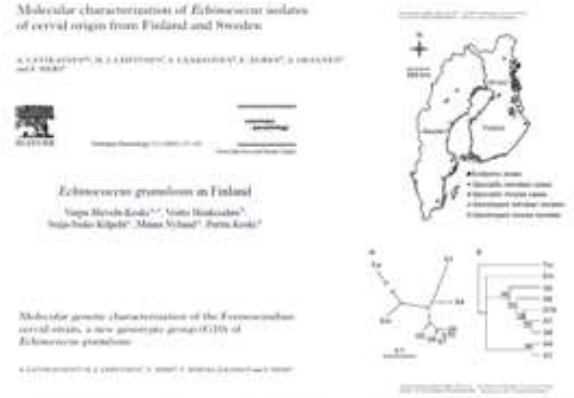
Echinococcus granulosus G10 (*Echinococcus canadensis*) is endemic in Finland

Cystic echinococcosis used to be common within reindeer husbandry of northern Fennoscandia (northern parts of Norway, Sweden and Finland), with up to 10 % of reindeer infected in parts of the Finnmark County, Norway, during the 1950s. Human infections were commonly seen in reindeer herders before eradication campaigns were introduced. The aim of the Norwegian campaign was to cut the parasite life cycle both by deworming herding dogs with arecoline and, later, praziquantel, and by banning feeding of dogs with uncooked reindeer offal.

The measures against echinococcosis were not that strict in Sweden and Finland, but the parasite became soon virtually eradicated from all the three countries. Apparently, the most important reason to that was the mechanization of reindeer husbandry from the 1960s on, making the herding dog redundant. After the introduction of snowmobiles and helicopters for reindeer round-ups and abandonment of nomadism, the number of dogs in reindeer husbandry soon declined. The latest autochthonous case of human cystic echinococcosis in Finland was diagnosed during the late 1960s. Decades passed without any findings in the mandatory reindeer meat inspection. The parasite involved in this life cycle was never genotyped, but we think it is reasonable to assume that it was the same genotype as identified later (see below).

Renaissance of the parasite was seen beginning in the early 1990s, but now circulating between the reindeer (and moose, European elk, *Alces alces*) and the wolf. This is a life cycle difficult to control, but the smallish population size of the wolf has limited the public health importance of the parasite. The infection has so far mostly been confined to the Finnish-Russian border zone where the wolf population is dense as the wolves have good survival possibilities utilizing hunting bans. All parasite isolates identified so far have belonged to the genotype G10. Reindeer meat inspection, sampling by moose hunters, and examination of all (legally) killed wolves form the basis of infection surveillance.

Reindeer husbandry area of Finland (Paliskuntain yhdistys)



The future of the parasite is dependent on the wolf welfare. From public health point of view, eradication of the infection totally would be desirable. However, it clearly is a part of the northern boreal ecosystem biodiversity, and it has been concluded that hydatid echinococcosis in the Canadian moose will predispose the intermediate host to predation.

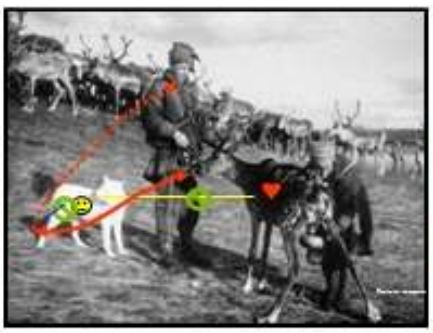
Echinococcus multilocularis has so far never been detected in Finland

Echinococcus multilocularis (Em) causing alveolar hydatidosis is known to be present in the neighbouring areas, the Baltic states Estonia, Latvia and Lithuania, Denmark, Spitzbergen (Norway), and northern tundra and taiga areas of Russia. However, it has never been diagnosed from Finland (or Fennoscandia), possibly partly because of the scarcity of definitive hosts caused by intensive hunt for fur and persecution due to game management during the early 1900s. After that, the red fox population revived, and in Finland there are now about 150,000 red foxes (autumn population). In addition, there is another excellent definitive host species, the raccoon dog, originally an eastern Siberian canid species introduced to the western parts of the former Soviet Union, and subsequently spread also to Finland. The current autumn population of this animal is about 230,000 individuals.

The arvicoline rodent populations in the country are certainly high enough to sustain the *E. multilocularis* life cycle. Because of no parasite finding in spite of surveillance, Finland is currently believed to be free of Em. Therefore, Finland wishes to maintain a 'derogation' from the EU regime to require dogs to be imported to be treated against *E. multilocularis*.

The Em surveillance is based on both definitive and intermediate hosts. Definitive hosts are collected by hunters in different parts of the country, with special emphasis on the south-eastern border region. This is actually because of rabies surveillance, but, according to our understanding, it serves also Em surveillance excellently. The potential definitive hosts are subject to autopsy at Finnish Food Safety Authority Evira. The rectal contents are collected for coproantigen ELISA at University of Zürich (courtesy of Prof. Peter Deplazes). The ELISA positive animals are then confirmation tested by egg flotation and worm sedimentation methods. No positive animal has been found.

The Finnish national rodent monitoring program, run by the Finnish Forest Research Institute, includes some 30 permanent study areas. Some of these are sites with extensive year around, long-term studies, whereas most are trapped twice a year for monitoring purposes. Data sets cover 30 – 60 years. Trappings cover optimal forest (*Myodes*) and field (*Microtus*) habitats. All animals are dissected by professional experienced mammalogists. Data gathered cover basic ecological parameters, but also detailed parasitological information including *Taenia* type cysts and larvae in liver, thorax and coelome. The annual number of voles studied depends on the cyclic phase, and varies usually between 1,000 – 5,000 individuals. *Taenia* larvae and cysts have been common, but in 60 years no Em has been found.



Echinococcus control program in Norway included both anthelmintic treatment of dogs and the ban of feeding them with raw offal.

Sampling for *Echinococcus multilocularis* during the first decade of the millennium in Finland			
Year	Foxes n	Raccoon dogs n	Voles n
2000	9	0	2000
2001	13	3	3000
2002	116	3	3000
2003	104	97	650
2004	340	337	1650
2005	291	219	3000
2006	209	169	2100
2007	264	217	2300
2008	411	143	2100
2009	184	175	900

A research van half full of fox carcasses

H. WAHLSTROM

Potential consequences of introduction of *Echinococcus multilocularis* into Sweden

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Conclusions

Potential consequences and estimated costs associated with endemicity of *Echinococcus multilocularis*(*EM*) in Sweden are significant and of major concern. Compulsory treatment of dogs entering Sweden from regions with *EM* with anthelmintics would therefore be a proportionate measure to manage the EM risk.

Introduction

Five EU member states, including Sweden, consider themselves free from *EM*. The regulation (EC) No 998/2003 allows these countries, over a transitional period, to maintain their national requirements for the entry of pet animals. Dogs and cats entering Sweden are treated against *EM* by a veterinarian with anthelmintics before entry. Furthermore, many endemic countries consider alveolar echinococcosis as an emerging disease. However, the EU Commission has indicated that due to the cost and inconvenience the present requirements are considered disproportionate.

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Increased costs of deworming

Method: The Swedish dog population was assumed to be 729 000 dogs with an average weight of 15 kg. All dogs were assumed to be de-wormed monthly. The annual number of introduced dogs was estimated to be 30 000, based on data from 2003. Costs for de-worming were 2.2 €/5 kg. Cats were not included, due to lack of data.

Results: Annual costs for de-worming dogs in Sweden, estimated to be 56 million €. With the present system costs for de-worming of all introduced dogs is approximately 0.2 million € per year (excl veterinary costs).

Altered outdoor activities

Background: The tradition of out-door activities such as mushroom- and berry-picking is strong among Swedes thanks to the right of public access. The law gives everyone the freedom to enjoy the countryside and pick berries and mushrooms in almost any non cultured area.

