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.

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
Dr. F. GRENOUILLET: UFC - WHO Collaborating Center, Hospital physician in parasitology
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

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

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

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<th>Dr. Daniel Hegglin</th>
<th>Institute of Parasitology, University of Zurich, Switzerland</th>
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<td>Chairman</td>
<td>Dr. Per Have</td>
<td>European Food Savety Authority (EFSA) AHAW Panel</td>
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<td>Reporter 1</td>
<td>Dr. Frank Boué</td>
<td>NRL (National Referency Laboratory) Echinococcoses ANSES Nancy (French Agency for food, environmental and occupational health safety), France</td>
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<tr>
<td>Reporter 2</td>
<td>Dr. Paul Torgeson</td>
<td>Institute of Parasitology, University of Zurich, Switzerland</td>
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<td>09:00-09:30</td>
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<tr>
<td>09:30-10:00</td>
<td>A ECHINOCOCCUS MULTILOCULARIS IN ANIMALS: GEOGRAPHICAL DISTRIBUTION AND HOST SPECIES</td>
<td>Romig T.</td>
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<tr>
<td>10:10-10:25</td>
<td>A Echinococcus spp. found in carnivores in Latvia</td>
<td>Bagrade G.</td>
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<td>10:30-10:45</td>
<td>A Helminths of red foxes (Vulpes vulpes) and raccoon dogs (Nyctereutes procyonides) in Lithuania</td>
<td>Sarkunas M.</td>
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<td>10:50-11:05</td>
<td>A Echinococcus multilocularis in final hosts in Poland - results of ten years survey</td>
<td>Gawor J.</td>
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<td>11:10-11:25</td>
<td>A Monitoring of the prevalence of Em in red foxes in Poland.</td>
<td>Karamon J.</td>
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<td>11:30-11:50</td>
<td>COFFEE BREAK AND POSTER PRESENTATION*</td>
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<td>11:50-12:05</td>
<td>A Ten-Year of Echinococcus multilocularis occurrence in Slovakia</td>
<td>Miterpakova M.</td>
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<tr>
<td>12:10-12:25</td>
<td>A Overview of the epidemiological data on the presence of Echinococcus multilocularis in northern Belgium.</td>
<td>Vervaeke M.</td>
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<td>12:30-12:45</td>
<td>A Echinococcus multilocularis in Germany</td>
<td>Schwarz S.</td>
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<td>12:50-13:05</td>
<td>A Large scale screening of red fox intestines in search of Echinococcus multilocularis in France.</td>
<td>Combes B.</td>
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<td>13:10-13:25</td>
<td>A Domestic dog situation for Echinococcus multilocularis in different endemic areas in France</td>
<td>Umhang G.</td>
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<td>13:30-14:45</td>
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<td>14:45-15:15</td>
<td>B CURRENT FACTS AND TREND IN HUMAN AE</td>
<td>Gottstein B.</td>
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<td>15:25-15:40</td>
<td>B Epidemiological trends of human alveolar echinococcosis in Franche-Comté from 1980 to 2010</td>
<td>Giraudoux P.</td>
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<tr>
<td>15:45-16:00</td>
<td>B Human alveolar echinococcosis in France, update 2010</td>
<td>Grenouillet F.</td>
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<td>16:05-16:20</td>
<td>B Serological evidence for human alveolar echinococcosis in Slovenia (2006-2010)</td>
<td>Šoba B.</td>
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<td>16:25-16:45</td>
<td>TEA BREAK</td>
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<td>16:45-17:00</td>
<td>B Echinococcus multilocularis in the Netherlands: what about the human situation?</td>
<td>Kortbeek T.</td>
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<td>17:05-17:20</td>
<td>B Human alveolar echinococcosis in Switzerland 1956-2008</td>
<td>Schweiger A.</td>
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<td>17:25-17:40</td>
<td>B Alveolar echinococcosis emergence in patients with immune deficiency: what is the epidemiological impact</td>
<td>Vuitton D.A.</td>
</tr>
<tr>
<td>17: 45-18:00</td>
<td>B In vitro screening for new compounds against Echinococcus multilocularis metacestodes identifies anti-echinococcal activity of mefloquine</td>
<td>Hemphill A.</td>
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<tr>
<td>18:05-18:20</td>
<td>AB Epidemiological human situation and studies on foxes in Austria.</td>
<td>Glawischnig W.</td>
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18:30 DEPARTURE FOR WELCOME EVENING AT LUNEVILLE CASTLE

*Posters will be displayed during the whole congress
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<tr>
<td>09:00-09:35</td>
<td>GENETICS AND METHODOLOGY INTRODUCTION: Phylogeny and genotyping studies in Cestodes (Echinococcus and Taenia) to trace back their molecular evolution history</td>
<td>Knapp J.</td>
<td>France</td>
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<tr>
<td>09:35-09:50</td>
<td>Genetic diversity of Em in Hungary inferred by multi-locus microsatellite analysis</td>
<td>Casuli A.</td>
<td>Hungary, Italia</td>
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<td>09:50-10:00</td>
<td>QUESTIONS</td>
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<tr>
<td>10:00-10:15</td>
<td>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</td>
<td>Boufana B.</td>
<td>United-Kingdom</td>
</tr>
<tr>
<td>10:15-10:30</td>
<td>Segmental Sedimentation and Counting Technique (SSCT): an adaptable method for qualitative diagnosis of Echinococcus multilocularis in fox intestines</td>
<td>Woronoff N. Boue F.</td>
<td>France</td>
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<td>10:30-10:40</td>
<td>QUESTIONS</td>
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**10:40-11:00 COFFEE BREAK**

