National Veterinary School of Alfort

Year 2012

VULPINE ECHINOCOCCOSIS IN EUROPE SINCE 2000: DETECTION METHODS AND ASSESSMENT OF EXTENSION

Review of Literature and Presentation of Information Exchanged during the European Symposium on December 8th and 9th of 2010

Thesis

For Doctorate of Veterinary Medicine

Publicly Presented and Defended Before the Faculty of Medicine of Créteil

On…………..

By

Anne-Laure, Jeannine, Maryse DESCLOIX

Born on January, 22 1987 in Saulieu (Côte-d'or)

Jury

President: Pr.

Professor at the Faculty of Medicine at Creteil

Members

Director: Jacques GUILLOT

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Table of Contents

Summary of Figures

Summary of Tables

List of Abbreviations and Acronyms Used in the Text

ANSES: National Agency for Sanitary Security of Food, Environment, and Labor

DGAL: General Directorate for Food

DNA: Deoxyribonucleic Acid

DVD-Rom: Digital Versatile Disc - Read Only Memory

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Authority

ELISA: Enzyme-Linked Immunosorbent Assay

ERZ: Entente Rage Zoonoses

ESCCAP: European Scientific Counsel Companion Animal Parasites

IST: Intestinal Scraping Technique

LNR: National Reference Laboratory

OIE: World Organization for Animal Health, formerly International Office of Epizootics

WHO: World Health Organization

PCR: Polymerase Chain Reaction

SCT: Sedimentation and Counting Technique

SSCT: Segmental Sedimentation and Counting Technique

SVT: Shaking in the Vessel Technique

INTRODUCTION

The red fox was longtime feared and hunted for its association with the risk of rabies. Since 2001, France has been free of vulpine rabies due to intense mobilization efforts and *per os* vaccination campaigns conducted by aircraft. The fox population has subsequently increased, and foxes remain carriers of many other agents of zoonoses, including the cestode *Echinococcus multilocularis,* which is the focus of this thesis.

The first human cases of Alveolar echinococcosis (AE) were discovered in Germany and Switzerland in the early 1850s. The disease was then called “colloidal cancer of the liver.” A few years later, Rudolf Virchow stressed the disease’s parasitic nature, believing that it acted as the same parasite responsible for Cystic echinococcosis (CE). Several cestodes or tapeworms were then identified within the *Echinococcus* genus, including *E. multilocularis,* which is responsible for AE. The first case in France, at a farm in Thonon (Haute-Savoie), was described in a doctoral thesis in 1890. A lethal parasitic disease when left untreated, AE is presently among the emerging diseases of Europe. *Echinococcus multilocularis* has as its principal host the red fox, but it has also found hosts in domestic carnivores. The larval form is hosted in the liver of rodents and hosted accidentally by other mammals, including humans.

The geographic distribution of *E. multilocularis* is confined to the northern hemisphere. This seems, however, to be evolving, and it has not been studied uniformly in each country. To chart the situation, a variety of resources and detection techniques have been implemented. On the 8^{th} and 9^{th} of December in 2010, a European symposium, organized by the Entente Rage Zoonoses (ERZ), was held in Nancy in order to discuss the subject.

This thesis offers a synthesis of present information concerning the *E. multilocularis* tapeworm. The methods permitting its detection and the assessment of the parasite’s expanded geographic distribution will in particular be analyzed. Firstly, the 2010 European symposium is presented. The second section provides a review of literature on the subject of *E. multilocularis*, which has been updated by new elements accrued during the conference. The recent data have been transcribed within boxes throughout the text. While certain discussions at the conference remain unresolved, a consensus was reached on other points. These points of consensus have been transcribed in boxes containing two borders.

First Section

PRESENTATION OF THE EUROPEAN SYMPOSIUM

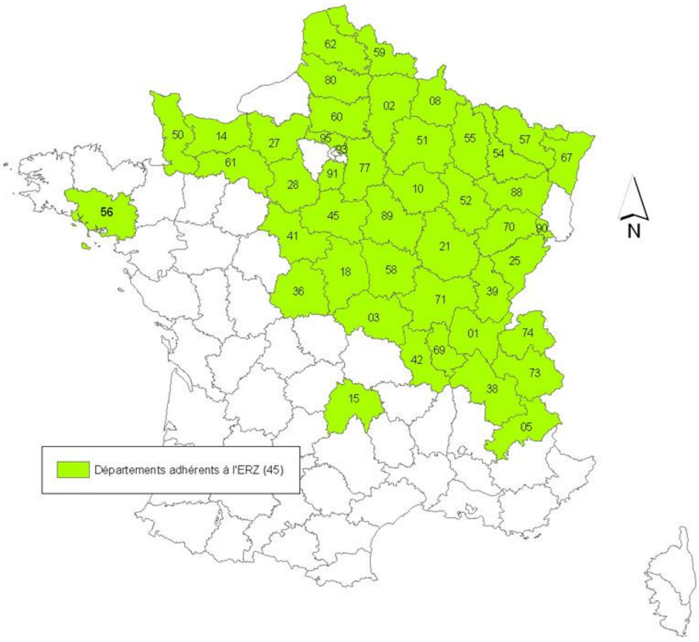
December 8th and 9th of 2010

1. Origin and Organization of Conference

The Interdepartmental Coalition for the Fight against Rabies and Zoonoses (L'Entente Interdépartementale de lutte contre la rage et les zoonoses), also known as "Entente Rage et Zoonoses," planned and oversaw the conference. Created in 1973 at the initiative of several General Councils and the former director of the Laboratory for Studies and Research on Rabies and Wild Animal Pathology (LERRPAS), which is currently the National Agency for Sanitary Security of Food, Environment, and Labor (ANSES), ERZ functions as a public establishment of interdepartmental cooperation. ERZ's work is financed by the General Council, along with the Ministry of Agriculture, Food, Fishing, and Rural Affairs. Its seat is situated in the periphery of Nancy, in the community of Pixérécourt.

The Entente currently has 45 department members, the majority of which are located in eastern France (figure 1).

Figure 1. ERZ Member Departments in France, 2010
(according to ERZ, Synopsis 2010)



When a case of zoonosis becomes a threat to public health, ERZ pursues a course of action with the goal of understanding the disease and reducing its impact on humans. The work of ERZ centers principally on the transmission of zoonosis by wild animals. Its role consists of conducting field experiments and informing scientists, elected officials, and the general population of its results. ERZ works closely with ANSES-NANCY and the University of Franche-Comté.

ERZ has done extensive work on rabies, particularly as it relates to oral fox vaccination campaigns. Since 2001, upon obtaining a vulpine rabies-free status, other forms of zoonosis have fallen within its field of expertise, such as Hemorrhagic Fever with Renal Syndrome (HFRS). ERZ is the head of national field operations in France for AE. The Entente assumes the role of coordinating different studies. It is presently working on a vast national mapping program and countermeasures for echinococcosis, as well as investigating the eventual epidemiological effects related to the reduced fox population (by culling) in the greater metropolitan area of Nancy. Recently, two vulpine deworming experiments were conducted in and around the cities of Pontarlier and Annemasse. In addition, ERZ is presently interested in Leptospirosis and Borreliosis (Lyme disease).

In collaboration with ANSES-NANCY and the University of Franche-Comté, ERZ began an unprecedented national mapping project in 2010 on the presence of *E. Multilocularis* in red foxes within the organization’s member departments. Samples have been obtained with the help of The Hunting Federation, special hunting public officials called “lieutenants de louveterie,” certified trappers, and agents from the National Office of Hunting and Wild Animals (ONCFS). As project manager, ERZ is in charge of field operations. The University of Franche-Comté is the geo-spatial data analyst, and ANSES-NANCY takes care of the diagnostic aspects of the project, along with the improvement of analysis techniques in the laboratory. The initial work of the project centered on testing whether or not there was a relation between the prevalence of the parasite in the fox and the environment of the countryside. The extensive mapping project now includes 43 departments, and 100 foxes are collected uniformly in each department.

ERZ occupies a key role in the prevention of AE due to its widespread diffusion of information within member departments. Communication initiatives regarding this parasite are directed towards private individuals, governments, communities (General Council and local health authorities), and professionals (veterinarians, pharmacists, national parks, and hunting associations).

The results of ERZ’s studies are communicated regularly in executive summaries, which are readily accessible on the organization’s website: http://www.ententeragezoonoses.com. These executive summaries are directed particularly towards Regional Councils, governments, territorial authorities, and departmental hunting federations. ERZ also publishes articles in newspapers and specialized publications.

In 2007, ERZ partnered with the pharmaceutical laboratory Bayer, the General Council of Meurthe and Moselle, the Ministry of Agriculture and Fisheries, the Ministry of Youth Health and Sports (MSJS), and The National Social-Agricultural Mutual Fund (*CNMSA*), to develop an informational DVD on AE, entitled “c’est de l’ECHINO qu’on cause” (We’re talking about ECHINO!). This DVD is directd towards a wide audience, from children to health professionals. It presents the work of ERZ, but it also involves European parasitologists, biologists, doctors, and veterinarians. In total, 14,000 copies were distributed.

To veterinarians, ERZ distributed two posters concerning the prevention of the disease in humans (appendix 1) and the prevention of infection in dogs, in totals of 100,000 and 5000, respectively. Additionally, 350,000 total leaflets (appendix 2) were published.

ERZ presently hopes to enlarge its area of study to include new departments so as to extend its prospective scope towards the west of France. The organization would equally like to develop its technical capacity on a European scale. The results of the studies conducted in Europe during the 2000s showed indications that the geographic range of *E. multilocularis* had extended. Concomitantly, the first results obtained from ERZ's mapping study sparked the idea of comparing the results of investigations conducted in different European countries. At the instigation of its scientific advisers, ERZ thus decided to organize, in partnership with ANSES and the University of Franche-Comté, a European symposium in order to acquire a global perspective on the recent data concerning the *E. multilocularis* tapeworm in Europe. The previous European conference on the parasite had taken place in 2003 in Arc-et-Senans within the framework of the Eurechinoreg research program.

In September of 2009, ERZ announced that it would hold the conference on December 8-9, 2010 in Nancy. The important subjects to be discussed were agreed upon by ANSES and the World Health Organization Collaborating Centre on Prevention and Treatment of Human Echinococcosis in Besançon, France. Afterwards, the first outreach to possible lecturers was conducted.

The financial partners of the conference included the Region of Lorraine, the general administration of Meurthe and Mosellle, as well as the greater metropolitan area of Nancy. The goals of the conference were to update the epidemiological data concerning *E. multilocularis* in Europe, to harmonize the research methods to a standardized European level, and to optimize epidemiological monitoring and countermeasures for zoonosis in order to limit its impact on public health.

Experts hailing from numerous European countries were invited to come share their knowledge on the subject of AE. The experts were comprised of parasitologists, doctors, veterinarians, biologists, laboratory directors, and epidemiologists. A preliminary call for candidates was launched at the end of 2009. By early 2010, twenty countries had responded favorably to our request.

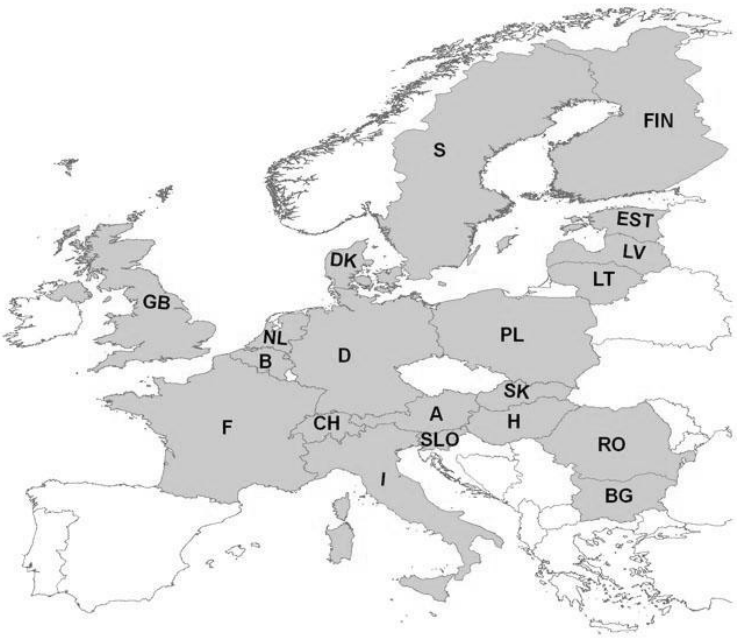
Each lecturer provided a written summary of their presentation in advance so that we could distribute a pamphlet to each of the participants. The website www.echino.edu/ and electronic communication served as tools for disseminating the information on the proceedings of the conference: the date, place, and guidelines for signing up. Finally, all participants could hang up a presentation poster outlining their activities on the subject during the conference.

2. Conference Proceedings

The conference took place on December 8-9, 2010 in Malzéville, a commune (municipality) in the greater metropolitan area of Nancy, in the conference hall of the agricultural secondary school of Pixérécourt.

A total of 75 scientists hailing from 20 European countries attended the conference (figure 2). Thirty-five lectures were given, and 11 posters were exhibited. Each participant received a pack consisting of a DVD entitled “c’est de l’ECHINO qu’on cause!” (We’re talking about ECHINO!), the conference program, two posters, a leaflet on the prevention of AE, as well as a pamphlet including the summaries of lectures and scaled-down versions of the exhibited posters.

Figure 2. Participating European Countries (in gray) at the December 8-9, 2010 Symposium
(according to http://www.echino.eu/francais/)



The two-day program was divided into three themes: animal and human epidemiology on the first day, followed by parasite detection techniques and control mechanisms, both of which occurred on the second day. The lectures, which lasted around 15 minutes apiece, were given with PowerPoint presentations, and a question and answer period, along with discussion, followed directly thereafter (appendix 4). An honorary conference member, a chairman of the session, and two reporters were designated for each of the three themes. The honorary conference member introduced the subject with a thirty-minute presentation.

The chairman of the session oversaw and respected the presentation times and gave the floor to members of the room. I also participated in the conference as the secretary of the session. The conference finished with the exhibition, by the reporters, of the adopted conclusions regarding the three main topics. These conclusions covered the key elements in each respective section.

Additionally, lecturers or other individuals who were unable to partake and exhibit their findings at the conference prepared posters covering the epidemiological situation in different European countries.

Constructive discussions took place following presentations, with each participant bringing valuable information to light concerning his/her work. The presence of speakers from different countries and professional backgrounds allowed us to compare and consolidate our information. Breaks and meals, where experts from different backgrounds could meet, also provided a venue for intense conversation.

A recording system was developed by ERZ in order to archive all of the lectures on audio and video files. These recordings served to help support our thesis work. Collectively, the presentations are available on the website www.echino.eu.

3. Projects Following the Conference

Following the conference, participants agreed upon the necessity of a wide diffusion of information. It was deemed necessary that the exchanged data at the European symposium be recorded and put into writing. To this end, ERZ made the audio recordings and summaries of the lectures available for free on the conference's website.