Results: If such outdoor activities would be restricted and associated with danger it might lead to changed behaviour on a long term basis. The tourism and berry picking industry might also be affected in a negative way.

Need for awareness campaigns

Background: In many countries with an endemic situation, the public and specific risk groups such as dog owners, farmers and hunters are given advice about how to minimize the risk to be infected. There are no such advice given in Sweden today and the awareness about the risks with *EM* and how to handle them is assumed to be very low.

Results: If *EM* is introduced to Sweden, there will be a need for continuous awareness campaigns for the public and risk-groups on how to handle potential risks..

Alveolar echinococcosis and costs for treatment

Method: The data in three separate publications (1, 2, 3) was applied on the Swedish population to estimate the expected number of human cases following an introduction and establishment of *EM* in Sweden.

Results: It was estimated that between 9 to 42 human cases of alveolar echinococcosis may be diagnosed annually. The costs of livelong treatment per patient has been estimated to be 300 000 €(3).

Increased surveillance

Method: During 2001 and 2007, between 200-300 wild red foxes have annually been investigated for *EM*; in total 1,987 animals. Currently, the surveillance of foxes costs around 50000 € per year (3).

Results: If *EM* is introduced to Sweden, there will be an increased need for surveillance in foxes and possibly intermediate hosts, to follow the situation and identify infected areas. A large increase in costs, compared to current surveillance (50 000 € per year), can be foreseen (3).

Photo: Gunilla Hallgren

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A. MARCINKUTE

Alveolar echinococcosis: an emerging disease in Lithuania

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Human alveolar ecchinococcosis is the most pathogenic zoonosis in temperate and arctic regions of the Northern Hemisphere. The fatality rate exceeded 90% within 10 years. Alveolar echinococcosis (AE) was a very rare human disease with various clinical syndromes mostly observed in the outcome stages in Lithuania for a long time.

In the last years national CDC represents an incidence rate of this infection to be 0,8 / 100 000 of population. It is noticed that the incidence rate of this disease is higher in the north part of Lithuania. In this particular region serological and ultrasound screening program is recommended. It is worth-while to integrate in the European Echinococcosis Registry.

Regional distribution of human autochthonous alveolar echinococcosis in Lithuania 1997-2010

Aim

- To analyze and present the epidemiological, clinical, morphological, laboratory, therapeutic characteristics and outcomes of alveolar echinococcosis in Lithuania during the period 1997-2010 years.
- To integrate in European Echinococcosis Registry (EurEchinoReg)

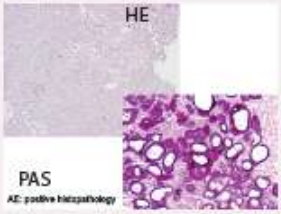
Methods

- There were analyzed patients with confirmed alveolar echinococcosis in the period of 1997-2010 years.
- Diagnosis was established by immunoenzyme assay, WB, PCR, morphological and instrumental methods.
- There were performed statistical analysis of epidemiological and clinical data, diagnostic and treatment peculiarities.

Case Definition

Diagnosis of alveolar echinococcosis is confirmed:

Positive histopathology
and/or
Typical liver lesion morphology identified by imaging techniques (ultrasound scan, computed tomography, and magnetic resonance imaging)
with/without
The detection of serum antibodies (serology)
P.Kern at all, 2003



AE: positive histopathology

Histologically, the irregular small cysts have a laminated membrane which is more visible in PAS stain then in usual hematoxylin-eosin. No nucleated germinal membrane or protoscolices are seen. Dr A.Sarkisdiene, RTC, 2006

Alveolar Echinococcosis: specific serology

- ELISA Em2plus
- EgHF-ELISA
- WB
- PCR

(~12% seronegative AE cases)

Results

- There were diagnosed and confirmed 128 new cases of AE in 1997-2010.
- High rate of AE has been observed in north- west and north-east parts of Lithuania .
- Most probably they have been accidentally infected with contaminated vegetables and berries and by hunting.
- The prevalence of E. multilocularis among red foxes in Lithuania is 57%.
- Increasing prevalence rates of foxes have been recorded in last 5 years in endemic areas.

In Lithuania, The Red Fox are the most important final host for E. multilocularis .

Epidemiological peculiarities of AE: 128 new cases in the period of 1997-2010

- A peak incidence was noted in 2002-2006 and in 2009 years (46% of all cases).
- A total number of patients - 128; male - 39% and female - 60,9% (gender ratio 0.6 : 1,0).
- The median age at first diagnosis was 57,7 years (range 21 - 91 years).

AE Epidemiology: potential risk factors

Hunting	26,7% (27/128)
Farmers/activity in agriculture	64,8% (83/128)
Picking wild berries/mushrooms	70,3% (90/128)
Dog	61,7 (79/128)
Cats	26,5 (34/128)

AE: clinical and diagnostical features

primary clinical diagnosis

- Hemangioma -11
- Liver tumour with/without mts -27
- Gynecological tumor with mts - 1
- Liver cyst -14
- Abscess of thoracic soft tissue -1
- Symptomic hypertension (renal) -2
- Biliary colic -30
- Gastritis, ulcer ? Anemia - 2
- TB hepatitis -2
- Icterus –20
- HCC - 2
- Intercostal neuralgia - 5
- During prophylactic examination - 7

78-93% of patient were in the final stage of the disease

AE: the main complains

- The pain in the right side of abdomen
- Fatigue
- Dyspepsia
- Icterus
- Itching
- Weight loss
- Anemia
- The pain in the epigastrium, severe reflux
- Liver damage: 128 patients; metacestodes in the bought lobes: 43 (33,5%) patients; right lobe: 57 (44,5%) patients;
- Larva also spreads into extrahepatic structures and even metastasizes to distant organs for 34 patients.
- The smallest focus of AE was 30 mm Ø up v. portae in 9 patients; the biggest - 15x17 cm Ø in 21 patient.

There were no correlations between the size of metacestode, rate of growth and antibodies level.

AE treatment

- Surgery:
  Radical
  Palliatyva: interventional procedures
  Combined arterial and portal vein embolization before extended resection
- Chemotherapy:
  2 years after radical resection
  Long life chemotherapy:
  - Not radical operation
  - Non operable case

AE Treatment in 128 patients

- 78,7% - surgical treatment;
- radical operations performed for 24,7% patients;
- 1 patients were indicated for liver transplantation;
- 1 patients - performed right nephrectomy
- 92,1% - benzimidazoles treatment:
- ALB,
- MBZ,
- ALB + P2Q 28 + 7 d. with 14 d. rest periods - 17 patients
- ALB without rest periods - 62 patients

- Negative serological reactions correlates with normal liver ultrasound, normal laboratory analyses and good patients condition.
- Is it possible to postulate that the disappearance of antibodies confirm radical surgery.

AE outcomes

- Survival rate from primary diagnosis until 2010m :
  15y – 1 case,
  14y – 1 case,
  13y – 2 case,
  11y – 4 case,
  9y – 6 cases,
  others < 5
- Mortality - 15,1 % in 2007, 10,7% in 2008-2010

A.Marcinkute, 2010

Conclusions

Conclusions

- Every case should confirm the Case Definition requirements.
- PAS stain should be used for routine histological examination.
- Histological AE lesion: multilocular, necrotic, multicystic cavities containing thick pasty material which is not surrounded by a fibrous wall.
- PCR is used for seronegative AE
- Serological and ultrasound screening is recommended in high incidence of AE in north-west and north-east parts of Lithuania to determine the disease in it's initial stage.
- It is noticed that the incidence rate of this disease is higher in the north part of Lithuania – endemic regions, probably determined by the increased population of red foxes (anamnestic data).
- The information about the new cases of AE should be sent to National CDC.