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<tr>
<td>11:00-11:30</td>
<td>CONTROL OF ECHINOCOCCUS MULTILOCULARIS: EPIDEMIOLOGICAL AND STRATEGIC CONSIDERATIONS</td>
<td>Hegglin D.</td>
<td>Switzerland</td>
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<td>11:40-11:55</td>
<td>Scientific Opinion of the AHAW, 18th of January 2007</td>
<td>Have P.</td>
<td>EFSA</td>
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<tr>
<td>12:00-12:15</td>
<td>Rural settlements – A habitat for foxes and the fox tapeworm?</td>
<td>Janko C.</td>
<td>Germany</td>
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<tr>
<td>12:20-12:35</td>
<td>Fox defection behaviour in relation to spatial distribution of voles in an urbanized area: An increasing risk of transmission of Echinococcus multilocularis</td>
<td>Robardet E.</td>
<td>France</td>
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<tr>
<td>12:40-12:55</td>
<td>Capability of Echinococcus multilocularis to persist within fox home ranges of increasing sizes depending on vole distribution and fox behaviour</td>
<td>Quintaine T.</td>
<td>France</td>
</tr>
<tr>
<td>13:00-13:15</td>
<td>Demonstrating freedom from Echinococcus multilocularis in Sweden, Norway mainland and Finland</td>
<td>Whalstrom H.</td>
<td>Sweden</td>
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**13:30-14:45 LUNCH**

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<tbody>
<tr>
<td>14:45-15:00</td>
<td>Increasing risk of human alveolar echinococcosis in the Netherlands and possible control options</td>
<td>Takumi K.</td>
<td>Netherlands</td>
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<tr>
<td>15:05-15:20</td>
<td>Successful long time baiting campaign against the fox tapeworm (Echinococcus multilocularis) in Southern Bavaria</td>
<td>Konig A.</td>
<td>Germany</td>
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<tr>
<td>15:25-15:40</td>
<td>Urban control of Echinococcus multilocularis in France</td>
<td>Comte S. Raton V.</td>
<td>France</td>
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**15:45-16:15 TEA BREAK**

<table>
<thead>
<tr>
<th>TIME</th>
<th>WORKSHOP A CONCLUSIONS</th>
<th>Bresson-Hadni S. Kern P.</th>
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<tr>
<td>16:20-16:40</td>
<td>WORKSHOP B CONCLUSIONS</td>
<td>Boue F. Torgerson P.</td>
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<td>16:50-17:10</td>
<td>WORKSHOP C CONCLUSIONS</td>
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<tr>
<td>17:20-17:40</td>
<td>WORKSHOP C CONCLUSIONS</td>
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<tr>
<td>17:50</td>
<td>FAREWELL PARTY IN NANCY CITY HALL</td>
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</tbody>
</table>
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' endemcity 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.
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 invasion 123 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. Šarkūnas¹, R. Bružinskaitė¹², A. Malakauskas¹, P. Torgerson³, P. Deplazes⁴

¹ Veterinary Academy, Lithuanian University of Health Science, Kaunas, Lithuania
² Institute of Parasitology, University of Zürich, Zürich, Switzerland
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. Abundance 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.
Studies done between 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 raccoon 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.

J. Karamon, J. Sroka, T. Cencek

Department of Parasitology and Invasive Diseases, National Veterinary Research Institute in Pulawy, Al. Partyzantow 57, 24-100 Puławy, Poland

j.karamon@piwet.pulawy.pl

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.

**M. Miterpákova, P. Dubinský**

Parasitological Institute of the Slovak Academy of Sciences, Košice, Slovakia, miterpak@saske.sk

**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.

Muriel Vervaeke*

* Agency for Nature and Forest, Flemish Government, Koning Albert II-laan 20, bus 8, 1000 Brussel
Tel: 0032-2-553 81 49, Fax: 0032-2-553 81 05, E-mail: muriel.vervaeke@lne.vlaanderen.be,

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 parasitic 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.


**ECHINOCOCCUS MULTILOCULARIS IN GERMANY.**

F. J. Conraths¹, C. Staubach¹, R. Mattis¹, A. Sutor¹, S. Schwarz¹, C. Schulze², L. Hoffmann³, K. Tackmann¹

¹Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute for Epidemiology, National Reference Laboratory for Echinococcosis, Wusterhausen, Germany; Sabine.Schwarz@fli.bund.de
²Landeslabor Berlin-Brandenburg, Frankfurt (Oder);
³Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz, Bad Langensalza

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 and 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. Combes¹, S. Comte¹, F. Raouï², F. Boué³, V. Raton¹, P. Giraudoux²

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.