ERZ hopes that its website acts as a means of communication, one that is regularly updated and easily accessible to all. ERZ envisions the English draft of a standardized technical guide for the detection and control methods of the parasite.

By allowing European experts to convene, the conference paved the way to realize a common work together. Scientists were in agreement on the idea of working together and communicating the results of their studies. A future symposium was envisioned in order to come to an agreement on the detection methods of the parasite.

Through the organization of this conference and the ensuing diffusion of information, ERZ became a key player in the pooling of information on a European scale.

The work of this thesis thus concentrates heavily on the 2010 European Symposium. The transcription of the information exchanged during the conference and the significant conclusions that resulted at the end of these two days of debate and discussion allowed us to update the bibliographic information on *E. Multilocularis,* which is the focus of the second part of this thesis.

SECOND SECTION

THE TAPEWORM *ECHINOCOCCUS MULTILOCULARIS* IN THE RED FOX, *VULPES VULPES*

REVIEW OF LITERATURE UPDATED WITH DATA COMPILED AT THE DECEMBER 2010 EUROPEAN SYMPOSIUM

1. *Echinococcus multilocularis*

1.1. Elements of Classification

The parasite E. multilocularis belongs to the following zoological groups (Thompson and Limbery, 1995):

✓ Phylum - Platyhelminthes Branch
- acoelomate, dorsoventrally flattened

- soft-bodied, triploblastic, with an external cell layer or ectoderm

- protonephridial excretory system

✓ Class - Cestoda
- endoparasite,

- body covered by a living syncytial tegument, an extraepidermal layer bearing microtriches,

- absence of digestive tract,

Cestoda are all parasites exhibiting a **heteroxenous life cycle**, whose hermaphroditic adult lives in the digestive tract of vertebrates.

✓ Sub-class - *Eucestoda*
- flatworm, composed of segments or proglottids,

- organs of attachment on the scolex.

✓ Order - *Cyclophyllidea*

- Four sucker disks encircling the scolex, rostellum armed with hooks,

- strobila with a series of proglottids at different stages of development,

- no uterine pore,

- round, non-operculated eggs containing a hexacanth embryo.

✓ Family - Taeniidae

- non-retractable rostellum bearing four crowns of dagger-shaped hooks,

- ovigerous segments that are more long than wide,

- uterus with a median stem and lateral branches and a regularly alternating genital pore,

- adults in the small intestine of carnivores and humans,

- intermediate hosts are Mammalia,

- larval type: cysticercus, coenurus, hydatid, or strobilocercus.

✓ Genus - *Echinococcus*

- worm size inferior to 5 mm,

- two to five segments,

- larval type: echinococcal.

In 2001, the World Health Organization (WHO) distinguished four species of the *Echinococcus* genus (Eckert *et al*., 2001) (figure 3)*:*

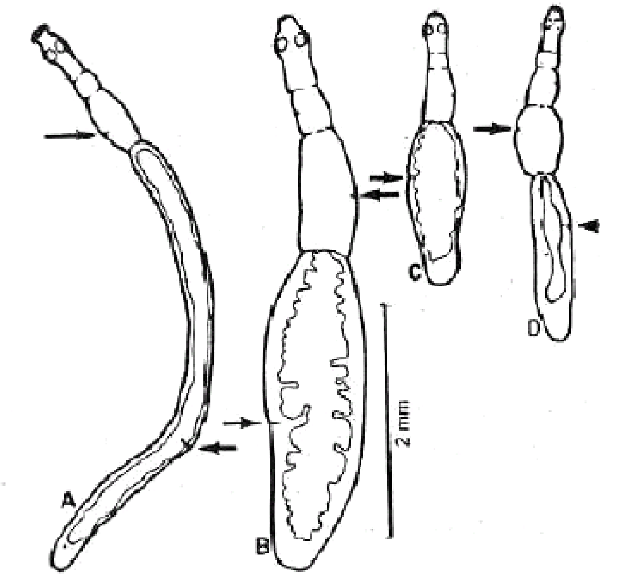
- *Echinococcus granulosus* (Batsch, 1786) is responsible for hydatidosis in numerous mammals (including sheep and sometimes humans) and for teniasis in dogs, its only definitive host. It took nearly 100 years of debate in order to determine whether *E. granulosus* and *E. multilocularis* constituted two separate species. It was only in the middle of the 20th century that the distinction between species was clearly demonstrated (Eckert, 1998).

- *Echinococcus oligarthrus* (Diesing, 1863) infects adult wild felids in America. Its intermediate hosts consist of rodents, such as the agouti (*Dasyprocta* sp.) and the lowland paca (*Cuniculus paca)*.

- *Echinococcus vogeli* (Rausch and Bernstein, 1972) is equally found in America. Its definitive hosts include the domestic dog and the bush dog (*Speothos venaticus*), while its intermediate hosts are the lowland paca *(Cuniculus paca),* the agouti *(Dasyprocta* sp.) and the spiny rat (*Proechimys* sp*.).*

- *Echinococcus multilocularis* (Leuckart, 1863) is responsible for AE. .

<u>Figure 3. Comparative Diagram of Different Species of *Echinoccocus* at the Adult Stage</u>
(according to Thompson and McManus, 2001)



A : *Echinococcus vogeli*

B : *Echinococcus granulosus*

C : *Echinococcus oligarthrus*

D : *Echinococcus multilocularis*

→ Pore génital

A fifth species was recently discovered on the Tibetan plateau:

- *Echinococcus shiquicus* (Xiao *et al*., 2005) has the Tibetan fox (*Vulpes ferrilata*) as its definitive host and the American pika (*Ochotona princeps)* as its intermediate host.

Molecular biology allows us to study the evolution of living organisms. The molecular evolution of *E. multilocularis* can be retraced with the help of genetic markers.

Phylogeny

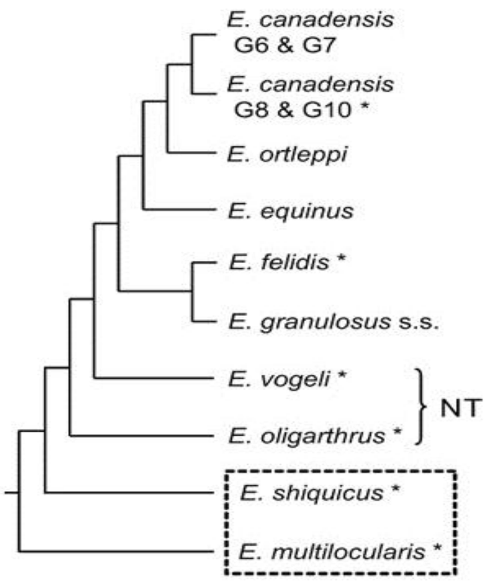
For each species of the genus *Echinoccocus,* it is possible to sequence five genes coding for proteins. The comparison of sequences and their analysis allows for the establishment of phylogenetic trees.

The evolution of *E. Multilocularis* and that of its hosts are directly related, and thus, it is possible to pinpoint the divergence of different species by studying the movements of the first definitive hosts. The divergence of the *Echinococcus* spp. occurred roughly 4.5 million years ago. It is therefore recent on the geological time scale.

Morphological and ecological criteria, as well as molecular markers, enable us to distinguish between species and to retrace their phylogeny (figure 4).

Figure 4. Phylogenetic Tree of Species of the Genus *Echinococcus* Established from Nuclear DNA

(according to Saarma *et al*., 2009)



At present, nine species can be distinguished in the genus *Echinococcus*: *E. multilocularis*, *E. granulosus, E. oligarthrus*, *E. vogeli*, *E. granulosus sensu stricto*, *E. felidis*, *E. equinus*, *E. ortleppi,* and the species of the *E. Canadensis* complex. The taxonomic position of the species of the *canadensis* complex is currently under review (Knapp).

Euroepan Symposium on Alveolar Echinococcosis, 2010

In Tibet, *E. multilocularis* and *E. granulosus* are co-endemic and live sympatrically with the newly described species, *E. shiquicus.* In Turkey and Switzerland, *E. multilocularis* and *E. granulosus* are also co-endemic.

In China, *E. shiquicus* has as its definitive host the Tibetan fox, which is equally a definitive host of *E. multilocularis* in this particular region. The zoonotic potential of this species, its transmission, and its specificity of hosts are presently not well-known. Co-infections with *E. multilocularis* and *E. shiquicus* in the American pika (*Onchotona princeps*) have also been demonstrated.

Since no diagnostic test was available, infection in Tibetan dogs by *E. shiquicus* was unknown until present, whereas these dogs have presented high infection rates of *E. multilocularis* and *E. granulosus* (Boufana).

Two sub-species exist within the species *E. multilocularis* (Thompson & Limbery, 1990):

- ✓ *E. multilocularis* var. *multilocularis* is present in Europe, and its principal definitive host is the red fox, *Vulpes vulpes*;
- ✓ *E. multilocularis* var. *sibiricensis* is present in the arctic regions in the arctic fox, *Vulpes lagopus*.

1.2. Morphology

The biological cycle of *E. multilocularis* includes three stages of development (Euzéby, 1971; Thompson and Eckert, 1983; Pétavy and Deblock, 1980, 1983; Deblock *et al*., 1986 ; WHO, 1981 ; Grisot, 1990): egg, hermaphroditic adult, and larva.

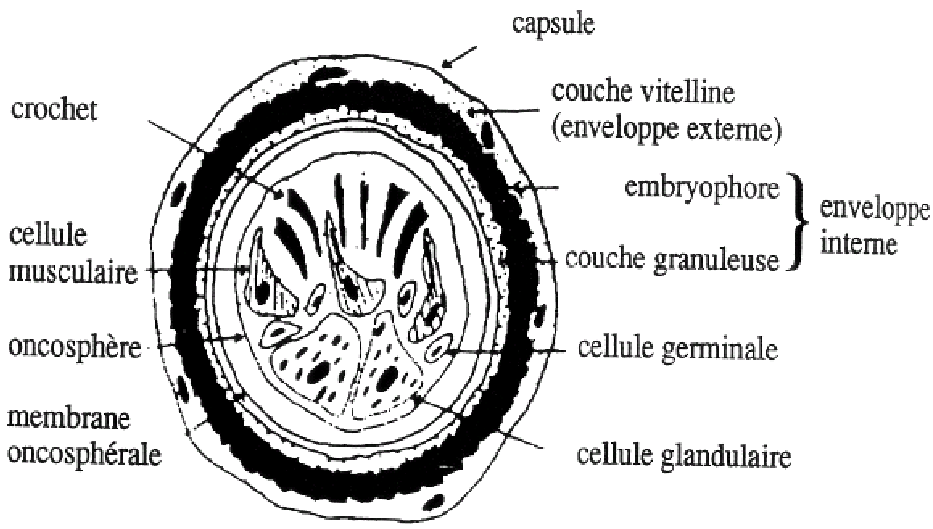
Egg

The *E. multilocularis* egg is spherically shaped, measuring 27-37 µm in diameter, and has a thick brown shell (figure 5). Inside it contains a spherical embryo, armed with six hooks at its anterior end. It is therefore a hexacanth embryo.

The egg of *E. multilocularis* **has no specific characteristic that would allow for it to be distinguished from other taeniid eggs** under an optical microscope. It is therefore impossible to perform a diagnosis of the species by simple observation of the eggs.

Taeniid eggs are **highly resistant** to adverse environmental conditions. They are also directly infective for intermediate hosts.

Figure 5. Diagram of the Taeniid Egg
(according to Romig *et al*., 1999)



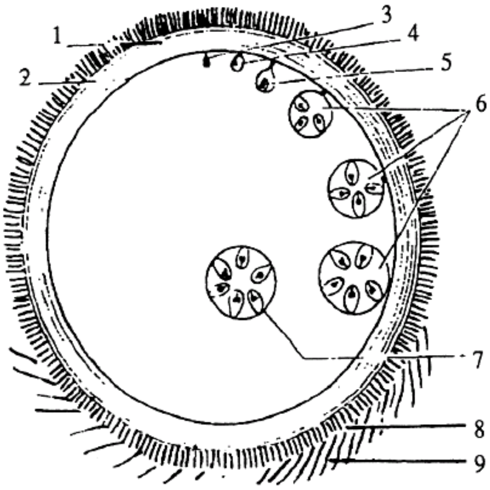
Larva

E. multilocularis has an echinococcal type of larva. It is a large vesicle bounded by a wall containing hydatid fluid within and from where its germinal elements originate (figure 6).

The wall is formed of two membranes. The outermost membrane, called the cuticular membrane, is stratified, acellular, hyaline, and of an irregular thickness. It is attached to the adventitial layer, the area where fibrous-connective activity takes place in host tissue. The inntermost layer, called the germinal membrane, is nucleated and in contact with the hydatid fluid. The germinal membrane proliferates by budding to form smaller proligerous vesicles. It is this assemblage of small vesicles that give the tissue its alveolar structure, whence the name of the disease originated.

Each proligerous vesicle forms 1 to 100 protoscolices, which are invaginated scolices. These protoscolices are what develop into adult cestodes in definitive hosts. This mode of proliferation explains the invasive character of the lesion, as it most often infiltrates the liver of the host by way of stolons. Its label as a “verminous cancer” is also reinforced by the fact that the germinal membrane can fragment and spread elsewhere through the lymphatic system, notably to the brain, lungs, and heart.

Figure 6. Diagram of *Echinococcus multilocularis* Larva in the Hepatic Tissue of an Intermediate Host
(according to Contat, 1984)



1 : Membrane cuticulaire

2 : Membrane germinative

3 à 6 : Vésicule proligère en formation

7: Vésicule proligère détachée

8 : Réaction fibro-conjonctive du tissu hôte (adventice)

9 : Parenchyme hépatique sain

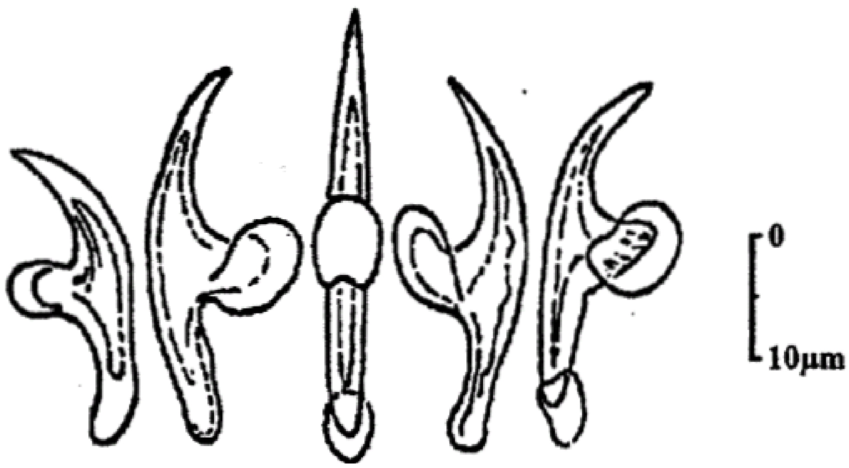
Adult

The adult of *E. multilocularis* is of a small size (0.9-4.5 mm long and 250 µm wide). It has a creamy white color, and its body is segmented (figure 9). The scolex attaches to the intestinal mucosa, and the body moves by way of crawling.