C. CRETU

European Symposium on Alveolar Echinococcosis
8-9 December 2010 Nancy, France

Echinococcosis/hydatidosis in Romania

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Bucharest, Romania

The neglected disease ECHINOCOCCOSIS (CE & AE), produced by the larval stage of tape worm Echinococcus spp. can determine diagnosis difficulties due to the limits of diagnosis tools, even in endemic areas.

- In spite the lack of official data regarding the registration of CE, Romania is an endemic territory for this disease.
- In Parasitology Department - Colentina Teaching Hospital Bucharest, Romania, an average of 25 new cases/month and about 800-900 cases/year (new and followed up) are registered.
- The disease in children represent about 16% of the total cases.
- Most of the cases are from the urban areas:
  - better addressability to the medical services
  - presence of stray dogs?
- The most common locations are liver and lung, but unusual locations, or cases in immune compromised hosts can also be present.

BUT,

ECDC Report 2006

49 communicable diseases and health issues

COUNTRY	TOTAL CASES	NOTIFICATION RATE % 000
Austria	28	0,31
Belgium	6	<0,1
Bulgaria	465	6,0
Cyprus	6	0,70
Czech Republic	3	<0,1
France	11	<0,1
Germany	124	0,10
Greece	6	<0,1
Hungary	7	<0,1
Ireland	68	1,0
Latvia	22	1,0
Lithuania	19	0,48
Netherlands	21	0,10
Poland	60	0,17
Romania		
Slovakia	6	0,11
Slovenia	3	0,15
Spain	123	0,30
Sweden	7	0,08
UK	16	<0,1
[illegible]	[illegible]	[illegible]

HOWEVER,

us screening for CE in rural areas in Romania, showed:

Ialomita County - October 2004;
Buzau County - May 2006
Teleorman County - November 2010

4146 screened people - prevalence between 3,7 and 4,9%

- 1.060 screened people
- 74 cases possible positive
- 3,77% out of the 1.960 screened people

- 1 710 screened people
- 76 cases possible positive
- 4,42% out of the 1.710 screened people

- 400 screened people
- 20 cases possible positive
- 4,94% out of the screened people

THE USEFULNESS OF ABDOMINAL US SCREENING DISCOVERED PATHOLOGY

Ialomita County (2004)

- Total group: 1710 persons

Abnormal US pattern: 840 persons (49,39%)
Normal US pattern: 870 persons (50,61%)

- CE suspicion
  - 76 cases
  - 4,42% out of the 1710 cases
  - 9,05% out of the 840 cases

Identification/confirmation of diseases revealed by US

PERCENTAGE OF IDENTIFIED CE ACCORDING TO THEIR NUMBER AND EVOLUTIVE STAGE

- Decreasing number of cases according to the involution of the cyst - WHO Classification
  - from the active stage (CE1, CE2)
  - through the transitional stage (CE3)
  - toward inactive stages (CE4 y CE5)

Conclusions:
- same trend as in endemic areas
- spontaneous involution of the cyst is rare
- study discovered mainly new cases

CE number discovered/person
- 1 cyst 48 (71,6%)
- 2 cysts 12 (17,9%)
- 3 cysts 1 (1,5%)
- multiple cysts 6 (9%)

Diameter: 10-49 mm

CASE PRESENTATION

B. C. - 8 years, female

17 CE discovered by means of US screening, confirmed later on by CT scan

ELISA CE intense positive ISR 10,4 (>5,1)

She gained 6 kg in weight and 10 cm in height

Rare locations of CE:
- spleen - 25 cases (2,79%)
- muscles/subcutaneous tissues - 14 cases (1,56%)
- kidney - 14 cases (1,56%)
- CNS - 6 cases (0,67%)
- heart - 6 cases (0,67%)
- bones - 6 cases (0,67%)
- ovary - 5 cases (0,56%)
- pancreas - 4 cases (0,45%)
- thyroid - 3 cases (0,33%)
- breast - 1 case (0,11%)
- mediastinum - 1 case (0,11%)
- retro-orbital - 1 case (0,11%)
- prostate - 1 case (0,11%)

Our results:

- 896 records of pts with CE
- 87 rare locations (9,7%)

Unusual locations (kidney, spleen, bones, soft tissues, CNS, pelvis/ovary, eye, heart, etc): Kinsul O A, et al., 2003
- 3,9-12,5% (WHO, 2000)
- 17,3% (Richerolle-Ellouroski M, et al, 2002)

Bone CE
6/896 cases (0.67%)

Ovary hydatid cyst
5/896 (0,56%)

before and after albendazole therapy

- 1-3% of cases (Richerolle-Ellouroski et al, 2004)
- 0,6% of cases (Lazar et al, 1978)

ALVEOLAR ECHINOCOCCOSIS

- Very few data….
- It seems that it was found only 1 case in animals - sheep (Ionescu, unpublished data)
- No reported cases in humans in spite the geographical conditions
- National Research Program PNCD 4 NR 52-161/2008 on echinococcosis - under development in this moment.
  One of the tasks is to look for AE in both humans and animals

All the countries around Romania mentioned AE cases.
In Romania mentioned cases in sheep and rodents ?
In humans infection seems to be under evaluated……

CONCLUSIONS

- Hydatidosis is an apparently benign disease, but it is malign due to its potential in evolution and to the risk of complications
- The success ⟹ good working team:
  - surgeon
  - parasitologist
  - laboratory

M. KIRJUSINA

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Latvia

PRELIMINARY RESULTS OF CATS AND DOGS HELMINTHOFAUNA INVESTIGATION IN LATVIA DAUGAVPILS AREA

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Daugavpils University, Institute of Systematic Biology

Introduction: The main objective of this study was to generate new data on the occurrence of internal organs helminths in domestic Canids and Felids including *Trichinella* in muscle. According to previous study of helmontofauna of wild life animals there is a risks of *Echinococcus* for pets (Fig. 1).

Figure 1. *Felis cati* and *Canis familiaris*

Materials and methods: Investigation of intestinal parasites of 24 dogs (*Canis familiaris*) and 39 cats (*Felis cati*) were carried out in Daugavpils area. Carcasses of animals were collected from veterinary clinics (19 cats and 9 dogs) and shelter (20 cats and 15 dogs) between January and May 2010. Information was obtained on the approximate age, sex, breed, and life mode of each animal. Carcasses were kept frozen until examination. Animals were studied by investigation of internal organs (heart, liver, lungs, kidneys, stomach, intestine, urinary bladder and gallbladder) examined according to conventional helminthological methods (sedimentation at necropsy). Muscle samples (25 g) from each animal were tested using magnetic stirrer artificial digestion to detect *Trichinella* spp. larvae. Examination was done in Daugavpils University Institute of Systematic Biology and Institute of Food Safety, Animal Health and Environment "BIOR".

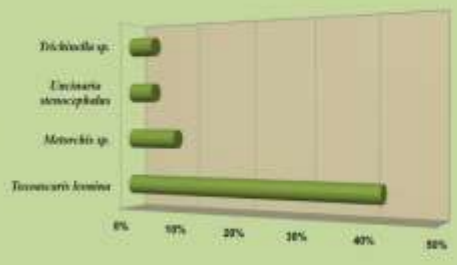

Figure 2. Prevalence (%) of helminths in dogs (n=24).

Results: Overall there were found four helminthes species in dogs: *Toxoascaris leonina* (prevalence 41,7%), *Uncinaria stenocephalus* (4,2%), *Trichinella* sp. (4,2%) and *Methorchis* sp. (8,3%) (Fig.2). *Trichinella* sp. was detected for 3 year old animal and construct 13,6 LPG (larvae per gram).