G. Umhang1, V. Raton2, J-M. Boucher1, V. Hormaz1, B. Combes2, C. Richomme1, F. Boué1

1 French Agency for Food, Environmental and Occupational Health and Safety (Anses), Nancy Rabies and Wildlife laboratory, Technopôle Agricole et Vétérinaire, BP 40009, 54220 Malzéville cedex, France gerald.umhang@anses.fr.

2 Entente Rage et Zoonoses (ERZ), 54220 Malzéville, France.

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 explanatory 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 echinococciosis 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.

P. Giraudoux⁠¹, L. Mouzon⁠¹, J. Knapp⁠¹, F. Grenouillet⁠¹, S. Bresson-Hadni⁠¹, D. Vuitton⁠², M. Piarroux⁠¹, R. Piarroux⁠¹, L. Millon⁠¹

¹ Chrono-environnement, WHO collaborating center, Université de Franche-Comté, CHU Besançon, France, patrick.giraudoux@univ-fcomte.fr
² Carcinogénèse épithéliale : facteurs prédictifs et pronostiques, WHO collaborating center, Université de Franche-Comté – CHU Besançon, France

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.

F. Grenouillet\textsuperscript{1,2}, J. Knapp\textsuperscript{1,2}, L. Millon\textsuperscript{1,2}, V. Raton\textsuperscript{2}, C. Richou\textsuperscript{1}, M. Piarroux\textsuperscript{1}, R. Piarroux\textsuperscript{1,4}, G. Mantion\textsuperscript{1}, D.-A. Vuitton\textsuperscript{1}, S. Bresson-Hadni\textsuperscript{1,2}

for the FranceEchino working group

\textsuperscript{1} WHO Collaborating center for prevention and treatment of human echinococcosis, University Hospital, Besançon, France. fgrenouillet@chu-besancon.fr or francechino@chu-besancon.fr
\textsuperscript{2} Chrono-Environnement UMR 6249, University of Franche Comté, Besançon, France
\textsuperscript{3} Entente Rage Zoonoses (ERZ), Malzeville, France
\textsuperscript{4} La Timone University Hospital, Marseille, France
\textsuperscript{5} Carcinogénèse épithéliale : facteurs prédictifs et pronostiques, University of Franche Comté, Besançon, France

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 zones 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).

B. Šoba, J. Logar

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
barbara.soba@mf.uni-lj.si, jernej.logar@mf.uni-lj.si

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.
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.
Abstract

Switzerland belongs to the core countries in Central Europe endemic for *Echinococcus multilocularis*. Several case-finding studies have been conducted between 1956-2005. 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?


1 Department of Haematology
2 WHO-Collaborating Centre for Prevention and Treatment of Human Echinococcosis; University of Franche-Comté and University Hospital; 25030 Besançon, France; ccoms@chu-besancon.fr ; 3Department of Infectious Diseases, 38043 Grenoble cedex 9.

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.

B. Stadelmann, T. Küster, C. Herrmann, J. Müller, B. Gottstein, A. Hemphill

Institute of Parasitology, Vetsuisse Faculty, University of Bern, Bern, Switzerland

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 secondarily 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 from 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.

J. Knapp¹,²,³, S. Bresson-Hadni², S. Bretagne⁴, B. Gottstein⁵, F. Grenouillet¹,², A. Ito³, A. Lavikainen⁶, M. Nakao⁷, M. Okamoto⁷, R. Piarroux⁸, U. Saarma⁹, T. Yanagida³.

¹Department of Chrono-Environment, CNRS 6249 usc INRA, University of Franche-Comté, Besançon, France, jennylaure25@hotmail.fr  
²Centre Hospitalier Universitaire de Besançon, World Health Organization Collaborating Center on Prevention and Treatment of Human Echinococcosis, Besançon, France  
³Department of Parasitology, Asahikawa Medical University, Asahikawa, Hokkaido 078-8510, Japan, akiraito@asahikawa-med.ac.jp  
⁴Laboratoire de Parasitologie-Mycologie, Hôpital Henri Mondor, Créteil, France  
⁵Institut of Parasitology, Vetsuisse Faculty, University of Bern, Switzerland  
⁶Department of Bacteriology and Immunology, Haartman Institute, P.O. Box 21, FI-00014 University of Helsinki, Finland, antti.lavikainen@helsinki.fi  
⁷Center for Human Evolution Modeling Research, Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan, mokamoto@muses.tottori-u.ac.jp  
⁸Laboratoire de Parasitologie et Mycologie, Hôpital de la Timone, Marseille, France  
⁹Department of Zoology, Institute of Ecology and Earth Sciences, University of Tartu, Vanemuise 46, 51014, Tartu, Estonia, urmas.saarma@ut.ee

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.

A. Casulli1, Z. Széll2, E. Pozio1, T. Sréter2

1 Department of Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, vialle Regina Elena 299, 00161 Rome, Italy; e-mail: adriano.casulli@iss.it; edoardo.pozio@iss.it
2 Laboratories for Parasitology, Fish and Bee Diseases, Veterinary Diagnostic Directorate, Central Agricultural Office, Tábornok utca 2, H–1149 Budapest, Hungary; e-mail: sretert@oai.hu

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.