Figure 7. Electron Microscopy View of *E. multilocularis* Scolex
(according to http://parasito.montpellier-wired.com)

The scolex (figure 7) is borne at the anterior end of the first segment and fixes itself to the intestinal mucosa of definitive hosts. It is formed of four suckers and a rostellum consisting of 26-36 hooks spread along two crowns. One of the crowns carries large hooks (25-34 µm), while the other bears smaller hooks (20-31 µm). All the hooks are comprised of an extended prong and shaft, which are separated by an oval-shaped protective sheath (figure 8). At the apical part of the rostellum is situated the rostellar gland, which permits, by adhesive secretions, attachment to the intestinal mucosa of the host. It also inhibits certain digestive enzymes of the host, thus preventing the rejection of the parasite.

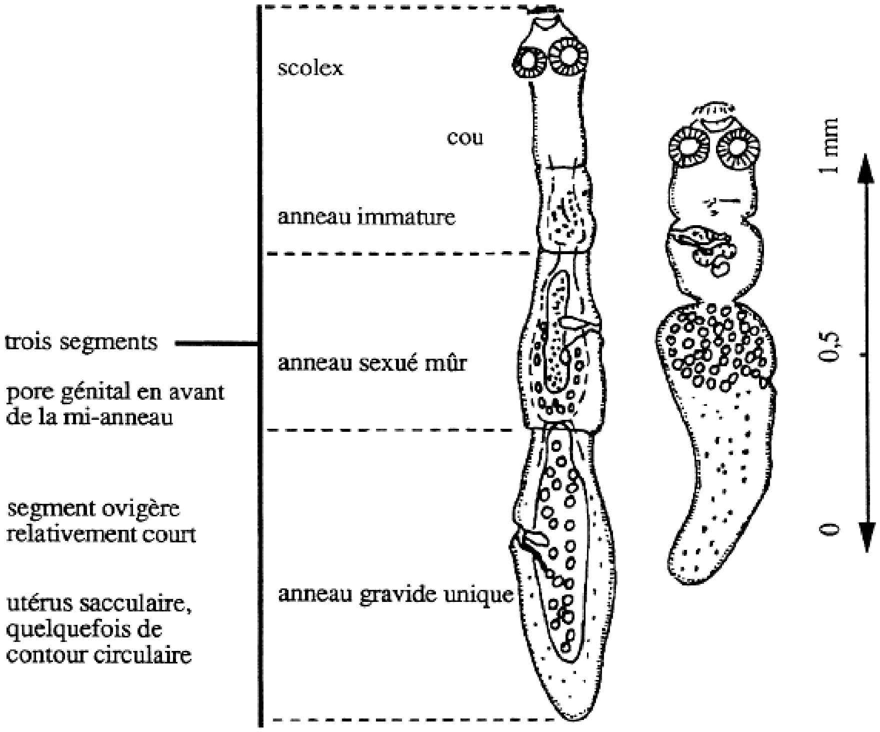
Figure 8. Morphology of Hooks of *Echinococcus multilocularis*
(according to Pétavy et Deblock, 1980)



Behind the scolex is situated the neck – much narrower than the scolex – where active histogenesis occurs; here, segments constituting the body are proliferated. The number of segments (proglottids) varies from three to five. Like all Cestoda, *E. multilocularis* is a hermaphrodite: the penultimate segment contains 16 to 35 testicles and a massive ovary that is markedly bilobed and kidney-shaped. The genital pores are regularly alternating. Male and female genitalia are respectively the cirrus and the vagina. The vagina opens up at the genital pore.

The last segment corresponds to the ovigerous segment. Its length varies from 480-700 µm, but its length is always inferior to half the length of the body. It consists of a vast sacciform uterus containing **50-500 eggs** according to the individuals and host species harboring them. The ovigerous segment is discharged every 14 days, and the preceding segment replaces it, which allows for the tapeworm to continually grow without increasing in size.

Figure 9. Morphology of *Echinococcus multilocularis* Adult
(according to Contat, 1984)



1.3. Life Cycle

Echinococcus multilocularis has a strictly heteroxenous life cycle. It thus requires an intermediate host and a definitive host and cycles between the two in the outside environment. As a result, the parasite has three phases:

- An adult stage in the small intestine of definitive hosts (domestic and wild carnivores);
- A free-living stage in the form of eggs in the outside environment;
- A larval stage in the hepatic parenchyma of intermediate hosts (micromammals).

Parasitic Phase

The intermediate host becomes contaminated by ingesting eggs. Under the effects of digestive juices of the host, the oncosphere gets rid of its cuticle and begins the process of eclosion. Only mature eggs can undergo eclosion. Due its hooks and secretion from penetration glands, the embryo subsequently perforates the wall of the small intestine then migrates through the vascular network up to the **hepatic parenchyma** to which it attaches itself. There, it transforms into larvae called metacestodes (Eckert *et al.*, 2001).

Figure 10. Liver of *Microtus arvalis* Infected by *E. multilocularis*

(according to http://antropozoonosi.it)

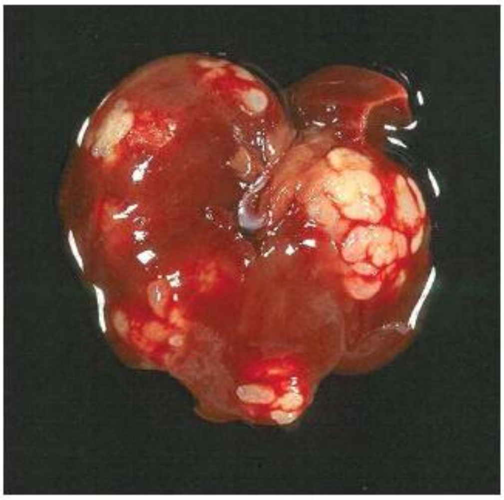


Figure 11: Macroscopic Section of Human Liver Infected by *E.* multilocularis
(according to ANOFEL, the French Association of Medical Parasitology Professors)

Larvae are observable in histological sections after 24 hours, and they can be seen from the naked eye after two to five days. The time period necessary for the development of numerous and voluminous vesicles depends upon the receptivity of the host. If the host is favorable (in the case of wild microtine rodents), vesicles are formed in two to three months; conversely, if the host is not very receptive to the larva (human cases), it may take a full year, at which point the larva will most likely be sterile. The duration of life for larvae depends upon the lifespan of the intermediate host, which generally does not exceed one year for micromammals. This asexual multiplication enables a significant proliferation of the parasite.

The lifespan of infective protoscolices contained in carcasses of intermediate hosts can last two months at 5°C and a week at 18°C (Deblock *et al.*, 1986). A definitive host can thus become contaminated by ingesting a carcass from several days to several weeks old.

The definitive host becomes contaminated by ingesting an intermediate host harboring infective larvae. Under the effects of gastric and intestinal secretions, along with bile salts, the larva becomes encysted and evaginates its protoscolices into the upper section of the duodenum. Due to the suckers and hooks of the rostellum, attachment to intestinal mucosa in the first sections of the digestive tube occurs rapidly. The rostellar gland prefers this deep attachment by its secretions (Thompson and Eckert, 1983). **The ingestion of a single intermediate host harboring fertile larvae is sufficient for the development of adult tapeworms in definitive hosts**.

Then, strobilation begins, as well as the production of immature, mature, and finally gravid segments. The first ovigerous segment is liberated between the 30^{th} and 35^{th} day. This results in a very short **prepatent period**, estimated at **28 days in dogs** (Thompson and Eckert, 1983). In foxes, this prepatent period is 26-29 days (Nonaka *et al*., 1996). The growth of *E. multilocularis* is thus much more rapid than that of *E. granulosus,* for which the prepatent period is estimated between 48-61 days.

The lifespan of the adult tapeworm is short (between 3-6 months) (Contat, 1984). However, successive infections lead to an increase in the infection duration of hosts (Euzéby, 1996).

The *E. multilocularis* tapeworm can coexist in the intestine with numerous other nematodes or cestodes (notably *E. granulosus*).

The ovigerous segment, containing several hundreds of eggs, detaches itself in the intestinal lumen and is discharged into the outside environment in the feces of the definitive host. It is estimated that an adult tapeworm produces an ovigerous segment around every 15 days (Thompson and Eckert, 1983).

Free-Living Stage

One to two days after its liberation into the outside environment, the ovigerous segment breaks up and liberates its eggs. Once **emitted into the outside environment, the eggs are embryonated (hexacanth embryo or oncosphere) and are thus directly infective for intermediate hosts.**

The resistance of E. multilocularis eggs is variable though very high in natural conditions: one to two years for temperatures between -5 and +25 °C (Schaeffer, 1986). Freezing eggs is inefficient since the eggs remain infective for several weeks at -51°C. **The inactivation of the oosphere is only obtained at temperatures lower than -70°C.** This explains why *E. multilocularis* is primarily found either in the northern hemisphere and mountainous regions of inferior latitudes or where hibernal conditions are severe.

Eggs are principally sensitive to desiccation (Euzéby, 1971). In fact, in a dehydrated environment, the infective power will only last an hour (Delattre *et al*., 1991). Furthermore, precipitation, such as snow (as it maintains a certain degree of humidity), provides excellent conditions for conservation. Buried eggs are also protected from dehydration. This relates to a higher prevalence of infection in rodents populating the edges of ploughed fields and grassy stretches traversed by bovines (Delattre *et al*., 1988).

Formalin does not permit the inactivation of eggs. The product Cresyl (at 20%) destroys embryophores after thirty minutes of contact (Chermette, 1983). Oxygenated water and a supersaturated solution in NaCl allows for inactivation (Contat, 1984).

Generally speaking, eggs are resistant in the outside environment from several months to two years. **Eggs therefore represent the parasite's form of resistance** and are only minimally dispersed due to rain, streaming, and trampling - by certain animals and by necrophage insects and coprophages, both of which constitute paratenic hosts.

By focusing on the cycle, we can deduce that:

- Definitive hosts are blood carriers since the presence of the parasite does not have an impact on their health.

- The presence of the larva in the liver of intermediate hosts and its infiltration into the adjacent organs is responsible for clinical signs. Humans can act as an accidental intermediate host. They are parasitic dead-ends because unlike micromammals, they are situated at the top of the food chain.

- *E. multilocularis* is the *Echinococcus* sp. that produces the least amount of eggs (200 eggs per proglottid compared to 1500 eggs per proglottid for *E. granulosus*). However, its short prepatent period and its multiplication at the larval stage explain its strong prolificacy.

- The resistance of eggs in the outside environment and the directly infective power of embryophores explain both the geographic distribution of the parasite and its danger for humans, who can become infected in many different ways.

- There exist different diagnostic methods: eggs of echinococcal tapeworms are sought in the feces of carnivores and adult tapeworms in the small intestine of carnivores. Regarding larvae, they are found in the hepatic parenchyma of rodents or accidentally in other mammals, such as humans.

Receptive Hosts

Definitive Hosts

Natural definitive hosts of *E. multilocularis* (table 1) belong to the families of Canidae and Felidae (Grisot, 1990; Rausch, 1995).

Among the Candidae, definitive hosts include **the red fox** (*Vulpes vulpes*), **the coyote** (*Canis latrans*), **the wolf** (*Canis lupus*), **the arctic fox** (*Alopex lagopus*), the corsac fox (*Vulpes Corsac*), the gray fox (*Urocyon cinereorargentatus*), the **domestic dog** (*Canis familiaris*), and the raccoon dog (*Nyctereutes procyonides*).

In the Felidae family, the **domestic cat** (*Felis catus*) and the wild cat (*Felis silvestris*) constitute definitive hosts of the parasite.

✓ Dogs

As with the red fox, the domestic dog is a definitive host of *E. multilocularis* and becomes infected through rodents.

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Infected dogs were examined in France (Comte), Switzerland, Germany (Hegglin), and China (Boufana).

The number of contaminated dogs in Europe, however, is not well-known. The proximity of contact that dogs maintain with humans is in fact an important element of the life cycle.

✓ Cats

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It has been established that cats do not constitute a receptive host and that the parasite is poorly suited for cats. In Germany and Switzerland, cats, however, were found with high parasite loads, though it was not known whether infected eggs had been excreted (Romig). The role of the cat is thus not totally clear, and their presence and proximity close to humans warrant further investigation in endemic zones (Giraudoux).

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Like the cat, the lynx does not seem to be a good definitive host. Amongst 146 animals analyzed in Latvia, none have been found to be infected (Bagrade).

We now describe **the red fox, which is the principal definitive host in Central and Western Europe** (Eckert *et al.*, 2001) (figure 12).

Figure 12. The Red Fox, *Vulpes vulpes*

(according to http://www.rnw.nl)

The red fox, *Vulpes vulpes*, measures between 90-112 cm in length and 38-41 cm in height. Its adult weight ranges from 3.6-6.8 kg (Blackourn, 1999). Its head is characterized by a pointed snout and triangular ears, and its tail is long and bushy. It has thick, predominantly red fur. More often than not, the fox's throat, abdomen, and the tip of its tail are all white, while the back of its ears, the tip of its snout, and the base of its paws are black.

The fox is considered to be an animal of the forest where it digs its burrow. It prefers, however, habitats created and frequented by humans, such as agricultural land and urban zones, where it may cohabit with humans without being recognized. The size of its home range is around 2 km^2 in a rural zone and 0.2-0.5 km^2 in the periphery of cities. The fox is able to travel 5-15 km in a given night to search for food or to mark its territory by way of urine, feces, and glandular secretions.

Foxes hunt from 6 P.M. to 6 A.M. in zones containing a strong density of rodents, notably on the edges of ploughed or trampled land. Their diet includes mice and voles (Muroidea), rabbits (Leporidae), eggs, birds, fruits, insects, and carrion. The fox requires 300-600 g of food per day, which represents around 20 voles (Artois *et al.*, 1986). Micromammals constitute 23% of its diet (Saint-Giron, 1973). The fox tends to prefer rodents inhabiting open areas, such as *Microtus arvalis* and *Arvicola terrestris* (Artois *et al*., 1991), as they are more easily accessible.

Foxes' hunting area corresponds to their feeding strategy, which favors areas with abundant prey; the fox is an opportunistic feeder. The fox holds a strong dietary preference for *M. arvalis* compared to *A. terrestris* (Delattre *et al*., 1985). This preference corresponds to the higher level of activity of *M. arvalis* on the ground surface, which consequently makes them easier to capture. In cities, the fox consumes hunted rodents, which constitute roughly 60% of its diet, both in green spaces and above all in human garbage (Meia, 2003).

The fox's reproduction period extends from mid-December to mid-March according to the climate. The gestation period lasts 53 days between March and May, at which point the female fox gives birth to five pups on average. Pup mortality is high. At the age of three months, the young are able to feed themselves and leave the burrow.