50% of investigated dogs were infected. One parasite species was detected for 91,7% of dogs, and three – for 8,3% of dogs. There was difference in prevalence of infection between male (63,6%) and female (38,5%) (Fig.3).

Totally four parasite species were detected in cats: *Toxocara cati* (prevalence 35,9%), *Trichinella* sp. (2,6%), *Dipylidium caninum* (7,7%) and *Methorchis albidus* (17,9%) (Fig.4). *Trichinella* sp. was detected for 12 year old animal and construct 160,5 LPG.

51,3% of investigated cats were infected. One parasite species was detected for 75% of cats, two – for 15% and three helminths species – for 10% of cats. Prevalence of infection was higher for male per 10% and amounted 58,3%. Similarly for cats and dogs males infection was higer than in females (Fig. 3).

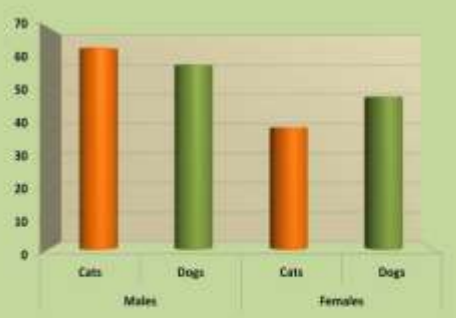

Figure 3. Prevalence of infection between males and females of cats and dogs in Daugavpils area.

Conclusions: Totaly were detected 7 helminths species in dogs and cats. Trematoda from *Methorchis* genus was mentioned first time in domestic animals in Latvia.
Preliminary results show that investigation was significant because three helminth species detected in cats and dogs were zoonotic: *T. cati*, *D. caninum* and *Trichinella* sp.

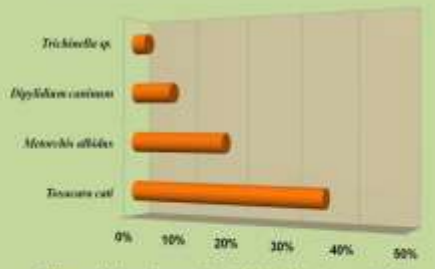

Figure 4. Prevalence (%) of helminths in cats (n=39).

Acknowledgement
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Authors are grateful for technical support from technicians of Institute "BIOR".

A. CASULLI

USING NICHE MODELS TO INFER POTENTIAL DISTRIBUTION OF *Echinococcus multilocularis* IN ITALY

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INTRODUCTION

The metacestode larval stage of *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), one of the most pathogenic helminthic zoonoses in the northern hemisphere (Vuitton et al., 2003). This parasite is endemic of central Europe and although it has been suggested that its geographical range has expanded in recent years, no prove of this theory is available because of the lack of adequate retrospective studies in the newly recognized endemic areas (Kenny et al., 2006).

In recent years, spatial analysis and Geographical Information Systems, have been further expanded using ecological models to predict the potential spatial spread of many diseases (Peterson, 2006). Niche models have been widely used in biodiversity studies, where the concern is usually the conservation of the habitat of a species, but few applications have been developed in the epidemiologic context (Peterson, 2006).

In this analysis, a model of fundamental niche is used as the basis for mapping the potential distribution of *E. multilocularis*, and to understand the environmental variables that determine its distribution in the alpine region of Italy.

MATERIALS AND METHODS

Between 2001 and 2004, Casulli and colleagues (2005) tested a total of 241 intestines from red foxes originating from Trentino Alto Adige region. Only 24 (9.96%) samples resulted positive to the molecular coprodiagnosis and thus the analysis was focused to this low endemic region (Figure 1). The 24 positive samples were georeferenced and used as input data for the subsequent analyses.

We characterized our study area using 4 environmental layers all re-sampled to a spatial resolution of 1 km^2: 1. land cover with six classes (artificial, agriculture, pasture, natural open areas, woodlands and forests, and natural pastures); 2. elevation; 3. climate with three variables (mean temperature of wettest quarter, precipitations of the driest month and of the warmest quarter); and 4. the host species distribution (three intermediate hosts, *Microtus arvalis*, *Microtus multiplex* and *Microtus arvalis*, and the definitive host, *Vulpes vulpes*) (Table 1). Using the environmental variables outlined above together with the 24 positive localities, we build a niche model for *E. multilocularis* using the maximum entropy approach as implemented in the Maxent software package (Phillips et al., 2006). Maxent predicts the distribution of a species inside the study area by finding the distribution of the maximum entropy subject to the constraint that the environmental characteristics measured in the observations, reflect the expectation (i.e. the potential distribution of a species). The final product is a map of the relative probability of presence for the species considered over all 1 km^2 pixels in the study area.

To evaluate if the model performs significantly better than random in predicting the distribution of *E. multilocularis*, a receiver operating characteristic (ROC) analysis, which characterizes the performance of a model using the area under the curve (AUC), has been used. Given that only presence data are available in our case, we performed a ROC analysis using the available presence localities and random localities using the procedure outlined in Phillips et al., 2006.

To map the risk of *E. multilocularis* being transmitted to the resident human population, we combined the niche model for the parasite with a map of human population density in 2001 (inhabitants/km^2, density rescaled from 0 to 100) obtained from the Italian National Institute of Statistics (ISTAT, www.istat.it). The human population density map has been multiplied by the map of probability of presence for *E. multilocularis*. Thus, we obtained a map of the relative risk of transmission for *E. multilocularis* based on the assumption that a higher human density coupled with a high probability of presence for the parasite are indicative of a higher probability to acquire this zoonotic infection.

RESULTS AND CONCLUSIONS

The resulting AUC was equal to 0.913 indicating an excellent model performance. The land cover classes "pasture" and "artificial" were the most important factors explaining the distribution of *E. multilocularis* followed by "precipitation of the warmest quarter", "woodlands and forests", and the presence of the intermediate host *M. arvalis*. The relative contribution of all other variables was negligible. The probability of presence for *E. multilocularis* increases for areas with increasing coverage in "pasture", higher suitability for *M. arvalis*, with a low coverage in "artificial" and with a medium value of "precipitation of the warmest quarter" and "woodlands and forests".

The spatial distribution model for *E. multilocularis* clearly outlines that the potential distribution of the species is limited to the valleys in the north east of the region, with only 5.4% of the region showing a probability of presence greater than 50% (Figure 2A). According to the environmental characteristics, a number of smaller areas with a relatively high probability of presence for *E. multilocularis* is scattered throughout the entire Trentino Alto Adige region mainly north-west and south-east), but with very few connections with the north-eastern core of the parasite distribution in Europe.

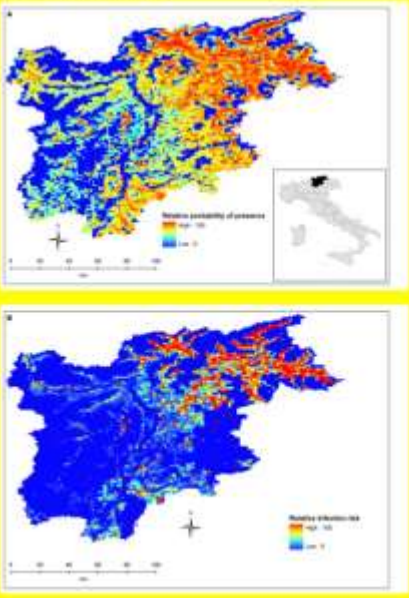
The highest risk of *E. multilocularis* transmission to the resident human population seems to be limited to the valleys of the north east of the study area, covering slightly more than 13% of the regional territory (Figure 2B).

Table 1.
Four environmental variables re-sampled to a spatial resolution of 1km^2: land cover, elevation, climate, and host species distribution. The environmental variables together with the available 24 positive points, we used to build a niche model for *E. multilocularis* using the maximum entropy technique.