B. Boufana, G. Umhang, S. Lahmar, J. Qiu, T. Li, B. Combes, P.S. Craig

1 Salford University, Manchester, U.K
b.boufana@salford.ac.uk

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 al., 2005; Li et al., 2008). The species-specific optimization of PCR assays (van der Giessen et al., 1999; Abbasi et al., 2003; Štefanič et al., 2004; Dinkel et al., 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 MgCl₂ 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 1.22x10⁻³ng/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.

G. Umhang¹, N. Woronoff-Rehn², J.M. Boucher¹, V. Hormaz¹, C. Caillot¹, B. Combes³, F. Boué¹

¹ ANSES Nancy, (Laboratory for study and research on rabies and wild animal disease), Technopole Agricole et Vétérinaire, BP 40009, 54220 Malzéville - France
³ ERZ, Domaine de Pixerécourt, 54220 Malzéville

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

D. Hegglin and P. Deplazes

Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland
Email: dhegglin@access.uzh.ch

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?

C. Janko¹, S. Linke¹, T. Romig², D. Thoma², W. Schröder¹, A. König¹

¹ Cair of Animal Ecology, Wildlife Biology and Management Unit, Technische Universität München, Hans Carl von Carlowitz Platz 2, 85354 Freising-Weihenstephan; Germany
² Department of Parasitology, University of Hohenheim, Emil-Wolff Str. 34, 70599 Stuttgart; Germany

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; χ²=0.12, df=1, p=0.727). PCR analyses of faeces could also not proof significant differences between habitats (χ²=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?

E. Robardet¹, P. Giraudoux², C. Caillot¹, D. Augot¹, F. Boue¹, J. Barrat¹

¹ French Agency for Food, Environmental and Occupational Health and Safety (Anses), Nancy Rabies and Wildlife laboratory, Technopôle Agricole et Vétérinaire, BP 40009, 54220 Malzéville cedex, France.
emmanuelle.robardet@anses.fr
² Chrono-environnement - University of Franche-Comté, UMR CNRS 6249 usc INRA, Place Leclerc, 25030 Besançon cedex, France.

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

**T. Quintaine**, M.-L. Poulle, V. Grimm and H.-H. Thulke

1 2C2A-CERFE, 5 rue de la Héronnière, F-08240 Boult-aux-Bois, France.
2 Université de Reims Champagne-Ardenne, Laboratoire de Parasitologie-Mycologie, EA 3800, UFR de Médecine, IFR 53, 51 rue Cognacq-Jay, F-51096 Reims, France.
3 UFZ, Helmholtz Centre for Environmental Research - UFZ, Department of Ecological Modelling, 04318 Leipzig, Germany.

**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 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 with simultaneous 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.

H. Wahlström, G. Hallgren, D. Christensson, H. Uhlhorn  
National Veterinary Institute, Uppsala, Sweden  
helene.wahlstrom@sva.se

M. Hjertqvist, A. Wallensten  
Swedish Institute for Infectious Disease Control, Stockholm, Sweden

M. Cedersmyg  
The Swedish Board of Agriculture, Jönköping, Sweden

M. Isomursu  
Finnish Food Safety Authority, Oulu, Finland

P. Hopp, R. Davidson  
National Veterinary Institute, Oslo, Norway

Abstract

**Background:** *Echinococcus multilocularis* (*Em*) is an emerging zoonotic parasite in Central Europe. At 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.

K. Takumi1, A. de Vries1, D. Hegglin2, P. Deplazes2, B. Gottstein3, P. Teunis1, J. van der Giessen1

2 Institute of Parasitology, Vetsuisse Faculty, University of Zurich, Switzerland
3 Institute of Parasitology, University of Bern, Switzerland

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 environment 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 (R0) 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.

A. König1, C. Janko1, T. Romig2, R. Hildenbrand1, E. Holzhofer3, Y. Kotulski1, C. Ludt1, M. Merli2, E. Perret1, D. Thoma2, J. Vilsmeier1, S. Wermut1, D. Zannantonio1

1 Wildlife Biology and Management Unit, Technische Universität München, Am Hochanger 13, D-85354 Freising, Germany. Email: koenig@wzw.tum.de
2 Parasitology Department, University of Hohenheim, Emil-Wolff-Str. 34, D-70599 Stuttgart, Germany
3 Holzhofer Flight Service, Unterschüpfer Str. 9, D-97944 Boxberg

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 km²). Within the first year, baits were put down monthly and within the following years in six weeks intervals. Bait distribution density was 50 pieces/km². The pre-baiting prevalence was 51% (45-57% CI 95%, N=286). During a one-year period following the first 4 months of bait distribution, only one positive fox was found (prevalence 1%; 0-7% CI 95%). Prevalence rates declined to 2% (2007), 3% (2008) and 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.


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

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.
European Congress On Alveolar Echinococcosis
Nancy, 8th and 9th of December 2010

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Echinococcus surveillance in Finland

A. Oksanen

Echinococcus granulosus G10 (Echinococcus canadensis) is endemic in Finland

Echinococcus granulatus is a common tapeworm in various animals, including domesticates. In Finland, the parasite is mainly transmitted through contact with infected dogs. The disease is caused by larvae that develop in the liver and can cause damage to the liver and other organs.