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Recently, this particular species underwent **population-density modifications in addition to a behavioral evolution.**

The fox population has increased in numerous European countries. Though rabies is still present in wild animals in certain European regions, its disappearance, due to oral anti-rabies vaccination campaigns, is the chief cause for the growth in vulpine population (Romig). Observations were made in Germany (Romig), France (Comte), the Netherlands (Takumi), Poland (Gawor), Lithuania (Sarkunas) and Switzerland (Hegglin). In Switzerland, the number of foxes found dead following accidents on public roads increased sevenfold by 1968 (Hegglin). In Bavaria, the density of the vulpine population increased by a factor of five during the 1970s, and it has tripled in Poland since 1995 (Gawor).

We are currently living in a world that is increasingly urban, and wild animals must adapt to this changing dynamic. Consequently, wild animals are closer in proximity to human habitations, and their contact with humans has become widespread. The same applies to **foxes, which are colonizing cities** (Hegglin). Their prevalence was also noted in Zurich (Hegglin); Nancy, Annemasse, and Pontarlier (Comte and Raton); and Berlin and Brussels (Vervaeke). In Zurich, Switzerland, the number of foxes that were found dead tripled from 1998 to 2000. This continued increase in population is foreseeable for years to come in numerous countries all across Europe (Hegglin).

Intermediate Hosts

Unlike the adult parasite, the larval form can be accommodated by a variety of species belonging to several different families (Grisot, 1991; Rausch, 1995). **The larval form is thus not very specific**. We cite here only the families or species present in Europe:

Insectivores: Soricidae, Talpidae;

Rodents: Sciuridae (the marmot *Marmota marmota*), Cricetidae, Arvicolidae (the common vole ***Microtus arvalis***, *Pitimys subterraneu*s, the European water vole ***Arvicola terrestris***, the muskrat ***Ondatra zibethicus***, the bank vole *Clethrionomys glareolus*), Muridae (the domestic mouse *Mus musculus*), Dipodidae, Ochotonidae;

Carnivores: Canidae (the domestic dog ***Canis familiaris***)

The intermediate hosts are for the most part micromammals. Accidental infections were observed in other species, notably in dogs, which carry the larval form, but not the adult form (Losson and Coignoul, 1997); primates (Brack *et al*. 1997); domesticated pigs (*Sus scrofa domesticus*); boars (*Sus scrofa*); coypus (*Myocastor copyus*); and, of course, humans. Humans represent an epidemiological dead-end.

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Below, we will describe the two principal intermediate hosts present in France, which are *Microtus arvalis* and *Arvicola terrestris*. These are two rodents of the Arvicolidae family.

✓ The common vole (*Microtus arvalis*)

Figure 13. The Common Vole, *Microtus arvalis*
(according to http://www.thomasgoelzer.de/glemstal/wanderungen/2005-09/microtus_arvalis_4779a.jpg)

The common vole is a rodent of a small size (83-178 mm in length) that weighs from 20-30 g as an adult. It is characterized by beige fur on its back and lighter fur on its abdomen. Its ears are hairless (figure 13). This microtine rodent inhabits **open areas**: mowed prairies, pastures, gardens, cultivated fields, ploughed land, embankments, and grassy border areas. It is an omnivore and nourishes itself on grains and young shoots. It digs burrows that are not very deep, and its tunnels lack earth mounds.

Its area of displacement does not extend beyond a radius of 10 m around its burrow. It is essentially a nocturnal animal that can, however, be observed during the day in periods of outbreak. In such a scenario, its displacements can reach up to 100 meters. Its reproduction rate is very high: the female reaches sexual maturity at 25 days old, and its gestation period is 19-25 days. The female can have on average 3-6 litters a year of eight baby voles (Nowak, 1991).

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The distribution range of *Microtus arvalis* in Europe overlaps that of *E. multilocularis*. It can be assumed that if *E. multilocularis* is well-adapted to a rodent species, the absence or presence of this intermediate host is determinant to the establishment of the parasite's life cycle (Romig).

- ✓ European Water Vole (*Arvicola terrestris*)

Figure 14. European Water Vole *Arvicola terrestris*
(according to http://www.raoulkonanz.com/Portfolio/CampagnolTerrestre.jpg)

In French, this microtine rodent goes by "grand campagnol" or large vole, though it is more commonly and erroneously referred to as "mulot." It is a rodent whose massive body weighs between 70-250 g. Its head is round while its tail is short. The length of its head added to its body measures between 120-220 mm. The rodent's fur is grayish-brown on its back and fairly beige on its abdomen (figure 14).

It lives principally in prairies, gardens, and orchards. As with *M. arvalis*, it is a **species that inhabits open areas**. It is vegetarian, nourishing itself on roots and the green part of plants. This rodent displaces itself on the surface and digs tunnels whose accumulated soil forms molehills. Most of its activity occurs at nighttime.

Its rate of reproduction is also rather considerable: sexual maturity sets in at six months, gestation lasts 21 days on average, and the female can have on average 4-6 litters per annum of six baby voles. The reproduction period generally occurs from March to October, but in certain years, it can start up in December, which triggers an outbreak (Nowak, 1991).

Table 1. Principal Intermediate Hosts and Definitive Hosts of *E. multilocularis* Present in Different Regions of the World

(according to Eckert *et al*. 2001)

Regions or Countries	Definitive Hosts	Intermediate Hosts
Central and Western Europe	Red Fox (*Vulpes vulpes*) Domestic Dog (*Canis familiaris*) Domestic Cat (*Felis catus*)	Common Vole (*Microtus arvalis*) European Water Vole (*Arvicola terrestris*) Muskrat (*Ondatra zibethicus*) Bank Vole (*Clethrionomys glareolus)* Domestic Mouse (*Mus musculus)*
Russian Federation	Arctic Fox (*Alopex lagopus)* Red Fox (*Vulpes vulpes*) Corsac Vox (*Vulpes corsac*) Wolf (*Canis lupus*) Wild Cat (*Felis silvestris*) Domestic Dog (*Canis familiaris*)	Common Vole (*Microtus arvalis*) Bank Vole (*Clethrionomys glareolus)* Musk Rat (*Ondatra zibethicus*)
China	Red Fox (*Vulpes vulpes*) Wolf (*Canis lupus*) Domestic Dog (*Canis familiaris*)	American Pika (*Ochotona princeps)* Mongolian Gerbil (*Meriones unguiculatus*)
Japan	Red Fox (*Vulpes vulpes*) Domestic Dog (*Canis familiaris*) Domestic Cat (*Felis catus*) Raccoon Dog (*Nyctereutes procyonoides*)	Brown Rat (*Rattus norvegicus*)
North America	Arctic Fox (*Alopex lagopus)* Domestic Dog (*Canis familiaris*) Red Fox (*Vulpes vulpes*) Coyote (*Canis latrans*)	Muskrat (*Ondatra zibethicus*) Domestic Mouse (*Mus musculus)*

Euroepan Symposium on Alveolar Echinococcosis, 2010

The ecology of intermediate hosts is not well-known. It represents, however, a determinant factor concerning the risk of *E. multilocularis* presence (Romig).

Euroepan Symposium on Alveolar Echinococcosis, 2010

In Nancy, ANSES conducted a study **in order to determine the impact of habitat type and the degree of urbanization on the density of intermediate hosts**, the density of fox feces, and the quantity of parasitic antigens in the feces of foxes (Robardet). The city of Nancy comprises three levels of urbanization, represented by the urban zone, the peri-urban zone, and the rural zone. In these three zones, there exist different habitats, including derelict land, parks, fields, pastures, and pathways (Table 2).

Table 2. Distribution of Different Habitats within Different Levels of Urbanization
(according to Robardet, European Symposium on AE, 2010)

	Derelict Land	Parks	Pathways	Pastures	Fields
Urban Zone	x	x			
Peri-Urban Zone			x	x	x
Rural Zone				x	x

At the local level, it appeared that *Microtus* spp. were more abundant than *A. terrestris,* and *Microtus* spp. were themselves more abundant in urban zones than rural ones, notably in derelict land. ***Microtus* spp. are therefore prey available to foxes in cities and could create the conditions for the local infiltration of *E. multilocularis*** since two categories necessary for the establishment of a life cycle are present. This is corroborated by the positive correlation observed between the density of rodents and the abundance of fox feces. By contrast, the quantity of antigens in the feces of foxes is independent of the level of urbanization and countryside.

The collection of data obtained in Nancy does not correspond to the data collected in the Swiss cities of Geneva and Zurich. There, *A. terrestris* is more abundant than *M. arvalis* and constitutes the principal prey of foxes. In this case, it is *A. terrestris* that could play a major role in the establishment of an urban cycle. Therefore, these are two different models of transmission providing favorable grounds for two distinct intermediate hosts. However, it is not entirely clear why *M. arvalis* is well-adapted to the urban zone of Nancy.

New host species have been recently discovered or introduced. This is notably the case for the **raccoon dog (*Nyctereutes procyonoides*)**, which was introduced into Western Europe from Asia. It acts as a potential definitive host in Lithuania, Latvia, Poland, Slovakia, and Germany. Its density is not well-known, but it is reportedly high in Central and Eastern Europe. In Poland, the raccoon-dog population has multiplied sixfold since 1995. In Germany, a recent study reports an infection prevalence of this canid species by the tapeworm between 6%-12%, but the actual prevalence is estimated between 20%-25%. It is 13% in the south of Lithuania. Certain observations indicate strong parasite loads in raccoon dogs in Germany, even greater than those observed in foxes. However, experimental infections indicate that the fox is the substantially more receptive of the two species.

In Slovakia, **the European polecat (*Mustela putorius*) and the European badger (*Meles meles*)** represent potential intermediate hosts. Mature protoscolices were found in these species, but their epidemiological importance has not been clarified (Miterpakova).

The **coypu *(Myocastor coypus*)**, originating from South America, constitutes an available intermediate host in Central and Southern Europe. Studies in Germany have provided conflicting results regarding the evolution of coypu populations and their capacity to harbor the parasite. The infection rate is approximately 10%, which means it could play a key role in the parasitic life cycle. The relative importance of the coypu with regard to the muskrat is also presently being discussed. Nevertheless, we have no scientific proof at our disposal concerning their predation by foxes – only observations reported by hunters. The absence of predation would make them sentinels of the increase in *E. multilocularis* prevalence. These species would have an essentially local role because they are a species that is ecologically restricted to a reduced territory. The fertility of *E. multilocularis* in the coypu has also not been determined. Several stages in this host would be necessary to produce infective larvae, and for the time being, transmission has only been proven in the fox and the raccoon dog (Romig).

Euroepan Symposium on Alveolar Echinococcosis, 2010

In Belgium, the level of *M. arvalis* infection is low, and the muskrat seems to play a preponderant role in the establishment of the life cycle. Ecological conditions in Belgium are more propitious to the muskrat (Vervaeke). In France, there is neither the raccoon dog nor the raccoon, both of which are definitive hosts of *E. multilocularis* (Giraudoux).

The role of semi-aquatic rodents in the establishment and perpetuation of the life cycle, as well as the prevalence of infection, is still poorly characterized and should be the focus of targeted studies. The ecology of these new hosts is in any case essential to understand (Giraudoux).

Despite the discovery of potential or verified new hosts, the fox remains the principal definitive host of *E. multilocularis* in Europe as it has the highest prevalence rates. Not all hosts carry the same level of importance in the establishment of the life cycle. In certain regions of Lithuania, for example, prevalence reaches 60% in foxes as opposed to 13% in raccoon dogs. Certain species can, however, serve as sentinels of the presence of *E. multilocularis*. This is the case for boars or pigs (Sarkunas).

Different Epidemiological Cycles

The study of different host species has allowed for four life cycles to be distinguished.

✓ Sylvatic Cycle

The sylvatic cycle, sometimes known as the "jungle" or "forest" cycle, involves exclusively wild species. Oncospheres are excreted into the environment from the feces of a wild carnivore, at which point they are ingested by a micromammal. The latter harbors the larvae in its liver. The ingestion of the larvae is the origin of a carnivore's contamination.

In Europe, the definitive host that is principally implicated in the sylvatic cycle is the red fox (*Vulpes vulpes*). Intermediate hosts can include the common vole (*Microtus arvalis*), the European water vole (*Arvicola terrestris*), the bank vole (*Clethrionomys glareolus*), the muskrat (*Ondatra zlbethicus*), the marmot (*Marmota marmota*), and the coypu (*Myocastor coypus*) (Grivet, 1999).

Humans can also act as an intermediate host in the sylvatic cycle when they become infected by ingesting eggs that are present on vegetables, in the ground, or on the fur of foxes.

✓ Rural Cycle

Wild voles are ingested by domestic dogs or cats – though not by foxes. The former two animals subsequently develop adult parasites in their intestine and excrete feces containing oncospheres that can cause direct infection. **Domestic carnivores**, when they freely roam in nature, **play the intermediary role between the sylvatic cycle and the rural cycle**. Given the proximity of domestic carnivores to humans, the risk of contact between humans and the parasite is much higher in the rural cycle than it is in the sylvatic cycle. It must also be noted that infected carnivores do not generally present clinical signs.

The prevalence of the parasite in domestic dogs is generally low and inferior to the prevalence found in foxes living in enzootic zones. However, in certain regions, such as China and the U.S. state of Alaska, the prevalence in dogs can exceed 10%. As a consequence, the rural cycle is especially important as it relates to human contamination (Deplazes *et al*., 1999). In enzootic zones, the rural cycle operates autonomously (Rausch *et al*., 1990).