ENVIRONMENTAL VARIABLE	SOURCE
Land Cover	http://terrestrial.eionet.europa.eu/CLC2000
Artificial	
Agriculture	
Pasture	
Open areas (natural)	
Woodlands and forests	
Natural pastures	
Elevation	http://dataservice.eea.europa.eu/download/download.agt?mode=G
Climate	Hijmans et al. (2005)
Mean T° of wettest quarter	
Precipitation of driest month	
Precipitation of warmest quarter	
Species distribution	Maiorano et al. (2007)
Vulpes vulpes	
Microtus arvalis arvalis	
Microtus multiplex	
Microtus arvalis	

Figure 1.
Locality of origin of red foxes (*Vulpes vulpes*) examined for *Echinococcus multilocularis*. A. North Italy. B. Trentino Alto Adige region. Red triangles represent positive foxes; black dots one or more negative foxes; grey dots the cities of Trento and Bolzano.

Figure 2.
Spatial distribution model for *Echinococcus multilocularis* in the Trentino Alto Adige region (Northern Italy), representing the potential distribution of the parasite (A) and the transmission risk map for the resident human population in the region (B). The inlet in A shows the location of the study area in Italy.



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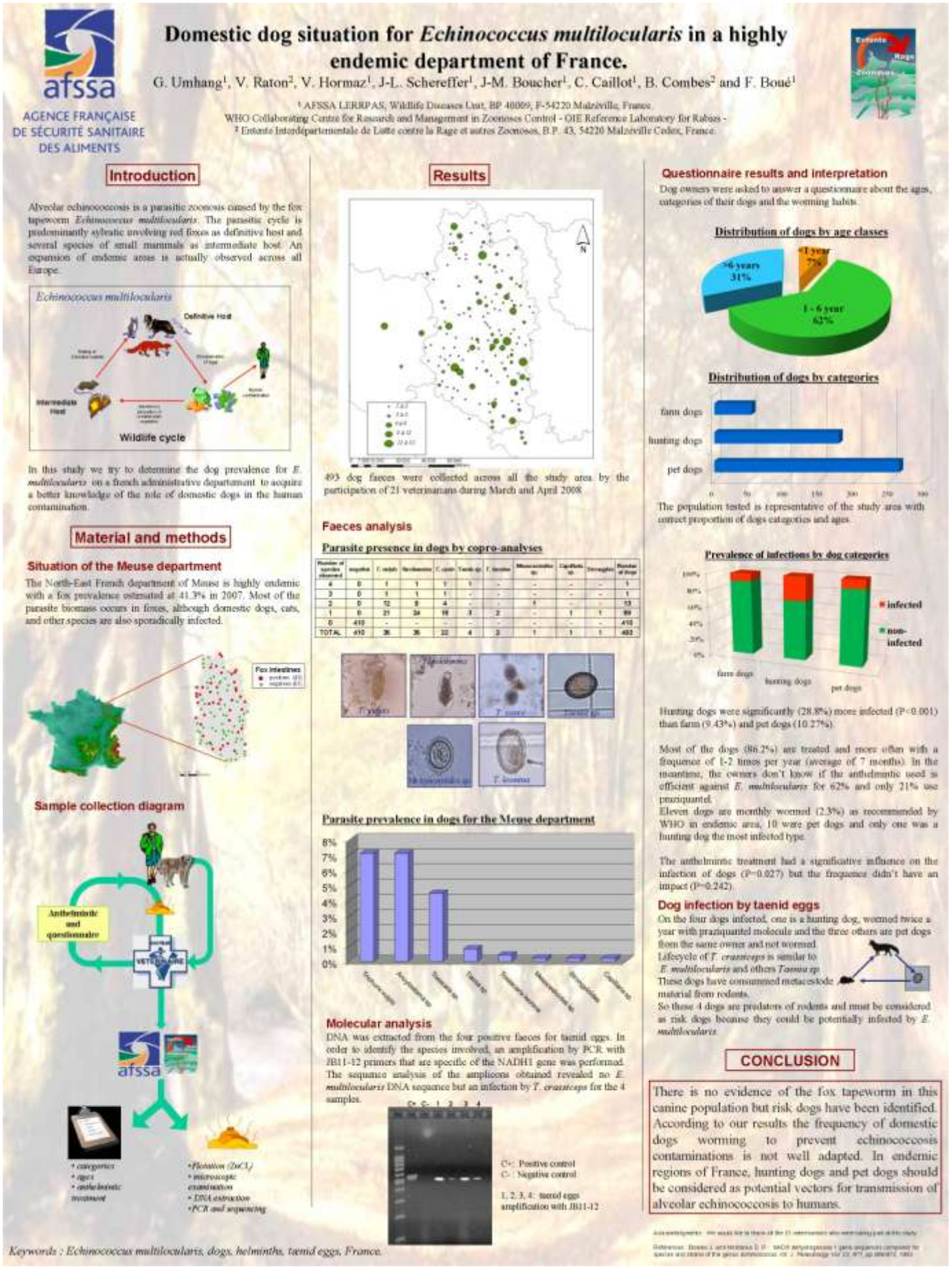
G. UMHANG



Domestic dog situation for *Echinococcus multilocularis* in a highly endemic department of France.

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AGENCE FRANÇAISE DE SÉCURITÉ SANITAIRE DES ALIMENTS

Introduction

Alveolar echinococcosis is a parasitic zoonosis caused by the fox tapeworm *Echinococcus multilocularis*. The parasitic cycle is predominantly sylvatic involving red foxes as definitive host and several species of small mammals as intermediate host. An expansion of endemic areas is actually observed across all Europe.

Echinococcus multilocularis

Definitive Host

Intermediate Host

Wildlife cycle

In this study we try to determine the dog prevalence for *E. multilocularis* on a french administrative department to acquire a better knowledge of the role of domestic dogs in the human contamination.

Material and methods

Situation of the Meuse department

The North-East French department of Meuse is highly endemic with a fox prevalence estimated at 41.3% in 2007. Most of the parasite biomass occurs in foxes, although domestic dogs, cats, and other species are also sporadically infected.

Fox intestines

Sample collection diagram

Anthelmintic and questionnaire

VETERINAIRE

afssa

- categories
- ages
- anthelmintic treatment

- flotation ($ZnCl_2$)
- microscopic examination
- DNA extraction
- PCR and sequencing

Results

N

493 dog faeces were collected across all the study area by the participation of 21 veterinarians during March and April 2008.

Faeces analysis

Parasite presence in dogs by copro-analyses

Number of species observed	negative	T. ovatus	Nematodes	T. canis	Taenia sp.	T. hydatica	Metacestodes sp.	Capillaria sp.	Strongyles	Number of dogs
4	0	1	1	1	1	-	-	-	-	1
3	0	1	1	1	-	-	-	-	-	1
2	0	12	9	4	-	-	1	-	-	13
1	0	21	24	18	3	2	-	1	1	69
0	410	-	-	-	-	-	-	-	-	410
TOTAL	410	36	36	22	4	2	1	1	1	493

T. ovatus *T. canis* *T. hydatica*

Parasite prevalence in dogs for the Meuse department

8% 7% 6% 5% 4% 3% 2% 1% 0%

Nematodes, Metacestodes sp., Strongyles, Capillaria sp.

Molecular analysis

DNA was extracted from the four positive faeces for taenid eggs. In order to identify the species involved, an amplification by PCR with JB11-12 primers that are specific of the NADH1 gene was performed. The sequence analysis of the amplicons obtained revealed no *E. multilocularis* DNA sequence but an infection by *T. crassiceps* for the 4 samples.

C+ C- 1 2 3 4

C+: Positive control
C-: Negative control

1, 2, 3, 4: taenid eggs amplification with JB11-12

Questionnaire results and interpretation

Dog owners were asked to answer a questionnaire about the ages, categories of their dogs and the worming habits.