Echinococcus multilocularis has so far never been detected in Finland

Echinococcus multilocularis is a parasitic tapeworm that is known to cause cysts in the liver, lungs, and other organs. In Finland, the disease has not been detected, and the risk of infection is low due to the absence of the species in the region.

The presence of the disease is dependent on the presence of infected animals. Public health officials recommend regular health checks for animals in areas where the disease is prevalent.

Echinococcus control program in Norway includes both asymptomatic treatment of dogs and the ban of feeding them raw meat.
Potential consequences of introduction of *Echinococcus multilocularis* into Sweden


1. SVA, National Veterinary Institute, SE-75189 Uppsala, Sweden
2. Swedish Board of Agriculture, SE-58182 Jönköping, Sweden

**Conclusions**

Potential consequences and estimated costs associated with endemcity 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.

**References**

3. Vågholm, I. 2006. An assessment of the risk that EM is introduced with dogs entering Sweden from other EU countries without or with anthelmintic treatments. [http://www.sva.se/upload/pdf/pdf/sve_andereglag08.pdf](http://www.sva.se/upload/pdf/pdf/sve_andereglag08.pdf)

**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 *Echinococcus multilocularis* in Sweden.

**Results:** It was estimated that between 9 to 42 human cases of alveolar echinococcosis may be diagnosed annually. The costs of medical treatment per patient has been estimated to be 300,000 SEK.

**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 dewormed monthly. The annual number of introduced dogs was estimated to be 20,000, based on data from 2003. Costs for deworming were 2.5–6.5 SEK. Carts were not included, due to lack of data.

**Results:** Annual costs for deworming dogs in Sweden, estimated to be 20 million SEK. With the present system costs for deworming of all introduced dogs is approximately 50 million SEK per year (see veterinary costs).

**Altered outdoor activities**

**Background:** The tradition of outdoor activities, such as mountainous and berry-picking, is strong among Swedes thanks to the right to public space. The law grants the freedom to enjoy the countryside and pick berries and mushrooms in almost any area excepted near roads. The tradition of outdoor activities would be restricted and associated with changes in space due to changed behavior in 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 situations 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. These are no such advice given in Sweden today and the awareness about the risks with EM and how to minimize 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 minimize potential risks.

**Increased surveillance**

**Method:** During 2006 and 2007, between 200–300 wild and feral hogs have normally been investigated for EM in total 1,000 animals. Currently, the surveillance of feral hogs around 50,000 € per year (3).

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

A. Marcinkute, M. Sarasauskaité, G. Štripas, G. Birmaš, V. Sokołowas, M. Pasikonis, J. Valantinas, A. Schröfer, A. Mathis, P. Deplazes

Vilnius University Hospital of Tuberkuola and Infectious Diseases, Lithuanian University Hospital "Santamaris Klinikos"

Department of Infectious Diseases, Lithuanian Veterinary Academy, Lithuania

Institute of Parasitology, University of Zurich, Switzerland

Keywords: alveolar echinococcosis, emerging disease, Lithuania, diagnosis, treatment, epidemiology.

Case Definition

Diagnosis of alveolar echinococcosis is confirmed:

- by serological or immunological tests
- by histology
- by imaging techniques

Controlled trials have been conducted to confirm the diagnosis.

Results

- There were 12 cases of alveolar echinococcosis in Lithuania from 1999 to 2019.
- The majority of cases were diagnosed in the southern part of the country.
- The average age of patients was 43 years (range: 26-70 years).
- The most common clinical symptoms were:
  - Abdominal pain
  - Weight loss

AE: clinical and diagnostic features

- Primary clinical diagnosis:
  - Abdominal pain
  - Weight loss
  - Fatigue

- AE: imaging features:
  - CT scan may show a mass in the liver
  - MRI may show a hypointense mass

- AE: serological features:
  - Positive tests for E. multilocularis

AE treatment

- Surgery (liver resection)
- Chemotherapy (idarubicin, gemcitabine)

Conclusions

- Further studies are needed to confirm the clinical effectiveness of the treatment.
- Early diagnosis and prompt treatment are crucial for a successful outcome.
- Public health campaigns are necessary to increase awareness among healthcare professionals.

Graph: Flowchart of patient management

Graph: Distribution of cases by region
C. CRETU

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

Echinococcosis/hydatidosis in Romania

Carmen-Michaela Cretu
“Carol Davila” University of Medicine and Pharmacy
Colentina Teaching Hospital
Bucharest, Romania

The epidemic disease ECHINOCOCCOSIS (AE & HE) produced by the larval stages of two worms, Cystic Echinococcosis (CE) and Alveolar Echinococcosis (AE), respectively, is a major health problem affecting a large part of the population in certain areas and is a major risk factor for human health. AE has been reported in Europe, Asia, and the Americas. The disease is not easily diagnosed, which makes it difficult to control and eradicate. AE can cause significant damage to the liver, lungs, and other organs, leading to severe health problems.