✓ Urban Cycle

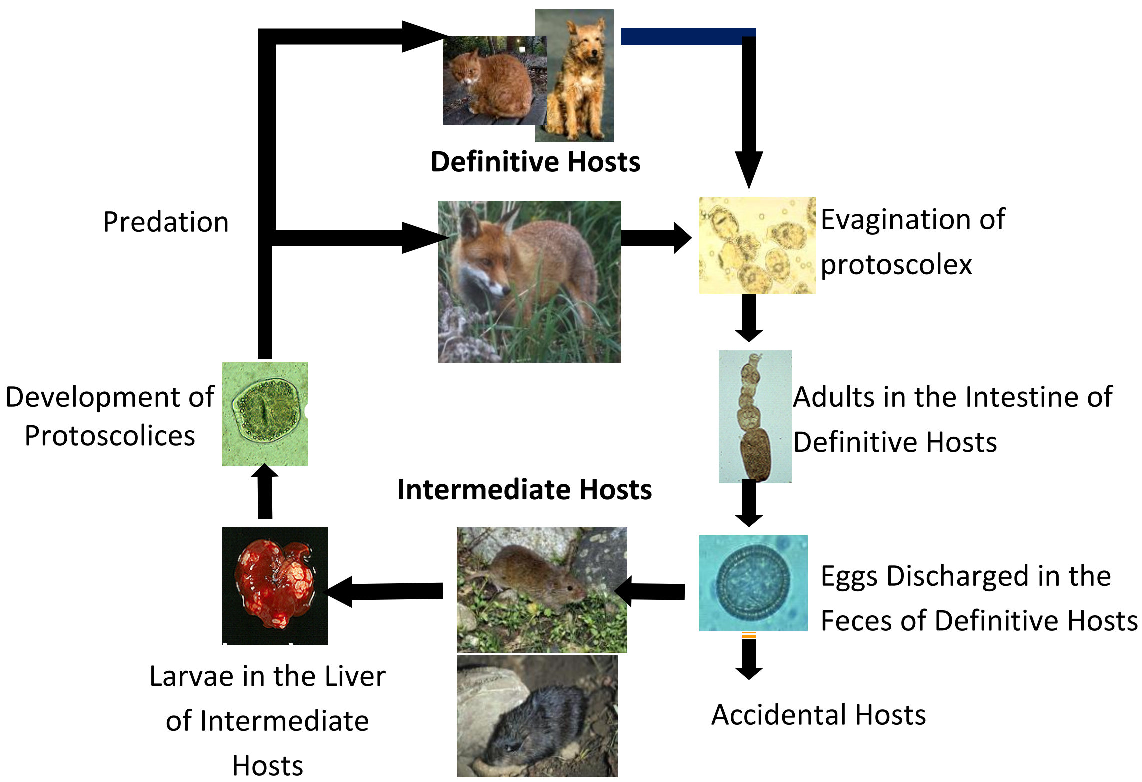
The urban cycle is actually the establishment of a sylvatic cycle - between micromammals and foxes - in urban areas. This recent phenomenon has coincided with the infiltration of foxes within cities. Foxes' presence in urban areas can be explained by their attraction to the alimentary resources or the relative calm of these locations; or they could have been imported into parks, as was the case in the United States (Houin and Liance, 2000). In the years to come, the presence of high densities of hosts that are well-suited for the parasite in urban areas, along with the possibility of close contact with humans, raises fear over an increase in human cases linked with this cycle. The risk of human contamination is heightened either by direct contamination or by the intermediary of domestic carnivores.

✓ Domestic Cycle

The domestic cycle involves the domestic mouse (*Mus musculus*) as the intermediate host and the domestic cat (*Felis catus*) as the definitive host (Eckert *et al*., 2001). Though the parasite has already been found in the cat and mouse (Pétavy *et al*., 1990; Prost, 1988), these animals do not seem to represent excellent hosts for *E. multilocularis*. The epidemiological importance of this cycle remains to be determined.

The different cycles of *E. multilocularis* are not independent, but instead, closely related by their common agents. Indeed, voles, domestic carnivores, and foxes constitute intermediaries in all four cycles.

Figure 15. Circulation of *Echinococcus multilocularis*
(according to Vervaeke, European Symposium on AE, 2010)



Euroepan Symposium on Alveolar Echinococcosis, 2010

➢ **The Interactions between Intermediate and Definitive Hosts**

The establishment of the *E. multilocularis* life cycle and the perpetuation of the parasite require both the predation by foxes of a sufficient amount of infected rodents and the proximity between contaminated feces and rodents. The latter depends upon the extent of foxes' geographic range and the spatial distribution of their defecations. Computer modeling of data indicates that foxes do not exhibit a tendency of defecating near rodents' burrows. Therefore, the proximity between the two types of hosts depends upon the extent of the inhabited area of foxes (Quintaine).

Though the *E. multilocularis* life cycle has been known for a long time, the relative importance of different putative hosts in the establishment of a life cycle and the circumstances of its establishment and perpetuation have not been elucidated. Multiple factors are implicated in the establishment of an *E. multilocularis* life cycle, notably the environment, the presence and concentration of intermediate hosts, and the hosts' ecology. Many questions still remain, such as the contaminant dose and factors explaining the resistance of certain hosts (Romig).

1.4. Immune Response to the Parasite

Immunity to the Adult Stage of *E. multilocularis* in Definitive Hosts

Studies were first carried out for *E. granulosus*. In 1995, Gemmell *et al*. infected dogs eight or nine times with *E. granulosus* and treated them with arecoline between infections. Half of the dogs showed a certain degree of resistance to the sixth infection. However, due to the small amount of dogs in the study, no conclusions could be drawn or validated. This study is corroborated by that of Deplazes *et al*. (1992). In their study, dogs were likewise repeatedly infected and treated with praziquantel between each infection. It appeared clear that the parasite load was diminishing and that the pre-patent period was progressively lengthening over the course of successive infections.

Similarly, *E. multilocularis* induces a certain immune response, demonstrated by **the production of circulating antibodies**. Among four infected foxes in an experiment, a decrease of coproantigen excretion was observed after three to four weeks, which indicates that a significant number of parasites had been expelled (Nonaka *et al*., 1996). Several studies showed the existence of higher prevalence rates and parasitic loads in young foxes in comparison to adults (Ewald, 1993; Wessbecher *et al*., 1994). These results, however, are controversial (Tackmann *et al*., 1998), and many points remain unclear.

There is thus a partial immunity with respect to adults of the *E. multilocularis* sp. The presence of an immune response consequently allows for a serological diagnosis through the identification of antibodies in the definitive host.

Additionally, as adult tapeworms of *E. multilocularis* are present in the digestive tract, definitive hosts excrete antigens into the intestinal lumen. This allows for a diagnosis by identifying *E. multilocularis* antigens in fecal matter (coproantigens).

Immunity to the Larval Stage of *E. multilocularis* in Intermediate Hosts

European hamsters (*Cricetus cricetus*) infected by *E. multilocularis* and subsequently treated with praziquantel 23-25 days after infection revealed a diminished parasite load (by 95%) during a second infection when compared to the control group. This demonstrates the acquisition of a certain degree of resistance with to respect to reinfections (Inohara *et al*., 1996).

Gemmell *et al.* (1995) also showed that intermediate hosts developed immunity to *E. multilocularis*, involving both humoral and cell-mediated immune responses. Though humans secrete the immunoglobulins IgG, IgA, and IgM abundantly, their protective effect could not be demonstrated (Vuitton *et al*., 1984). Regarding IgE, they are only present in half of sick patients and are without an established protective role. It is thus a cell-mediated immune response that enabled the resistance to the development of *E. multilocularis* infection.

Parasitic antigens are glycolipids of a proteinaceous nature and are of relevance when it comes to diagnosis (Persat *et al*., 1991).

Vaccination Trials

- ✓ In Definitive Hosts

Despite initial disappointments, studies on vaccinations to *Echinococcus* tapeworms have slowly progressed.

A short immune response is obtained experimentally in dogs after a parenteral injection of activated oncospheres of *E. granulosus* or other *Taeniid spp*. The number of adult tapeworms, their growth, the production of eggs, or all three of said parameters are subsequently affected. However, the antigen responsible for this form of resistance has not yet been identified (Hearth, 1986).

- ✓ In Intermediate Hosts

In Australia and in New Zealand, a vaccine composed of antigens issued from oncospheres that were used on sheep to fight against *Taenia ovis* served as a model for the development of a vaccine against *E. granulosus.* This recombinant vaccine produces levels of ovine protection at 97% to experimental infections by *E. granulosus* eggs. From this vaccine, a strong immunity persists for six months, and the vaccination of gestating mothers leads to an increased level of protection of lambs at birth (Hearth *et al*., 1996).

In conclusion, whereas certain elements of the epidemiological cycle of *E. multilocularis* have been known for a long time, others were only discovered more recently, and some still remain to be studied. The role of new hosts in the establishment of the life cycle represents a major area of interest to explore. The modifications of population and behavioral adaptations of the principal definitive host, the red fox, must also be taken into consideration.

2. Direct and Indirect Detection Methods of *E. multilocularis* in the Red Fox

2.1. Post-mortem Examination of Digestive Content

Collection and Conservation of Organs

Fox carcasses must be sealed in a hermetic plastic bag and transported as quickly as possible to the laboratory. It is then possible to conserve the carcasses or intestines by freezing them. ERZ has published a protocol for handling foxes (appendix 5).

Pre-treatment and Sanitary Precautions

In order for technicians to avoid all risk of contamination, carcasses or intestines must be **decontaminated by freezing them between -70°C and -80°C for at least one week.** The eradication of *E. multilocularis* eggs requires a period of four days at which point the entire frozen material reaches -70°C or two days to reach a temperature of -80°C (Blunt *et al.,* 1991; Veit *et al*., 1995).

The potentially contaminated material must be handled with care. Examinations must be performed in an autopsy room specifically reserved for this use and whose access is monitored. Personnel must wear clothing strictly assigned for this autopsy room, and they must undergo one serological examination per year in order to detect the presence of antibodies specific to *E. multilocularis* (Eckert *et al*., 2001).

Sedimentation and Counting Technique (SCT)

Up until the end of the 1980s, necropsy analysis was the only reliable technique for diagnosing *E. multilocularis* infection. It is still the reference technique recognized by WHO (Eckert *et al*., 2001). In the sedimentation and counting technique, the small intestine is first incised longitudinally. The macroscopically visible parasites are removed, and the intestine is cut into sections of 20 cm (Hofer *et al*., 2000; Rausch *et al.,* 1990). These sections are transferred into a glass bottle containing a liter of physiological saline solution. After having shaken the bottle vigorously for several seconds, the mucosal layer is pressed between two fingers in order to dislodge the potential parasites, at which point the intestinal segments are removed from the bottle. The solution containing the intestinal material is then prepared for sedimentation several times over fifteen minutes until the sediment is sufficiently clear. The sediment is finally transferred by aliquots of 5-10 mL into rectangular plastic dishes with built-in counting grids and is then examined under a stereomicroscope (Eckert *et al*., 2001).

Intestinal Scraping Technique (IST)

In the intestinal scraping technique, the intestine is placed in a metal tray then incised along its length. Large-sized parasites are removed, and then the mucosa is deeply scraped with microscope slides. The material adhering to the slides is delicately spread out in Petri dishes in thin layers by pressing the slides together. Five scrapings are performed on the proximal small intestine, five on its middle section, and five on the distal section. These fifteen samples must be performed at an equal distance. The scrapings are then carefully observed under stereomicroscope (Eckert *et al.,* 2001). This technique economizes time, and over these past few years, it was frequently used in large-scale studies.

In two thirds of parasitic foxes, *Echinococcus* tapeworms are observed in the last third of the small intestine. As for the other cases, worms are only observed in the anterior or middle of the small intestine. Counting of observed parasites allows for the classification of infection - in a subjective manner - as low, average, or high (Eckert *et al*., 2001).

Shaking in the Vessel Technique (SVT)

This technique is an adaptation of the sedimentation technique. The intestine is incised longitudinally and placed in a 1 L cylindrical plastic vessel. The vessel is covered with a lid, filled with water, and shaken before proceeding to the decantation process. The latter action is repeated until the decanted water becomes clear. To dislodge the parasites stuck in the mucosa, the vessel is then opened, and the intestine is pressed between two fingers. The vessel is filled one last time with water and shaken; then a maximum amount of water is drained from the container. The sediment is placed in a plastic container and stored at 4°C. Finally, the materials are placed in a Petri dish, and the parasites are counted by microscope (Duscher *et al*., 2005).

Identification of Parasites

Identification of adult tapeworms relies on morphological criteria and is generally easy to perform. The *E. multilocularis* species is characterized by its small size (< 4.5 mm), the constant presence of five proglottids, the position of the genital pore on mature segments, the length of the ovigerous segment inferior to half the length of the body, and the sac-like uterus.

The presence of *E. granulosus* is rare in the fox. It can be differentiated by the number of proglottids (most often equal to three), the ovigerous segment longer than half of the body, and a genital pore generally located from the posterior end to the middle of the segment.

Sensitivity and Specificity of Detection Techniques

By examining 170 foxes from an enzootic zone, Hofer *et al*. (2000) detected 87 positive animals with a parasite load ranging from one to nearly 57,000 tapeworms. While noting that the technique allows for the detection of low levels of parasitic loads, this study concluded that the sensitivity of the SCT was near 100%. Eckert (2003) estimates the sensitivity of the technique at 98%.

The characteristics of other detection techniques are evaluated in relation to SCT. The sensitivity of IST of mucus is 78% in comparison to SCT. The specificities of these two techniques are equivalent (Hofer *et al*., 2000).

Duscher *et al*. (2004) compared SVT to IST by analyzing 356 Austrian foxes, of which 26 were positive. Nineteen foxes were revealed positive by IST (with a sensitivity of 73%) and 25 by SVT (sensitivity of 96%). SVT thus represents an alternative to SCT for fox populations with low levels of infection. Conversely, in regions of a high endemicity, SVT and IST would be equivalent (Duscher *et al*., 2004).

Limits

The three previously described techniques are recommended by OIE for post-mortem diagnosis in domestic carnivores (Kamiya, 2007). SCT remains the reference technique for the detection of *Echinococcus* tapeworms (Deplazes and Torgeson, 2009). This technique, however, has certain limits. Karamon *et al*. (2010) evaluated the detection limit of SCT by using it on fox intestines experimentally enriched with a number of known tapeworms. Four samples of ten intestines, each containing 2, 5, 10 and 30 *Echinococcus* tapeworms respectively were analyzed. By setting the probability of obtaining a positive result at 60%, the limit of detection was revealed to be ten parasites per intestinal sample. SCT is therefore capable of detecting very low quantities of *Echinococcus* tapeworms, but it does not represent a perfect "gold standard." It has a limit which must be taken into account in prevalence studies, notably of fox populations with low levels of infection. Additionally, the significant variation of the number of counted *Echinococcus* tapeworms within the samples demonstrates the weak repeatability of this technique (Karamon *et al*., 2010).

Advantages

These three techniques allow for parasite load to be detected in autopsied animals. When the prevalence does not seem sufficient to characterize the epidemiological situation, it is necessary to obtain the parasite load in infected animals. Several highly infected foxes are indeed capable of contaminating - by themselves - a defined area from the numerous eggs that they are likely to excrete (Hofer *et al*., 2000).

The estimation of parasite load allows us to study the parasite's transmission. As the infection's intensity in new definitive hosts is better understood, we can learn more about their importance in the establishment of the parasite life cycle and in the contamination of the environment and estimate the contamination risk for intermediate hosts.

It would be interesting to know the parasite load of foxes in culling campaigns. Since culling in effect induces a modification of parasite pressure, it allows for the study of the influence of vulpine population density.

During ERZ's mapping project, SSCT (Segmental Sedimentation and Counting Technique) was employed. Only qualitative data indicating the presence or absence of *E. multilocularis* were thus available. The study of parasite load in foxes in a study of such magnitude would be interesting (Deplazes).

Although the ANSES laboratory of Nancy analyzed the intestines by the SSCT method, they also performed estimations on the degree of infection, which can provide valuable results. In earlier studies, this parasite load was very heterogeneous (though it is not presently known what this is dependent upon) (Boué).

In the Netherlands, the parasite is present in certain locations, but absent in others; knowledge of the parasite load would be able to explain why this is the case.