Distribution of dogs by age classes

Distribution of dogs by categories

The population tested is representative of the study area with correct proportion of dogs categories and ages.

Prevalence of infections by dog categories

Hunting dogs were significantly (28.8%) more infected ($P<0.001$) than farm (9.43%) and pet dogs (10.27%).

Most of the dogs (86.2%) are treated and more often with a frequence of 1-2 times per year (average of 7 months). In the meantime, the owners don't know if the anthelmintic used is efficient against *E. multilocularis* for 62% and only 21% use praziquantel.
Eleven dogs are monthly wormed (2.3%) as recommended by WHO in endemic area, 10 were pet dogs and only one was a hunting dog the most infected type.

The anthelmintic treatment had a significative influence on the infection of dogs ($P=0.027$) but the frequence didn't have an impact ($P=0.242$).

Dog infection by taenid eggs

On the four dogs infected, one is a hunting dog, wormed twice a year with praziquantel molecule and the three others are pet dogs from the same owner and not wormed.
Lifecycle of *T. crassiceps* is similar to *E. multilocularis* and others *Taenia sp.*
These dogs have consumed metacestode material from rodents.
So these 4 dogs are predators of rodents and must be considered as risk dogs because they could be potentially infected by *E. multilocularis*.

CONCLUSION

There is no evidence of the fox tapeworm in this canine population but risk dogs have been identified. According to our results the frequency of domestic dogs worming to prevent echinococcosis contaminations is not well adapted. In endemic regions of France, hunting dogs and pet dogs should be considered as potential vectors for transmission of alveolar echinococcosis to humans.

Keywords : Echinococcus multilocularis, dogs, helminths, taenid eggs, France.

F. BOUE

Range extension and contamination gradients of Echinococcus multilocularis in France.

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Introduction

Among the carnivore pathogens transmissible to human, the intestinal parasite members of Cestode class are very important. The Cestodes from *Echinococcus* genus are transmitted to human by accidental ingestion of infective eggs, distributed with faeces of the definitive host, that are particularly resistant in the environment.

In most European endemic areas the cycle of *Echinococcus multilocularis* (*Em*) is predominantly sylvatic involving red foxes as definitive hosts and several species of small mammals as intermediate hosts. Important factors enhancing the risk of human exposure include an increasing number of infective eggs of *Em* shed in the environment by foxes.

In view of the probable public health significance of *Em*, the causative agent of human alveolar echinococcosis, there is an urgent need for identification of new endemic areas. Knowledge of *Em* area repartition in France must be actualised to establish a pertinent program of fox surveillance and determine a baseline data to anticipate human risks of contamination in unknown endemic areas.

The aim of our study was to evaluate the emergence of the parasite, in a large area of Eastern France, and to determine its geographical distribution.

Material and method

Study area and sample collections

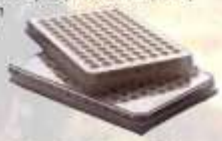
The monitoring was conducted to determine where *E. multilocularis* was present in 41 administrative departments of France, covering an area around 200 000 Km² in the North-Eastern territory.

In each department, using the multi-scale CORINE land Cover data, ecological areas with a percentage of grassland over 35% where selected. Inside these selected areas, between 10 and 50 sampling areas of 16 km² have been randomly selected in function of grassland covering. In the study area, a total of 1163 sampling areas have been defined. In collaboration with local collectors, from hunting associations or National Game Office, five fox faeces have been collected on each sampling area using an individual sample Kit.

ELISA coproantigen test

Specific *E.multilocularis* coproantigen detection was performed, by three similar ELISA techniques. The first collected samples were analysed by sandwich enzyme-linked immunosorbent assay (copro-ELISA) using the first generation of the commercial Chekit ® Echinotest (Dr Bommeli AG, liebefeld_bern, Switzerland) and for specificity reasons all the positive samples were validated by the original techniques developed by Deplazes et al (1999).

For all samples collected in the last years of the study the faecal extract were analysed by the new generation of the Chekit ® Echinotest.



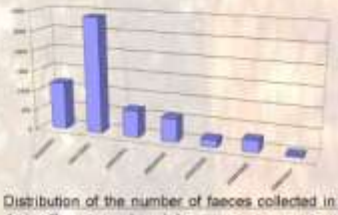
Keywords : Echinococcus multilocularis, Foxes, ELISA.

Results

During the period of 2000-2007, a total of 5854 fox faeces were collected from the field in 41 departments according to our sampling protocol. Each department has sent more than 90% of the expected number of samples. The majority of the faeces was collected during the two first winters 2000/2001 and 2001/2002, and correspond to 70 % of the total number of samples analysed.

Results of coproantigen ELISA detection in fox faecal samples collected in the 41 administrative departments

DEPARTMENTS	Number of samples	ELISA Faecal NEGATIVE	ELISA Faecal POSITIVE	Indeterminated or doubtful	% Pos
Ain	152	152	0	0	0,00%
Aisne	224	219	5	0	2,23%
Allier	146	132	1	13	0,71%
Ardennes	206	198	8	0	3,88%
Aube	158	148	5	5	3,16%
Doubs (Dm)	141	128	10	3	7,09%
Doubs	175	162	0	13	0,00%
Eure	158	158	0	0	0,00%
Eure-et-Loir	159	155	5	0	3,14%
Doubs	224	215	0	9	0,00%
Dordogne	214	205	0	9	0,00%
Essonne	54	50	4	0	7,41%
Eure	106	96	3	7	2,83%
Finistère-Sud	44	38	3	3	6,82%
Haute-Marne	208	179	7	14	3,37%
Haute-Marne	158	158	0	0	0,00%
Haute-Saône	142	134	0	8	0,00%
Haute-Savoie	94	89	0	5	0,00%
Indre	185	173	0	12	0,00%
Jura	196	179	7	10	3,57%
Loire	126	120	0	6	0,00%
Loiret	90	90	0	0	0,00%
Loir-et-Cher	48	39	2	9	4,17%
Marne	132	123	3	6	2,27%
Meurthe-et-Moselle	158	134	0	24	0,00%
Meuse	206	184	10	12	4,85%
Moselle	206	185	21	12	10,19%
Nièvre	128	119	3	6	2,34%
Nord	226	207	0	19	0,00%
Oise	208	207	0	1	0,00%
Pas-de-Calais	94	81	0	13	0,00%
Rhône	154	142	0	8	0,00%
Saône-et-Loire	150	142	0	8	0,00%
Savoie	108	98	0	8	0,00%
Seine-et-Marne	50	46	4	0	8,00%
Seine-Maritime	104	98	1	4	0,96%
Somme	108	98	4	6	3,70%
Territoire-de-Belfort	50	38	0	8	0,00%
Val d'Oise	50	46	2	2	4,00%
Vosges	198	182	4	12	2,02%
Yonne	150	139	0	8	0,00%
Total	5854	5149	221	484	4,13%



Distribution of the number of faeces collected in the field during the successive winters.

Localisation of the collected faeces, red spots are positive samples and green are negative samples

221 samples were considered as positive and 5149 as negative. We found positive samples in 38 departments out the 41 departments investigated, which cover a large east area of France. For some of these departments it was the first detection. The estimated apparent prevalence vary from 0.75% in Allier to 14.63% in the Territoire-de-Belfort and we where unable to detect *E. multilocularis* positive samples only in 3 departments.

Spatial analysis of the collected faeces:
Spatial variation of the probability that a collected faeces was positive. Risk decrease from high level in red to low level in pale yellow.
★ Statistical Border effect.