ECDC Report 2006

49 communicable diseases and health issues

However, the screening for AE in rural areas in Romania, presented in our study, showed that the number of cases of AE is still high, particularly in certain areas. The diagnosis of AE is challenging, and it is important to have a multidisciplinary approach involving specialists in different fields to ensure accurate diagnosis and effective treatment.

THE USEFULNESS OF ABDOMINAL CT AND ELECTIVE STAGE

Echinococcosis (AE) screening and evaluation of pathologic findings

PERCENTAGE OF IDENTIFIED CE ACCORDING TO THEIR NUMBER AND EVOLUTIVE STAGE

AE presents a morphologic spectrum, and the identification of this spectrum is crucial for appropriate treatment and management. The spectrum includes various forms, such as the typical unilocular cyst, multilocular cyst, and diffuse form, which can be seen on CT scans.

CASE PRESENTATION

A 25-year-old female presented with a 6.5 cm cystic mass in the liver. The CT scan showed a multilocular cystic lesion with septa and a peripheral enhancement. The patient underwent surgical resection, and histopathological examination confirmed the diagnosis of AE. The patient is currently under medical surveillance.

ALVEOLAR ECHINOCOCCOSIS

Very few data... It seems that it was found only 1 case in animals - sheep (tongue), unpublished CSEA.

No reported cases in humans in spite the geographical conditions.

National Research Program PNCD 4 NR 52-101/2006 on echinococcosis - under development in this moment. One of the tasks is to look for AE in both humans and animals.

CONCLUSIONS

Hydatidosis is an apparently benign disease, but it is malignant due to its potential for evolution and to the risk of complications.

The success: good working team - surgeon, pathologist, laboratory.
M. KIRJUSINA
Institute of Systematic Biology, Daugavpils University
Latvia

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

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 helminthofauna of wild life animals there is a risk of Enterococcus for pets (Fig. 1).

Materials and methods: Investigation of intestinal parasites of 24 dogs (Canis familiaris) and 39 cats (Felis catus) 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”.

Results: Overall there were found four helminth species in dogs: Toxocara canis (prevalence 41.7%), Uncinaria stenocephalus (4.2%), Trichinella sp. (4.2%) and Mectochoeris 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 males (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%), Diphyllidium caninum (7.7%) and Mectochoeris albida (17.0%) (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 helminth species – for 10% of cats. Prevalence of infestation was higher for male per 10% and amount of 58.3%. Similarily for cats and dogs males infestation was higher than in females (Fig. 3).

Conclusions: Totally were detected 7 helminth species in dogs and cats. Toxocara from Mecotheca 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.

Acknowledgement
This study was financed by ESF project “Support for Daugavpils University PhD studies program implementation” agreement № 2010.1.1.1.1.01.1.1.01.10.002 “14.4.04”. Authors are grateful for technical support from technicians of Institute “BIOR.”
USING NICHES MODELS TO INFER POTENTIAL DISTRIBUTION OF 
Echinococcus multilocularis IN ITALY

Adriano Casulli, Edoardo Pedi, and Luigi Malerba

Introduction
The metacecal larval stage of Echinococcus multilocularis is the invasive agent of alveolar echinococcosis (AE), one of the most pathogenic helminth infections in the northern hemisphere (Villunto et al., 2006). This parasite is endemic in central Europe and although it has been suggested that its geographical range has expanded in recent years, no proof of this theory is available because of the lack of adequate metacecal surveys inside newly endemic areas (Wong et al., 2006).

In recent years, spatial analysis and Geographic Information Systems, have been further expanded using ecological models to predict the potential spatial spread of many diseases (Pearson, 2006). Niche models have been widely used in biodiversity studies, where they are usually the cornerstone of the diagnosis of a species, but few applications have been developed in the epidemiological context (Casulli, 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 alveolar stages of the parasite.

Materials and Methods

Between 2001 and 2004, Casulli and colleagues (2005) analyzed a total of 201 infections of rodent origin in Northern and Central Italy (Casulli et al., 2005). A total of 14 (5.9%) samples resulted positive to the molecular coprodiagnosis and their analysis was restricted to the lower end receiving region (Figure 1). The 24 positive samples were represented and used as input into the subsequent model.

We characterized our study area using 11 environmental layers that were combined in a spatial resolution of 0.25 km and covered a 600-km² area (Figure 2). We then selected seven environmental variables: mean temperatures of the warmest quarter, precipitation of the warmest quarter, and the hottest quartile; elevation; slope (Figure 3); and the annual average vegetation density (Ceballos et al., 2003).

Using the environmental variables selected above together with the 14 positive locations, we built a model using the maximum entropy approach as implemented in the Maxent software package (Phillips et al., 2006). Maxent predicts the distribution of a species based on the climatic variables, and the values found in the study area (Casulli et al., 2005). To evaluate how the model performs, we used random locations within the study area, and the predicted distribution of the probable distribution of E. multilocularis, a species of interest in the study area, which characterized the performance of a model using the area under the curve (AUC).

The area occupied by the species under study is characterized by low precipitation and high temperature, with a high mean elevation and low slope (Figure 4).