It must be noted that it is not worth calculating the average parasite load. As certain foxes are highly parasitic, this figure is not representative. In Hungary, for example, 10% of foxes carry by themselves 99.9% of the parasite load. It is more judicious to classify the infection intensity in four or five classes (Romig).

Euroepan Symposium on Alveolar Echinococcosis, 2010

In Poland, as in Hungary and The Slovak Republic, the presence of *E. multilocularis* in the red fox was researched by SCT. Even though it is more time-consuming (5-10 samples per person per day instead of 20-25), this method was chosen in Poland instead of IST because the lack of sensitivity of IST did not allow for the detection of cases in their pilot study. Through SCT, the study was able **to estimate the level of infection in Poland and to separate infections into four classes** (Gawor).

Optimization of Necropsy Analysis

As part of the national mapping project of AE, which consisted of testing 100 foxes per department, ERZ consulted the French Association of Directors and Managers of Public Veterinary Laboratories of Analysis (ADILVA) in order to optimize SCT and to minimize the overall costs of the project. The challenge was to put in place a simple, reliable, and rapid technique in order to save time, while maintaining a satisfactory level of sensitivity. The method of sedimentation, though very sensitive and specific, is actually costly due to the prolonged time required for technicians to count the parasites in all of the intestines. This technique required approval from the National Reference Laboratory for *Echinococcus* of ANSES in Nancy. Building upon previous observations, it became apparent that we could economize time by selecting intestinal segments representative of *E. multilocularis* presence. Indeed, Thompson and Eckert (1983) had already described the preferential localization of tapeworms in the posterior or distal section of the intestine of definitive hosts.

The Segmental Sedimentation and Counting Technique (SSCT) is an adapted version of the sedimentation method that divides the small intestine into specific segments. The samples of fox intestines are set aside to freeze after the identification of the duodenum by a double ligature. The total length of the intestine is then measured, and the intestine is divided into five parts of equal length. The segments are labeled S1 to S5 in increasing order from the anterior to distal end of the intestine. As the duodenum consistently measures 20% of the total length of the intestine, it corresponds to S1 (Umhang *et al*., 2011).

The study conducted on 358 foxes consisted of evaluating the sensitivity obtained after assessing a single segment or two combined segments and comparing it to that which was obtained after an analysis of the entire intestine by the reference method. In total, 227 foxes were analyzed by the Departmental Veterinary Laboratories and 131 by LNR of ANSES.

The Departmental Veterinary Laboratories perform a segment-by-segment qualitative analysis beginning with S4. If the parasites are observed there, the result is positive; if no parasite is found, S1 is analyzed. This qualitative analysis provides a positive or negative response to the presence of *E. multilocularis,* and the process ends with the identification of the first tapeworm.

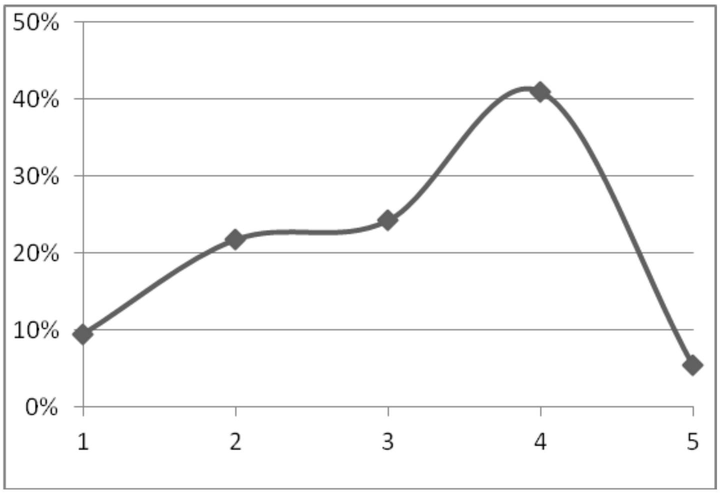
The ANSES Laboratory also evaluates the parasite load and classifies the positive samples in five categories, running from very weak to very strong parasite load. Each segment is treated independently in order to obtain independent results for the five intestinal sections (Umhang *et al*., 2011).

The Departmental Veterinary Laboratories detected the presence of *Echinococcus* tapeworms in 117 foxes through the sedimentation technique. The analysis of S4 on 227 foxes was able to reach a sensitivity of 94.3%; combining it with the analysis of S2 or S1, the sensitivity is 98.3%. The ANSES laboratory obtains a sensitivity of 100% with combinations S4-S1 or S4-S2 for parasite loads greater than 1000 tapeworms. Sensitivity seems to be dependent on the degree of infection, and only the couple S1-S4 allows for the detection of *Echinococcus* tapeworms with low parasite loads. Additionally, for eight positive samples, infection was localized in the anterior part of the intestine. The combination of S1 or S2 thus makes it possible to detect infections and to maintain a sensitivity of around 100%, while minimizing observation time with respect to the reference technique. The decrease in analysis time is estimated at 66% by ANSES (Boué *et al.*, 2010).

Segment 4 is a carrier of 40.2% of the parasite load (figure 16). This preferred localization is explained by the favorable physiological conditions to the establishment of the parasite (Thompson *et al*., 1983). This justifies the prioritized observation of S4.

Figure 16. Distribution of Average Parasite Load of *E. multilocularis* based on Intestinal Segments of Foxes

(according to Umhang *et al*., 2011)



A similar method to optimize IST was established by Tackmann *et al.* (2006). It consists of dividing the intestine into three parts and performing twelve scrapings in the second section and twelve in the third section. The sensitivity is near 100% (Tackmann *et al*., 2006).

Specificity is unchanged in comparison to the sedimentation technique and remains at 100% in regions where *E. granulosus* is not co-enzootic. This is because the identification of *E. multilocularis* relies on morphological observation of adult parasites (Hofer *et al*., 2000).

SSCT therefore represents an excellent compromise between saving time and maintaining high predicative values in large epidemiological studies that are aimed at following the prevalence of infected foxes in zones where it is either low or unknown. This technique has a disadvantage in that it does not provide **any data on the subject of parasite load.**

The reference method by sedimentation and counting (SCT) was optimized by the ANSES laboratory of Nancy and the Departmental Veterinary Laboratories (LVD) in order to economize time and money during the mapping campaign led by ERZ. The study required the testing of 100 fox intestines by department, with the largest costs coming from tapeworm counting. This adaptation of the SCT method makes it possible to obtain a **qualitative diagnosis** (positive-negative) of the presence of *E. multilocularis* in fox intestines (Woronoff).

In order to validate the SSCT technique, ANSES performed the SCT method for each intestine (Boué).

This technique is carried out through the preferential localization of adult stage *E. multilocularis* in the posterior part of the intestine. This was demonstrated by several previous studies conducted in Franche-Comté.

In comparing the length of the duodenum to the total length of the intestine, it appeared as if the duodenum consistently measures 20% of the length of the intestines. The intestines are therefore divided into five equal segments, the first of which corresponds to the duodenum.

While evaluating parasite load in each segment, it has been observed that 40% of worms are localized in the fourth segment (S4) whereas only 10% are situated in S1. It thus appeared possible to limit the time of analysis by choosing the intestinal segments most representative of the parasite's presence.

However, the analysis of S4 alone can only reach a sensitivity of 93.2% in comparison to the 100% sensitivity obtained by SCT. The combination of two segments thus appears indispensable to increasing this sensitivity.

The best value is obtained by combining S4-S1 or S4-S2 (Boué) (Table 3).

Table 3. Sensitivity of the Detection for Each Individually Tested Intestinal Segment and for Each Segment Couple

(according to Boué, European Symposium on AE, 2010)

Segment	1	2	3	4	5
1	56.40%				
2	72.60%	70.10%			
3	83.80%	82.90%	80.30%		
4	98.30%	98.30%	97.40%	93.20%	
5	73.50%	79.50%	85.50%	94.30%	58.10%

In order to determine the most judicious order in which to test the segments, eight member departments of ERZ analyzed a total of five segments of 250 intestines. The results that were obtained for the segments 1 and 5 are represented in Table 4.

Table 4. Intestinal Segments Testing Positive among 104 Contaminated Intestines (according to Woronoff, European Symposium on AE, 2010).

	Segment IV Positive	Segment IV Negative
Segment I Positive	57	9
Segment I Negative	37	1

The study of different possible orders of analysis between S1 and S4 has indicated that the analysis of S4 followed with that of S1 – only if S4 is negative – makes it possible to reach the highest rate of detection of *E. multilocularis*. The sensitivity achieved by LVD is 99%. The risk of a false-negative is thus inferior to 1% (Woronoff).

The sensitivity achieved by ANSES is near 98%. This can be considered in relation to parasite load: the higher the parasite load, the greater the sensitivity. This sensitivity of 98% is associated with 0.8% false negatives for two segment couples. The specificity is identical to that of SCT because the conditions are identical; it is therefore 100%.

To save time, while also conserving a proper level of sensitivity, it was decided to analyze S4 first, followed by S1 if no tapeworm were found in S4. Through this technique, only 607 intestinal segments need to be tested, compared to 1790 as required by the SCT method. This results in a decrease in analysis time of 66% (Boué).

The optimization of the SCT method by SSCT reduces the required time to analyze intestines by 66% , while maintaining maximal specificity and only minimally reducing the sensitivity to 98%. **This improved method constitutes a very rapid, reliable technique for epidemiological studies conducted on large scales, particularly in zones where *E. multilocularis* prevalence is low or unknown (Boué).**

2.2. Detection of Coproantigens by ELISA

Principal Method

ELISA (*Enzyme Linked Immunosorbent Assay*) is a method enabling the detection of *Echinococcus* antigens excreted in feces. ERZ has published a protocol for gathering feces (appendix 6).

Allan *et al*. (1990) and Deplazes *et al*. (1992) first employed polyclonal antibodies from hyperimmunized rabbits with *E. granulosus* antigens in order to test infection in carnivores. Deplazes *et al.* (1999) then developed a Sandwich Elisa method using polyclonal rabbit and chicken antibodies that were directed against *E. multilocularis* coproantigens and somatic adult worm antigens, respectively. Feces can be fresh, frozen, or preserved in formalin. It is possible to demonstrate the presence of parasite antigens in feces during the prepatent period, from four days post-infection onwards (Deplazes *et al*., 1999). Parasite forms that are detected can be mature or immature.

In an experiment conducted on four foxes infected experimentally with 15,000 *E. multilocularis* protoscolices and with a parasite load ranging from 3720 to 9240 tapeworms per animal, the coproantigens were detected after 4-6 days post-infection and up to 125 days thereafter; there was, however, a decrease in the quantity of detected antigens occurring around the third to fourth week (Nonaka *et al*., 1996).

Sensitivity and Specificity

Studies on red foxes have shown a high specificity (95%). Results are even better (99%) on large groups of dogs or cats, even if these animals are infected by intestinal nematodes. Nevertheless, cross-reactivity occurred in 16% of dogs co-infected with *E. granulosus*. This creates problems for detection in regions where the two parasites live sympatrically.

Sensitivity is dependent upon parasite load (Raoul *et al*., 2001). It was evaluated at 84% in a study conducted on 55 foxes infected with *E. multilocularis.* It reached 95% on 37 foxes carrying more than 100 tapeworms, but fell to 61% on 18 foxes harboring less than 100 tapeworms (Deplazes *et al*., 1999).

Predictive Values

In a population where the prevalence is low, detection of parasite coproantigens by ELISA is characterized by a high negative predictive value and a low positive predictive value.

In this case, positive ELISA results must be confirmed by other techniques, such as PCR. In a study conducted on dogs in South America, Guarnera *et al*. (1999) showed that confirmation of results by immunoblot assay allowed for sensitivity and specificity to approach 100%

Value

With regard to large sample sizes, the detection of parasitic coproantigens by ELISA proves valuable in studying the prevalence of a certain region or to track the performance of a deworming program. Raoul *et al.* (2001) compared the ELISA coprotests with the necropsy method in two regions in eastern France that exhibited high and low endemicity, respectively, as well as a third non-endemic control area in the western part of the country. The results obtained from ELISA are representative of the parasitic distribution in the population. Therefore, the ELISA method is an alternative to the sedimentation method for identifying different degrees of endemicity. Even if the ELISA method **does not provide a precise estimate regarding prevalence and is not usable in countries or regions where multiple *Echinococcus* spp. cohabit**, such as China for example, it does allow for the estimation of a certain level of parasitic pressure and for monitoring the arrival of the zoonosis in urban areas by the intermediary of foxes (Raoul *et al*., 2001) It necessitates, however, the knowledge to interpret data analyses for diagnosis on the scale of populations – and not of individuals. (Deplazes *et al.* 1999)

Analysis can be performed on feces that are fresh, frozen or preserved in formalin. It is, however, advised that tested samples be used either from the large intestine during an autopsy or from fresh fecal material. Also, the study of coproantigens can be performed on either dead or live animals, as in the case of PCR assays. The sensitivity of this technique is less high than that of IST and shows a less significant parasite load than with detection by way of the sedimentation technique. It does enable the analysis of 200 specimens per person per day, whereas the necropsy techniques only allow for an analysis of 10-20 animals per person per day.

2.3. Detection of Parasite DNA by PCR

Method

PCR (Polymerase Chain Reaction) permits detection by the amplification of *E. multilocularis* DNA contained in fecal matter. PCR was first used by Bretagne *et al.* for detecting the DNA of *E. multilocularis* eggs found in the feces of foxes. The method was then modified and improved (Deplazes and Eckert, 1996; Dinkel *et al*., 1998; Monnier *et al*., 1996)

Bretagne *et al*., (1993) chose a target sequence of around 1300 bp (base pairs) repeated in tandem in the *E. multilocularis* genome around 50-100 times. The DNA is extracted by chemical lysis with potash and is purified with resin. After amplification, electrophoresis, and coloration by ethidium bromide, the presence of *E. multilocularis* eggs is detected by the presence of a fragment characterized by 337 bp.

At the ANSES laboratory in Nancy, trials were performed in order to simplify and improve the technique. These trials led to the conclusion that classic PCR, with a simplified method, was unusable due to its low sensitivity (24%). The improvement of its sensitivity requires a dilution combined with a supplemental amplification step on the PCR products, provided by new sets of primers. With these adjustments, the sensitivity was raised to 65% (Monnier *et al.*, 1996)

At the University of Zurich's Institute of Parasitology, the addition of a preliminary step of egg concentration in the feces by sequential sieving of the samples and flotation of the eggs in a zinc chloride solution substantially improved the results (sensitivity 93%).

Dinkel *et al.* used a method that does not require a concentration of eggs, but rather, an optimization of the DNA-extraction step achieved through alkaline hydrolysis. Their method targets a genetic fragment coding for mitochondrial ribozyme RNA, 12S. In using three different primers enabling the amplification of the same gene, Boufana *et al.* demonstrated that the use of less-conserved genes maximizes specificity while limiting cross-reactivity with other *Echinococcus* spp. Their technique allows for the detection of DNA contained not only in eggs, but also, in proglottids.