CONCLUSIONS

According to these observations results we postulate that *Em* could be considered as an emerging parasite in the fox population in the Western part of France but this expansion cannot really be proven due to the lack of adequate retrospective studies in newly recognized endemic areas. This phenomenon could be related to the move of infected red foxes that have the potential to spread infective *Em* eggs far from the area of detection to new areas where the cycle could easily be established. However, taking into account the level of infection in red foxes and the presence of landscapes favourable for the common voles, that are considered as major intermediate hosts in West of France, it is not surprising to find new positive areas.

K. VUTOVA

Alveolar Echinococcosis and Echinococcosis in Bulgaria

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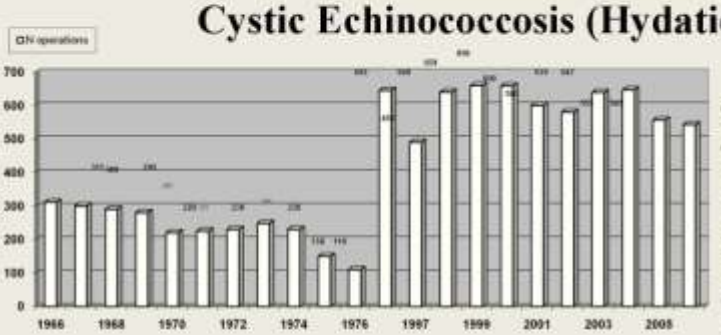
Department of Infectious Diseases, Parasitology and Tropical Medicine

Cystic Echinococcosis (Hydatid disease)



Human echinococcosis

•**1950 - 1962** ► high rates – 5,0 ÷ 6,6 ‰o

•**1948 and 1965 decree against rabies**

1970 - 1984 ►**1,2 ÷ 2,7 ‰o**

1985 - 1995 ►increasing trend - **3 ÷ 6,8 ‰o**

1996 - 2004 – 6,8 ÷ 8,4 maximum 8,47‰o in 1998

Varies between 6,25 and 7 ‰o during the last 5 years

2004 – 2008 ► program against echinococcosis

2007 - 543 operated patients

Animal echinococcosis: herding dogs 78%, Straying dogs 57%, House dogs 31%, Hunting dogs 16%;

Slaughterhouse post-mortem:

Cattle - 17,9%, Sheep - 7,5%, Pigs - 2,19%

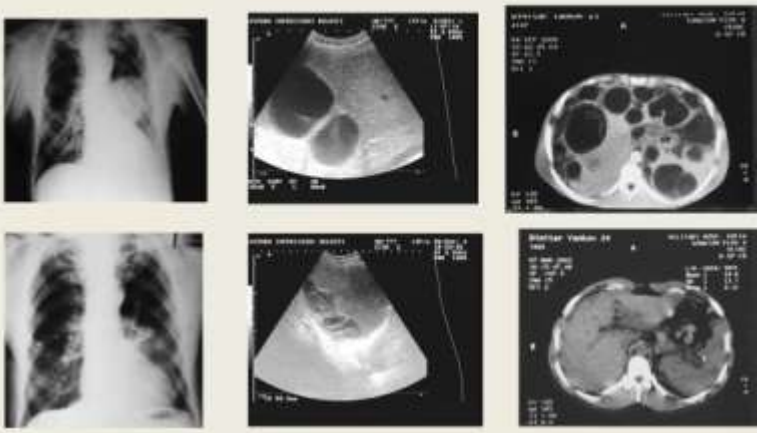
Chimiotherapy of cystic echinococcosis in Bulgaria started from 1979 with benzimidazoles: Albendazole and Mebendazole

Patients with: - multiple cysts and multiorgan involvement; - secondary disseminated cysts, relapses , - inoperable cases and with contraindications for surgery, - cases intraoperatively ruptured or residual cysts

1979 - 2008 г. - 308 patients with cystic echinococcosis treated with: ABZ - 92 patients and MBZ - 42 patients; 67 (male), 67 (female); mean age of the patients was 42 years (range 5-72)

Localisation: Hydatid cysts were located in **single organ** (66 patients 21,4 %); **Multiple** (152 49,7%) - 2 organs involved in 94 patients (30,5 %); - 3 organs - in 41 patients (13,3 %); - 4 organs and tissue -11 (3,6%); - 5 - 2 (0,6%) and 6 organs – 1 patient (0,3%). The distribution of patients according to the organs involved was as follows: in 240 (77,9%) patients the cysts were located in liver; in lungs – 147 (47,7%) ; 22 (14,3%) – in spleen, abdominal cavity – 7,5%, bones–11 (7,1%), kidneys - 13 (4,2%), brain – 5 (1,6%), heart – 8 (1,3%) .

Therapeutical effectiveness



Overall effectiveness of treated patients

➢84,41 % were cured

➢13,64 % - partial succsess

➢treatment failure was between 1,95 %

Alveolar Echinococcosis (AE)

T. P. Genov, D. K. Svilenov, 0. T. Polyakova-Krusteva. OCCURRENCE OF ALVEOCOCCUS MUTTILOCULARIS IN THE MICROTUS NIVALIS IN BULGARIA. Comptes rendus de I'Acadömie bulgare des Sciences, v. 33, N" 7, I9B0. After continuous study of wild mammalian helminths in Bulgaria we found the Algeococctls multilocuiaris larva in liver of vole (Microtus rligalis Mart.) from the Northern part of the Pirin Mountain (intermediate host).

CONCLUSIONS

We observed high therapeutic effectiveness of benzimidazoles in the treatment of hydatidosis. Chimiotherapy is effective and alternative in patients with single, multiple cysts and relapses.

There is no cases of human AE.

V. SNABEL

GENETIC VARIATION IN *ECHINOCOCCUS MULTILOCULARIS* FROM EUROPE ASSESSED BY DNA SEQUENCING

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INTRODUCTION

Echinococcus multilocularis, induces one of the most serious zoonotic disease in northern hemisphere, alveolar echinococcosis. The disease is of increasing public health concern in Europe, with high fatality rates and poor prognosis if managed incorrectly. Until the beginning of the 1990´s, the geographical range of *E. multilocularis* in Europe appeared to be restricted to southern Germany, northern Switzerland, and neighbouring regions of France and Austria. Over the past 15 years, the known range of the parasite has markedly extended accros Europe and it is assumed to be currently distributed over most of territory with the exception of the British Isles, Fennoscandia and the Mediterranean region (Romig, 2009). In addition to this report, *E. multilocularis* was recently confirmed in Romania in north-western Transylvanian counties (Barabási et al., 2010) and in Ukraine Kharchenko et al. (2008). To examine genetic variability and indicate transmission patterns in *Echinococcus multilocularis* from Europe, isolates from a contiguous territory of 8 European countries were subjected to analyses of DNA sequences.

MATERIAL AND METHODS

Nucleotide compoeition of 25 *E. multilocularis* isolates collected from 8 European countries and 18 capturing sites were examined in the study (Tab. 1). Samples originated from six intermediate host species and red fox definitive host. Following isolation, DNA of four mitochondrial targets (*cox1*, *nad1*, *rrnS*, *atp6*) and one nuclear target (*actII*) was amplified. For amplification of *cox1* and *atp6* regions, used primers were those described in Xiao et al. (2005), for *cox1* and *nad1* in Bagrade et al. (2008), for *actII* intron in Bart et al. (2004). PCR was conducted under following conditions: an initial denaturation step at 94°C for 3 min, followed by 30 cycles of 95°C for 30 sec, 56°C for 30 sec, 72°C for 1 min, final extension step 72°C for 7 min, except for *actII* with the shorter extension time to 50 s to increase the stringency of amplification. Amplicons were then purified using a Nucleospin Extract II kit (Macherey Nagel, Düren, Germany) and directly sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). Nucleotide sequences were aligned using CLUSTAL W program (Thompson et al., 1994) and compared with GenBank data using BLAST system. Resulting trees were constructed using MEGA 3.1 program (Kumar et al., 2001).