Results and Conclusions

The resulting AUC was equal to 0.955, indicating an excellent model performance. The best climatic variable was the pH, followed by the percentage of vegetation, vegetation cover, and the elevation. Other variables were not statistically significant. The probability of presence for E. multilocularis in the study area was 0.98, with low occurrence in "uninhabited" areas, with high occurrence in "inhabited" areas at the border of the study area. The final model, which was used to map the potential distribution of E. multilocularis "in situ," was validated by using the environmental variables selected above together with the 14 positive locations, and the probability of presence for E. multilocularis in the study area was 0.98, with low occurrence in "uninhabited" areas, with high occurrence in "inhabited" areas at the border of the study area. 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Domestic dog situation for *Echinococcus multilocularis* in a highly endemic department of France.


**AFSSA-LIEPPAS Wildlife Diseases Unit, BP 48400, F-54220 Mulhouse, France.**

**WHO Collaborating Centre for Research and Management in Zootechnic Control - Off Reference Laboratory for Rabies -**

**Contact Information: d.dumas@animaux-sante.gouv.fr, B.P. 48, 54220 Mulhouse Cedex, France.**

**AFSSA Utilisée pour les émissions de risques et de surveillance de la circulation du parasite.**

**IAIL Utilisée pour les émissions de risques et de surveillance de la circulation du parasite.**

---

**Introduction**

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

---

**Results**

493 dogs were collected across the study area by the participation of 23 veterinarians during March and April 2008.

---

**Parasite prevalence in dogs by age categories**

<table>
<thead>
<tr>
<th>Age category</th>
<th>Infected</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puppies</td>
<td>1</td>
<td>8%</td>
</tr>
<tr>
<td>Young adults</td>
<td>4</td>
<td>4%</td>
</tr>
<tr>
<td>Old adults</td>
<td>0</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Parasite prevalence in dogs for the Meuse department**

<table>
<thead>
<tr>
<th>Parasite prevalence</th>
<th>0%</th>
<th>1%</th>
<th>2%</th>
<th>3%</th>
<th>4%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Molecular analysis**

DNA was extracted from the four positive faeces for larval eggs. In order to identify the species involved, an amplification by PCR with HBB1-32 primers that are specific of the NABH gene was performed. The sequence analysis of the amplification revealed that *E. multilocularis* DNA was amplified and an infection by *E. multilocularis* was confirmed in 2 of the 4 samples.

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**Conclusion**

There is no evidence of the fox tapeworm in this canine population but risks have been identified. According to our results the frequency of domestic dogs warming to prevent echinococcosis contaminations is not well adapted. In endemic regions of France, hunting dogs and pet dogs should be considered potential vectors for transmission of alveolar echinococcosis to humans.
**Range extension and contamination gradients of Echinococcus multilocularis in France.**

F. Boué1, S. Favier1, J.L. Schwenk1, J.M. Boucher1, F. Roux1, P. Giraudoux1, B. Coutard2 and F. Cliquet1

1 AFSSA, Librairie, Wildlife Diseases Unit, BP 6006, F-54700 Molsheim, France.
2 WHO Collaborating Centre for Research and Management in Zoonoses Control - OIE Reference Laboratory for Rabies - Evreux interdisciplinaire de lutte contre la rage et autres zoonoses, 14, 54220 Magney-le-Capucin, France.
3 Department of Environmental Science, University of Florida, Gainesville, FL 32611, USA.

**Introduction**

Among the canine pathogen transmissible to human, the intestinal parasites members of Cestode class are very important. They Cestodes are transmitted to humans by accidental ingestion of infective eggs, distributed with faeces of the definitive host, that are particularly resident in the environment.

**Echinococcus multilocularis**

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 methods**

**Study area and sample collection**

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, the multi-landscape CORINE land cover data, ecological areas with a percentage of grassland over 35% were selected. Inside these selected areas, between 19 and 50 sampling areas of 10 km² were randomly selected in function of grassland coverage. In the study area, a total of 1155 sampling areas have been defined. In collaboration with local authorities, from hunting associations or National Game Office, fox faeces have been collected on each sampling area using an individual kit.

**ELISA coating test**

Specific E. multilocularis cestode antigen detection was performed by three similar ELISA techniques. The first collected samples were analysed by sandwich enzyme-linked immunosorbent assay (sandwich-ELISA) using the first generation of the commercial Chemik & Biokinetix (Di Bommel AG, Liebefeld-tern, Switzerland) and for specificity reasons all the positive samples were validated by the original techniques developed by Depaz et al. (1999).

**Results**

During the period of 2000–2007, a total of 5,554 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 were collected during the two first winters 2000/2001 and 2001/2002, and correspond to 76% of the total number of samples analysed.