Sensitivity and Specificity

In comparing the results obtained from the technique used by Bretagne *et al.*, namely the necropsy examination of 55 red foxes, specificity was evaluated at 100% and sensitivity at 94%. Two false-negatives were observed in foxes harboring immature worms. The sensitivity of PCR is superior to the examination of intestinal content (Bretagne *et al.*, 1993).

A significant volume of fecal material is necessary in order to obtain a satisfactory sensitivity. The presence of numerous **enzyme inhibitors** can equally reduce sensitivity. One solution is to perform PCR on isolated taeniid eggs in order to detect patent infections. Flotation is more sensitive to detect these eggs in comparison to the McMaster method (Mathis *et al.*, 1996). To increase sensitivity, it is equally possible to repeat fecal sampling while using PCR techniques that do not rely solely on isolating eggs, but rather, on detecting prepatent infections (Al-Sabi *et al.*, 2007). Parasite DNA in feces comes from either excretion of protoscolices in the first days following infection or from immature segments at the end of the prepatent period. This excretion is dependent upon the metabolic activity of the parasite. A trial conducted on 58 dogs infected by *E. granulosus* only detected 15 positive dogs during the prepatent period, which corresponds to a sensitivity of 26% (Lahmar *et al.*, 2007).

Dinkel et *al.* (1998) observe that sensitivity is correlated to the number of parasites and the degree of maturity of the parasitic worms. After an analysis in Germany of 250 foxes, of which 165 were infected, an average sensitivity of 89% was obtained; this sensitivity reaches 100% when more than 1000 adult tapeworms are present. PCR sensitivity is superior to the necropsy method (76%).

Predictive Values

Predictive values obtained with a sensitivity of 94% and a specificity of 100% are very high. The positive predictive value is 100%, and the approximate negative predictive value is equally 100% in populations with a low prevalence (Mathis *et al.*, 1996).

Advantages and Disadvantages

PCR can be performed on fresh fecal material placed in 70% ethanol or after the inactivation of eggs (by freezing them), two conditions that provide a certain degree of protection for the lab technician. Additionally, the fecal material preserved in formalin is no longer usable since DNA degrades therein (Al-Sabi *et al*., 2007). **Costly and time-consuming**, PCR cannot be used in large-scale studies, but instead, only for confirmation of positive results or individual assays in human medicine (Mathis and Deplazes, 2002). However, Dinkel *et al*. (1998) presented PCR as an alternative method to IST. The technique notably enables the distinction between the morphologically non-differentiable eggs of the *Taenia* or *Echinoccous* spp. (Torgerson *et al*., 2007).

Microsatellite genotyping also enables the study of the spatial and temporal extension of the parasite in different host populations (Knapp *et al*., 2007)

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- PCR Application in Search of *E. multilocularis* in Domestic Dogs in France

In France, from 2008 to 2010, the ANSES laboratory of Nancy, in collaboration with ERZ and veterinarians from hyperendemic departments, such as the Meuse and the Haute-Saône, endeavored to identify the prevalence of the parasite in dogs. Given the expected low prevalence rate in comparison with other European countries, numerous fecal samples were required (a target was set at 1000 specimens per department). These samples were gathered after deworming had been completed, so that sensitivity was enhanced and dog owners were convinced to bring in fecal material. In total, 493 and 367 fecal samples were collected in the Meuse and Haute-Saône, respectively (Umhang).

After decontamination, the flotation technique modified by Mathis was effectuated, and the eggs were then observed under microscope (Table 5).

Table 5. Principal Parasites Observed in Dogs in Haute-Saône and in the Meuse
(according to Umhang, European Symposium on *E. multilocularis*, 2010)

Principal Intestinal Parasites Tested in Coproscopic analysis	**Infection Prevalence in Haute-Saône (%) (N=367)**	**Infection Prevalence in the Meuse (%) (N=493)**	**Total % (N=860)**
Ancylostoma spp.	13.90	7.10	10.00
Trichuris vulpis	8.72	6.90	7.67
Toxocara canis	4.63	4.46	4.54
Capillaria spp.	5.18	0.20	2.33
Taenia spp.	0.82	0.81	0.81

In 20% of cases, feces contained at least one type of parasite egg. Taeniid eggs were observed in seven dogs, four of which resided in the Meuse and three of which were located in the Haute-Saône (Umhang).

DNA was then extracted and tested by 2 PCR assays, the first comprising specific primers of *E. multilocularis* and the second to determine other *Taeniid* spp. (Table 6).

Table 6. Results of PCR Assays on 7 Dogs whose Feces Contained Taeniid Eggs

(according to Umhang, European Symosium on *E. multilocularis*, 2010)

PCR Results	**Location**	**Dog Type**
T. crassiceps	Meuse	companion
T. crassiceps	Meuse	companion
T. crassiceps	Meuse	companion
T. crassiceps	Meuse	hunting
T. crassiceps	Haute-Saône	hunting
Inhibition	Haute-Saône	hunting
Inhibition	Haute-Saône	hunting

Among these seven samples, two were inhibited, and the other five tested positive for *T. crassiceps.* **No dog was thus found positive for *E. multilocularis* in the hyperendemic departments of the Meuse and the Haute-Saône.**

However, the sample size of this study is too small, and these preliminary results must be confirmed (Umhang).

The tapeworm *T. crassiceps* has the same life cycle as *E. multilocularis*. This implicates rodents as intermediary hosts and carnivores as definitive hosts. Dogs infected by *T. crassiceps* are predators of rodents, and in such endemic zones, every dog contaminated by a tapeworm that was transmitted following the consumption of rodents is considered at risk for *E. multilocularis*.

Among these five dogs infected by *T. crassiceps*, three were companion dogs or pets, and two were hunting dogs. The presence of contaminated household dogs indicates that all categories of dogs are concerned (Umhang).

Euroepan Symposium on Alveolar Echinococcosis, 2010

- Application of PCR in Search of *E. multilocularis* in Domestic Dogs in China

In Tibet, where *E. multilocularis, E. granulosus*, and *E. shiquicus* live sympatrically, PCR was used to highlight the presence of *E. shiquicus* in dogs (Boufana).

- Optimization of PCR by the University of Salford in Manchester

In Tibet, *E. multilocularis, E granulosus,* and *E shiquicus* live sympatrically. Tibet is the unique region in the world where all three *Echinococcus* spp. cohabit. Here, AE and Cystic echinococcosis have the highest combined local prevalence in the world, at roughly 10%.

Previously available PCR assays used localized tandem repeats on the RNA 12S after extracting the genetic material from feces. No test has yet been conducted for *E. shiquicus* DNA. In an endemic ecosystem as complex as that of Tibet, **simple and specific assays enabling the differentiation of the three *Echinococcus* spp.** were crucial - hence the idea of producing an optimization of the PCR technique.

Thus, a new gene, NDI, was targeted. NDI possesses a genetic polymorphism, which makes the identification of *Echinococcus* spp. possible - as well as other *Taeniid* spp. - through copro-DNA. This technique needed to be validated, and Nancy's ANSES laboratory was able to accomplish this.

DNA can be extracted from feces after the deworming of dogs, from tissue during an autopsy of foxes or from larvae contained in human tissue.

In order to test the specificity, the assay was conducted with other *Taeniidae* in dogs. No cross-reactivity was observed. ANSES did observe a single cross-reaction between *E. multilocularis* and *E. shiquicus*. The technique is equally specific to DNA extracted from feces as it is to DNA extracted from tissue. **For the first time, PCR has made possible the detection of *E. shiquicus* in a dog co-infected with *E. multilocularis*.**

The limit of detection for DNA extracted from feces was estimated from one to five eggs by ANSES and at 12 pg of DNA by the University of Salford for DNA extracted from tissue. DNA is concentrated by ethanol precipitation upon extraction in order to increase the technique's sensitivity. However, precipitation has the disadvantage of concentrating other species of DNA as well. The PCR assay performed on tissue that has been obtained following an autopsy allows for an 89% increase of sensitivity for *E. shiquicus*.

The optimization of PCR allows for the detection of prepatent infections. Primers permit the detection of DNA liberated by immature forms.

The optimization of PCR produces assays having a high diagnostic value in the complex region of Tibet. It enables the detection of *E shiquicus* in the dogs and foxes of Tibet that are co-infected with *E. multilocularis*. This technique opens a new avenue for additional research concerning the role of dogs as primary hosts of *E. shiquicus* and the modes of transmission of *Echinococcus* spp. in Tibet (Boufana).

2.4. Serological Screening

Implementation

The indirect method consists of detecting the presence of *E. multilocularis* antibodies in the blood of foxes through ELISA. Various antigens issued from *E. multilocularis* can interact with the immune system of the host and direct that host to produce specific antibodies (Deplazes *et al.*, 1997). The antigens are produced by adult tapeworms, juvenile intestinal forms of the parasite, or oncospheres (Lightowlers and Gottstein, 1995). First developed for detecting antibodies specific to *E. granulosus* in dogs (Gasser *et al*., 1988), this technique was subsequently adapted to test foxes infected by *E. multilocularis* (Gottstein *et al*., 1989, 1991). This serological test is performed on serum obtained by cardiac puncture of or on pleural fluid from fox carcasses.

The employed antigen is the purified homologous larval antigen called EM2 (Gottstein, 1985). In this initial test conducted on 400 red foxes, originating from populations infected by *E. multilocularis*, 12%-60% of animals were found positive for the presence of circulating antibodies against EM2 antigens. Moreover, none of the 98 raised foxes, infected naturally or experimentally by helminths other than *Echinococcus* tapeworms, contained specific antibodies (Gottstein *et al*., 1991).

Advantages and Disadvantages

Seropositive results occur in animals infected by *E. multilocularis*, as well as foxes from the same enzootic area that are absent of observable parasites during the examination of digestive content. **Therefore, the presence of antibodies does not allow for the differentiation between presently infected foxes and previously exposed foxes that had** immediately eradicated *E. multilocularis* (Gottstein *et al*, 1991). Additionally, even if the presence of antibodies in a population is closely correlated with the presence of *E. multilocularis*, there is not a demonstrated link between the seroprevalence of antibodies and the prevalence of *E. multilocularis* infection in a population (Ewald, 1993).

Additionally, the possibility of cross-reactivity between other *Taeniidae* diminishes the reliability of the results (Gasser *et al*. 1993)

The automation of the ELISA technique allows for the analysis of a great many samples and constitutes an alternative to IST for epidemiological studies. The use of serum diminishes the risks for the lab technician since he/she is not in contact with the eggs of the parasite. The serological testing, therefore, represents a viable pre-selection test in regions where the status of *E. multilocularis* is thus far unknown. Nevertheless, the results necessitate confirmation by way of PCR (Eckert *et al*., 2001).

Sensitivity and Specificity

The detection of antibodies by ELISA is neither very sensitive nor specific (Le Guenic, 1991)

Table 7. Characteristics of Different Diagnostic Methods for Foxes

(according to Eckert, 2003)

Technique	Reference Technique Used for Comparison	Sensitivity (%)	Specificity (%)	Number of Animals Examined per Person per Day
Sedimentation	Reference technique	100	100	10
IST	Sedimentation	78	100	20
ELISA Copro-antigen Detection	Sedimentation	84	96-99	200
PCR from Fecal Samples	Sedimentation	94	100	15

Each test has its advantages and drawbacks with regard to sensitivity and specificity; the nature and quantity of requisite samples; and the duration, complexity, and cost of analysis (table 7). The carefully performed sedimentation technique approaches the “gold standard,” which means that the sensitivity and specificity of said method approaches 100%. This technique thus lets us precisely estimate if an individual is infected at any stage of the infection. It is the only method recognized by WHO. However, this technique is costly. A test of high sensitivity will allow for the detection of a maximum number of cases in a screening operation, yet the positive results must be confirmed by a test demonstrating a higher specificity. It is therefore necessary to choose tests to implement based on the nature and objective of the envisioned study.

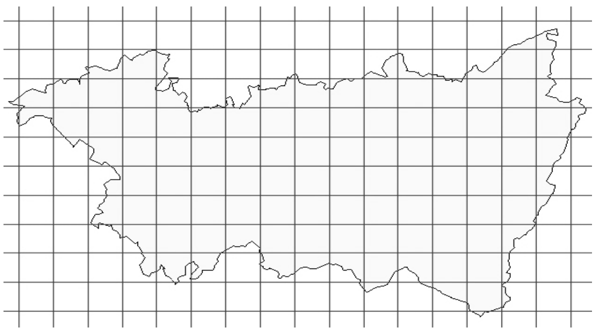
- **Methods for Researching the *E. multilocularis* Tapeworm in European Countries**

- **Sampling**

In Europe, the number of examined animals differs according to the size of countries, the rapidity of techniques used, financial resources, and the focus of the study.

Between 2008 and 2009 in Hungary, around 1% of the vulpine population was collected across 100% of the country (Casulli). In Poland, a country with extensive territory, over 3500 foxes were gathered homogenously - at 250 animals per region - over the entire country between 1993 and 2002 (Karamon). Approximately 5000 foxes were examined in 10 years in Slovakia. This represents a high density of samples given the country's small size (Miterpakova). In France, as part of ERZ's mapping project, over 3500 foxes were analyzed in a homogenous manner over 45 departments in a five-year span. Each department was divided into 100 square regions, and one animal per region was collected (Combes). The grid for collecting foxes in a department is diagrammed below in figure 17.

Figure 17. Division of a Department into 100 Squares of 8 km^2
(according to ERZ)



In contrast to these very intensive campaigns, other more simple efforts were led, for example, in Latvia where 53 foxes have been examined since 2003 (Bagrade).

The duration of campaigns differs from country to country. Certain have led investigations spanning 10 years while others have conducted campaigns of 1-2 years. Some countries concentrate on the principal definitive host while Poland (Gawor) and Slovakia (Miterpakova) have included numerous intermediate host species in their research.

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In Germany, the strategy for sampling varies across the country, differing from region to region. Certain regions analyzed over 2000 foxes while others collected only three. Regarding the latter case, it is impossible to interpret the results obtained from such a small sample size. The samples are evenly distributed in certain provinces, yet in others, they only come from a small portion of the territory. Only modeling enables the analysis of this set of data.

It must also be noted that the higher number of samples and the increased homogeneity of their distribution lead to more indicative data, yet the associated costs of such campaigns are higher as well.

Many countries have recognized that combining their research of *E. multilocularis* with other parasites results in both time and money saved (Schwarz).

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- *Historical Precedent for Researching the Parasite:*

In historically high-endemic areas, the tapeworm *E. multilocularis* has been researched for decades.

Investigations are much more recent in countries on the periphery of the known geographical range of the parasite, such as those countries in Eastern Europe. Consequently, a certain number of European countries only recently commenced their investigations in the 2000s. This is the case, for example, for Slovakia (Miterpakova), Poland (Karamon and Gawor), and Slovenia (Soba).

Therefore, not all countries possess historical data, and advancements in research and the optimization of detection techniques are variable from country to country.