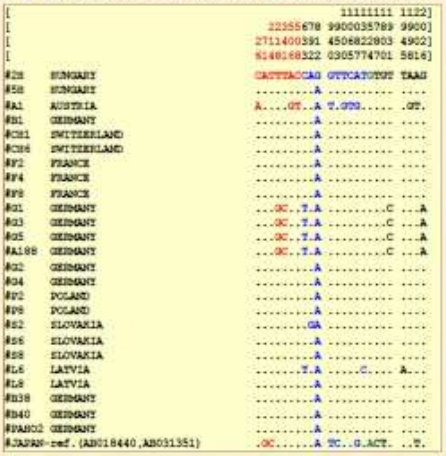
RESULTS

A total of 2 715 nucleotide sequences in five gene targets were examined in every isolate (*nad1* - 589 bp, *cox1* - 789 bp, *12S rRNA* - 362 bp, *atp6* - 516 bp, *actII* - 459 bp). Gathered data showed consistent differences in isolates allocated to the major European variant compared with reference *E. multilocularis* sequences from Japan, revealing 1-4 nucleotide substitutions in four targets - in 3 positions in *cox1* and *atp6*, 2 positions in *nad1*, and 1 position in *12S rRNA* (variable sites given in Fig. 1). In *atp6*, the major European variant shared two of 3 polymorphic sequences with isolate from north-central China (FmV2) analyzed by Yang et al. (2005), which differs from an American/Japanese form in a single site. Several additional genetic forms were detected within the European sample. Specifically, an Austrian human isolate A1 displayed 1-4 mutations in *cox1*, *nad1*, *atp6*, *actII* compared with remaining European isolates. Interestingly, all 4 substitutions in *cox1* compared with the European variant were identical to isolates from North America (N1, N2) described in Nakao et al. (2009), and 2 of these sites were corresponding to an Asian form in the Nakao´s study (A1-A10). Four isolates from the locality Feldstetten in southern Germany produced haplotype with 5 mutations compared to the main European form - one mutation in *cox1*, and two mutations in both *nad1* and *atp6*. The substitution in *cox1* was previously found also in France by Nakao et al. (2009) (Fig. 2). One Latvian isolate displayed the two nucleotide substitutions in *cox1*, one of which was identical with German isolates from Feldstetten. One of Slovak and Hungarian isolates had one specific mutation in the *cox1* target. In the *actII* target, variable sequences of which are not depicted in Fig. 1 due to the absence of any reference sequence, French isolate from Jura department and the above Austrian isolate displayed one nucleotide substitution. Among 26 substitutions in the whole European set, 18 were transitions and 8 transversions. In coding regions, 16 non-synonymous (inducing aminoacid changes) and 7 synonymous substitutions were detected. Noteworthy, in *nad1* all 6 mutations were non-synonymous. In dendrogram based on concatenated sequences (Fig. 3), three major clades in European analyzed sample were seen, one forming by Austrian isolate, second by German isolates from Feldstetten and third main clade consisted remaining European samples.

Tab. 1. Geographical origin of isolates

Code of isolate	Geographical origin	Host
A188	Germany (Feldstetten, Baden-Württemberg)	water vole
G1	Germany (Feldstetten, Baden-Württemberg)	water vole
G3	Germany (Feldstetten, Baden-Württemberg)	water vole
G5	Germany (Feldstetten, Baden-Württemberg)	water vole
G2	Germany (Westerheim, Baden-Württemberg)	common vole
G4	Germany (Westerheim, Baden-Württemberg)	common vole
B1	Germany (Münsingen, Baden-Württemberg)	common vole
B38	Germany (Münsingen, Baden-Württemberg)	muskrat
B40	Germany (Münsingen, Baden-Württemberg)	muskrat
PAHO2	Germany (Grünwald, Upper Bavaria)	muskrat
F2	France (Eulmont, Meurthe et Moselle dept.)	red fox
F4	France (Jura dept.)	coyou
F8	France (Jura dept.)	wild boar
CH1	Switzerland	patient
CH6	Switzerland	patient
A1	Austria	patient
2H	Hungary (Nagybajcs, north-western Hungary)	red fox
5H	Hungary (Penc, north-central Hungary)	red fox
S2	Slovakia (Prešov, eastern Slovakia)	red fox
S6	Slovakia (Nitra, western Slovakia)	red fox
S8	Slovakia (Dolný Kubín, north-central Slovakia)	red fox
P2	Poland (Ustrzyki Dolne, Sub-Carpathia)	red fox
P8	Poland (Kartuzy, Pomerania)	red fox
L6	Latvia	red fox
L8	Latvia	red fox

Fig. 1. Polymorphic sites in examined mitochondrial gene targets



red colour *nad1* nucleotides, blue *cox1*, velvet - 12S, black - *atp6*.
Dots indicate identity with the above sequence.

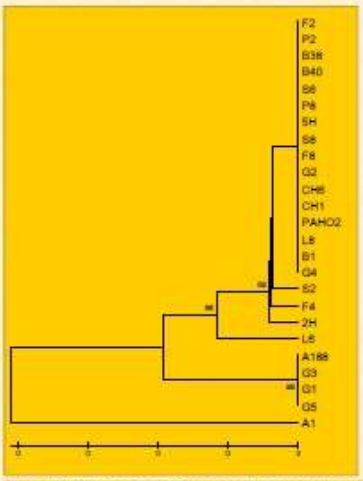


Fig. 3. UPGMA tree inferred from concatenated sequences in 5 DNA regions. Numbers at nodes represent percentage occurrence of clades in 1000 bootstrap replications.

Position (cox1)	12	21	40	44	203	223	251	351	354	402	411	438	476	468	738
Position (concatenated)	601	610	629	633	792	812	850	940	953	991	1000	1025	1065	1077	1327
MEV	A	T	G	C	A	A	G	G	T	C	T	A	C	A	T
G1,G3,G5	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
S2	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
A1	.	.	.	.	.	.	.	T	.	.	G	.	T	G	.
H2	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.
L6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
Japan ref.	.	.	.	.	.	.	.	T	C	.	.	.	.	G	.
E1	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.
E3	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
A1-A10	.	.	.	.	.	.	.	T	C	.	.	.	.	G	.
N1	G	.	.	.	.	.	.	T	T	.	G	.	T	G	.
N2	.	.	.	.	.	.	A	T	T	.	G	G	T	G	.

Fig. 2. Nucleotide substitution in *cox1* gene relative to the most common variant detected in Europe (MEV). By blue colour are indicated substitutions published in a study of Nakao et al. (2009) in relation to this study. Origin of isolates from Nakao´s study: E1 - Austria, E3 - France, A1-A10 - Asian isolates (Japan, Kazakhstan, China), N1 - North America (St. Lawrence Island), N2 - North America (Indiana, South Dakota).

CONCLUSIONS

In obtained DNA sequences, genetic diversity of *E. multilocularis* was higher in historically documented central European endemic zone (0.082% of polymorphic sites) than in territories presumed to be invaded by parasite more recently (0.048%). Coupled with the commonly distributed transversal profiles of sequencing patterns even in apparently newly endemic parts of Europe, the data support Knapp et al. (2007) model inferred from microsatellite patterns suggesting that parasite focus in Europe is governed by "mainland-island" transmission where ancestral foci supply hitherto non-endemic areas by dispersal generated by fox mobility and migration. The occurrence of the two further genetic forms found in was confined to the restricted territory (southern Germany, Austria) to former endemic European zone (established until 1990´s), where the higher genetic structuring is more expected. Due to high affinity of the Austrian isolate from patient to North American form of *E. multilocularis* it is likely that the respective Austrian genotype represents an ancestral form of *E. multilocularis* from glacial refugia maintaing in the historical endemic zone of Europe.

ACKNOWLEDGEMENTS

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