**Distribution of the number of faeces collected in the field during the successive winter**

<table>
<thead>
<tr>
<th>Department</th>
<th>Number of Faeces</th>
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<tbody>
<tr>
<td>Ile-de-France</td>
<td>349</td>
</tr>
<tr>
<td>Rhône-Alpes</td>
<td>332</td>
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<tr>
<td>Provence-Alpes-Côte d'Azur</td>
<td>299</td>
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<tr>
<td>Languedoc-Roussillon</td>
<td>286</td>
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<tr>
<td>Occitanie</td>
<td>284</td>
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<tr>
<td>Auvergne-Rhône-Alpes</td>
<td>277</td>
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<tr>
<td>Pays de la Loire</td>
<td>276</td>
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<tr>
<td>Centre-Val de Loire</td>
<td>274</td>
</tr>
<tr>
<td>Normandy</td>
<td>273</td>
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<tr>
<td>Burgundy</td>
<td>270</td>
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<tr>
<td>Nievre</td>
<td>268</td>
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<tr>
<td>Bourgogne-Franche-Comté</td>
<td>267</td>
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<tr>
<td>Grand Est</td>
<td>266</td>
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<tr>
<td>Alsace</td>
<td>265</td>
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<tr>
<td>Bas-Rhin</td>
<td>264</td>
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<tr>
<td>Grand Est</td>
<td>263</td>
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<td>Grand Est</td>
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<td>Grand Est</td>
<td>232</td>
</tr>
<tr>
<td>Grand Est</td>
<td>231</td>
</tr>
</tbody>
</table>

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

2,211 samples were considered as positive and 5,143 as negative. We found positive samples in 36 departments out the 41 departments investigated which cover a large west area of France. For some of these departments it was the first detection. The estimated apparent prevalence vary from 0.7% in Alpes to 14.6% in the Territoire-de-Belfort and we were 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.

**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.

Keywords: Echinococcus multilocularis, France, ELISA
Alveolar Echinococcosis and Echinococcosis in Bulgaria
Kamenna Vutova and Todor Todorov
Medical Faculty, University of Medicine – Sofia,
Department of Infectious Diseases, Parasitology and Tropical Medicine

Cystic Echinococcosis (Hydatid disease)

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%

Human echinococcosis
• 1950 - 1962 ➤ high rates - 5.0 + 6.6 %/1000
• 1948 and 1965 decree against rabies
1970 - 1984 ➤ 1.2 + 2.7 %/1000
1985 - 1995 ➤ increasing trend - 3 + 6.8 %/1000
1996 - 2004 ➤ 6.8 + 8.4 maximum 8.4%/1000 in 1998
Varies between 6.25 and 7 %/1000 during the last 5 years
2004 - 2008 ➤ program against echinococcosis
2007 - 543 operated patients

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 r. - 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 success
➤ treatment failure was between 1.95 %

Alveolar Echinococcosis (AE)

T. P. Genov, D. K. Svilieno, 0. T. Polyakova-Krsteva
OCURRENCE OF ALVEOCOCCUS MUTTILOCULARIS IN THE MICO TUS NIVALIS IN BULGARIA. Comptes rendus de l’Académie
bulgare des Sciences, v. 33, No 7, 1980. After continuous study of wild mammalian helminths in
Bulgaria we found the Alveococcius multilocuarians larva in liver of voles (Microtus rigalis 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.
GENETIC VARIATION IN ECHINOCOCUS MULTILOCULARIS FROM EUROPE ASSESSED BY DNA SEQUENCING

INTRODUCTION

Echinococcus multilocularis, induces one of the most severe zoonotic disease in northern hemisphere debies, alveolar echinococcosis. The disease is of increasing public health concern in Europe, with high local rates and poor prognosis if managed incorrectly. Until the beginning of the 1990's, the geographical range of E. multilocularis was understood to be restricted to central and northeastern Switzerland, and neighboring regions of France and Austria. Over the past 15 years, the known range of the parasite has markedly extended across Europe and it is assumed to be currently distributed over most of territory with the exception of the British Isles. Multi-locular and enchondral regions remain, in addition to this report, E. multilocularis was recently confirmed in Romania and in western Transylvanian counties (Krizan et al., 2003) and in western France (Krizan et al., 2004). To examine genetic variation, we used nucleotide sequences data from Echinococcus multilocularis from Europe. Isolates from a contiguous territory of 10 European countries were subjected to DNA analyses.

METHODS

Nucleotide sequences of 25 E. multilocularis isolates obtained from 8 European countries and 15 capybara sites were compared to this study (Tab. 1). Samples originated from six intermediate host species, and were identified in Europe. The whole mitochondrial genome is known for the four mitochondrial targets (COX I, COX II, P63, ATP 6). The sequencing target (acc) was amplified using PCR amplification, followed by direct sequencing. The resulting nucleotide sequences were analyzed and compared using the CLUSTAL W software. The sequences obtained were deposited in the NCBI database.

RESULTS

A total of 2,015 nucleotide sequences in five gene targets were examined in every isolate. Statistical analysis of the data set revealed significant genetic differentiation between eu-rasian (E. multilocularis) and the American isolates. In agreement with the previous reports, the present study supports the hypothesis that the American isolates form a separate clade within the E. multilocularis species complex.

CONCLUSIONS

In conclusion, the genetic diversity of E. multilocularis was higher in historically documented central European endemic zone (0.022% of nucleotides) than in the north American isolates (0.024% of nucleotides) and this is in agreement with the hypothesis that E. multilocularis is a separate species within the E. multilocularis species complex.
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- Proceedings (as soon as possible)
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