Each country has a sampling plan (number of collected animals and extent of analyzed territory) **and a detection technique**. Their plan depends on the resources of the country, the length of time for which *E. multilocularis* has been researched in the country, and the political interest directed towards AE. Consequently, there is **a significant heterogeneity of diagnostic methods used in Europe, which makes it rather difficult to compare results and compile data in order to establish a comprehensive map for the presence of the parasite.**

Use of the sedimentation technique is widespread in a multitude of European countries despite its cost and the prolonged duration of time it takes to perform. PCR is widely employed for dogs since it permits the identification of parasites in living animals.

There is not one single technique that is superior to its counterparts; nor are there standardized procedures. Techniques and sampling must be adapted to the goals and questions at hand. Each tool has its advantages and its disadvantages in terms of costs, time, and reliability of results.

Research subjects cannot be identical in zones with different epidemiological statuses. Moreover, in **historically endemic zones**, where the prevalence is high, such as Franche-Comté, the situation can quickly change. Thus, efforts must be performed regularly, but the frequency at which such work should be conducted is unknown. Frequency must be higher in **emerging zones**, such as Poland. In **parasite-free** regions and **regions of unknown status**, strategies must be adjusted accordingly.

However, a harmonization of sampling procedures and diagnostic techniques must be performed on a European scale. Studies can be conducted in a homogenous and standardized manner in countries with equivalent epidemiological statuses.

3. Molecular Biology

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Molecular Biology allows us to study the evolution of organisms. The evolution of *E. multilocularis* can be retraced using genetic markers.

The use of a polymorphous genetic marker enables us **to retrace the process of human contamination.** Since AE has a long latency period before its associated clinical signs become manifest, it is essential that the marker is stable over time.

Similarly, there is also a human immunogenetic predisposition associated with the severity of the disease, which raises the question of whether or not transmission is linked to certain particular genotypes of the parasite or if the virulence depends upon genetic profile.

The microsatellite marker EmsB possesses a rather high polymorphism. It has a sequence that is tandemly repeated in the *E. multilocularis* genome. The number of repeats depends on the isolate. Analysis of the fragments' length allows for different profiles of EmsB to be obtained for *E. multilocularis* in Europe, North America, and Asia. Important similarities in acquired profiles from distinct hosts, such as that which was observed on Ile de Saint Laurent, indicate contamination from a distinct strain.

As part of the EchinoRisk project, profiles of parasites coming from patients and from foxes, whose geographic range was known, were compared. From the parasites arising in foxes or issued from human cases, nine profiles are shared.

EmsB is an excellent marker for retracing the history of human contamination by *E. multilocularis*. Nevertheless, it would be useful to study other markers as well (Knapp).

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Hungary, where different prevalences were observed in the north (16%) and the south (4%) of the country, the polymorphism of the microsatellite marker EmsB was employed in order to test the genetic diversity of *E. multilocularis*.

No link was able to be established between the genetic and the geographic ranges. By obtaining a majority genetic profile, it seems clear that Hungary constitutes a zone on the periphery of the *E. multilocularis* geographic range. The migration of foxes accompanies the extension of the parasite (Casulli).

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Genotyping can provide useful information for understanding the epidemiology of AE. More concretely, it lets us demonstrate the existence of a former endemic hotbed in Italy and rule out the hypothesis of the parasite's introduction from abroad. This technique enables the distinction of *actual* increases in prevalence from *observed* increases following an intensification of research. The existence of a particular profile in a patient makes it possible to retrace the origin of contamination (Knapp).

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It is legitimate to question whether the increase in prevalence of observed infection of foxes since the 1980s was not in fact due to the increase in sensitivity of detection methods. It does seem that this evolution in prevalence is very real since the acquired results from different European countries have all moved in the same direction, without one decrease in prevalence having thus far been noted (Romig).

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This problem brings to light the difficulties of interpretation, which derive from the use of techniques showing different characteristics over time and from country to country. The research subjects and sampling programs must be harmonized according to the varying epidemiological situations (Giraudoux).

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ERZ's mapping project demonstrated that the use of standard protocol on a national scale (including collection of samples, research of the parasite, and confirmation of the results) permit the acquisition of uniformly distributed, homogenous, and thus comparable information in a given country. With such protocol, it is possible to highlight certain factors correlated to the presence of the parasite, such as the nature of the geographical landscape. Similar operations conducted on the European scale and in a regular manner would make it possible to better understand the epidemiology of the parasite and to work on decreasing the risk of human contamination (Combes).

To obtain comparable results and to effectively detect *E. multilocularis*, **a consensus must be reached concerning the methods used in different research programs. A standard, simple procedure that is both inexpensive and accessible to all must be developed** and approved by a competent organization. The exchange of information and the mobilization of countries in a common direction are both necessary.

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EFSA, European Food and Safety Authority, is interested in all aspects of health and animal protection. The role of this European institution consists of the following: the provision of scientific advice, the collection of data, and the communication of scientific information to member countries.

EFSA recognizes that there presently exists no coordination between monitoring systems in different countries. The available sets of data oftentimes concern small geographical areas studied over short periods of time and are not comparable with one another. **EFSA also recognizes that the current available diagnostic methods are not adapted to monitoring the disease on a large scale.** Most of these methods are labor-intensive and are ill-suited for domestic animals.

Having observed a problem in reliability and harmonization of data, EFSA established animal-health workgroups, composed of European scientists, in order to improve the monitoring of several parasites, including *E. multilocularis*. Advice was notably provided on the subject of data collection. A document drafted from this meeting is available on EFSA's website. For the time being, no recommendation has been provided with regard to detection methods. As the epidemiological situation differs in each country, instituting a universal method has proven especially difficult.

EFSA strongly endorses the implementation of rapid detection methods that are simple, validated, and able to be used on humans, as well as on live animals or carcasses.

By sending out recommendations to European countries, EFSA was able to play a critical role in standardizing detection techniques for *E. multilocularis* (Have).

Euroepan Symposium on Alveolar Echinococcosis, 2010

However, it is not clear to European authorities that they should enforce a detection method (Romig). It is the task of scientists to come to an agreement on the best technique. It is incumbent though on EFSA to coordinate action, to gather data, and to disseminate information.

In conclusion, there does not exist at present a preferred detection method used by the entirety of European countries. In reality, the varying sampling plans and diagnostic techniques possess a high degree of heterogeneity from country to country. This makes the comparison of results and the compilation of data on a European scale extremely difficult. It is absolutely necessary to agree upon a common research method for *E. multilocularis* for all European countries, without of course forgetting to take into account each epidemiological situation

.

4. Epidemiology of *Echinococcus multilocularis* Infection

4.1. Descriptive Epidemiology

Affected Species

- ✓ Definitive Hosts

In Europe, species infected by *E. multilocularis* include **the red fox (*Vulpes vulpes*), the domestic dog (*Canis familaris*)**, the raccoon dog (*Nyctereutes procyonides*), the domestic cat (*Felis catus*), and the wildcat (*Felis sylvestris*).

All of the definitive hosts possess different capacities to multiply and disseminate the parasite. In descending order, the largest receptivity is present in the arctic fox, the red fox and coyote (equivalent receptivity), the wolf, and the corsac fox.

Dogs play an essential role in maintaining the cycle and in human contamination in highly endemic zones (Vuitton *et al.*, 2003). In China and the U.S. state of Alaska, dogs represent the predominant definitive hosts (Shantz *et al.*, 1995; Eckert *et al.*, 2000).

The cat seems to be a less favorable definitive host for the parasite, yet its capacity to transmit infection has not presently been clarified. Cases of cats naturally infected with *E. multilocularis* were recently documented in several enzootic regions in North America, Europe, and Asia (Yagi *et al.*, 1984). In France, between 1987 and 1996, three cats out of 81 were confirmed as infected (Petavy *et al.*, 2000). Furthermore, cats can be carriers of the mature adult forms of the parasite, which means that they can excrete infected eggs. In Japan, a case was reported of a cat releasing eggs (Yagi *et al.*, 1984; Eckert *et al.*, 2001; Thompson *et al.*, 2006).

However, artificial inoculation trials prove that the receptivity of cats is more variable than that of foxes or dogs (Zeyhle *et al.*, 1982). Secondly, the development of the parasite in cats is protracted (Thompson and Eckert, 1983). Therefore, the tapeworms do not achieve a maturity in the intestines of felines, and the number of eggs excreted into the environment is lower compared to other definitive hosts. Consequently, cats only play a minor role in the parasite maintaining its presence in an enzootic area. They represent, however, a non-negligible actor in the cycle because of their hunting activities and close proximity to humans.

- ✓ Intermediate Hosts

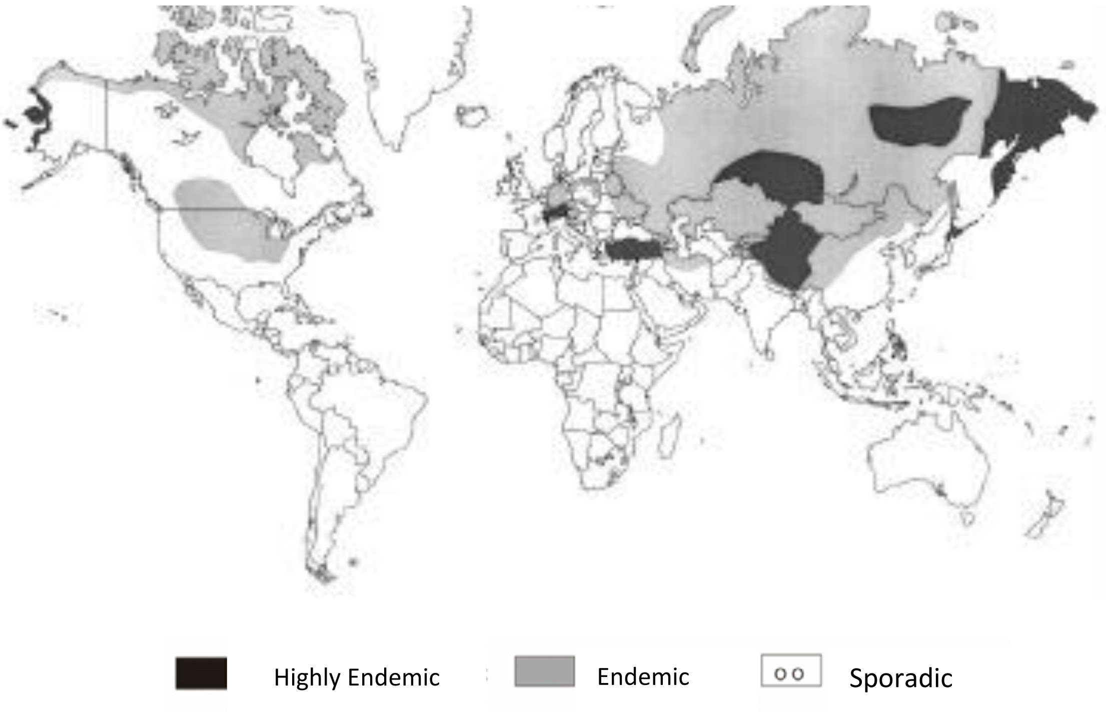
All intermediate hosts do not have the same epidemiological importance, and certain seem to be more favorable for enabling the parasite's establishment. The intermediate hosts considered to be the most important in Central Europe are **the common vole (*Microtus arvalis*), the European water vole (*Arvicola terrestris*), and the muskrat (*Ondatra zibethicus*)** (Eckert, 1998). The high sensitivity of the coypu (*Myocastor coypus*), imported from South America, was also confirmed in Germany. The populations of these last two species drastically grew over several years, and their epidemiological role could increase in the years to come (Machnicka-Rowinska *et al.,* 2002; Berke *et al.*, 2008)

The domestic mouse (*Mus musculus*) is a poor host for *E. multilocularis*, as larvae are oftentimes sterile and only rarely lead to the infection of definitive hosts (Petavy, 1997). It can nevertheless be considered as a sentinel indicating the presence of the parasite.

This diversity of hosts and the recent discovery of certain species strongly implicated in the life cycle demonstrate the existence of a certain adaptability of the parasite. The receptivity of the hosts is not the only criteria to consider. One must also be attentive to the density of these species and to the existence of cohabitation between intermediate hosts and definitive hosts, which is necessary to predation and the establishment of a life cycle.

Global Geographic Distribution, Prevalence, and Incidence

Figure 18. Geographic Distribution of *E. multilocularis*
(according to Eckert *et al*., 2000)



The geographic distribution of *E. multilocularis* is confined to the northern hemisphere (Raush, 1995). The parasite is found in cold regions as determined by either latitude or altitude (Eckert *et al*., 2001). The parasite is said to have originated in Alaska and to have successfully spread throughout the rest of the U.S. by way of Canada, extending into Central Europe and Eurasia thereafter (Raush and Schiller, 1954; Eckert *et al.*, 2001) (figure 18).

The prevalence of *E. multilocularis* infection is difficult to estimate since it does not have the same value depending on the local geographical scale considered. It is generally low across wide territories, but it can prove to be rather high in small habitats of several hectares, which correspond to hyper-enzootic hotbeds (Eckert *et al.*, 2001). Therefore, geographical maps only reflect an average parasitic prevalence.

Additionally, the estimated prevalence depends on numerous factors, such as the diagnostic method used and the number of tested animals (Colas and Deiller, 1987; Eckert *et al.*, 2001). The maps illustrating the global distribution of the parasite are issued from data collected uniformly in diverse countries, and this renders the comparison between different regions especially difficult (Vervaeke *et al.*, 2006).

Finally, though the prevalence is known rather well in Western Europe, Japan, China (notably on the Tibetan plain), Central Asia, and North America, other regions have not conducted studies and lack sufficient data. AE principally affects people living in rural areas, though studies are not very advanced in certain poor regions such as that of China (Houin *et al.*, 1992). Therefore, global geographic representations are mere approximations.

Four global endemic hotbeds are described below:

China and Central Asia

Three principal high-endemic zones or hotbeds have been identified in China; they are situated in the northwest, center, and northeast of the country (figure 19). The first local studies were published in 1960, but their dissemination did not take place until the 1980s when the country "opened its doors." The first discoveries of the parasite in animals date back only to 1980 (Craig, 2006). The sparse available data indicate to us that in the province of Sichuan, there was a prevalence of 59.4% for 32 tested foxes (Schantz *et al.,* 1995) and a prevalence of 14.3% for 28 tested dogs (Schantz *et al*., 1995). In intermediate hosts, the highest rates of prevalence reside in the American pika (*Ochotona princeps*) in the Sichuan province (4.2%) and in Brandt's voles (*Microtus brandti*) located in Mongolia (2.4%) (Craig *et al.,* 1996). In 1999, 25% of Chinese scrub voles (*Pitymys Irene*) that were studied in Sichuan had been found infected (Jiamin *et al.*, 2005).

Figure 19. Distribution of *E. multilocularis* (in gray) in Chinese Provinces
(according to Ito *et al*., 2003)

A significant endemic hotbed was recently discovered with prevalence rates running as high as 15% of the human population in certain rural zones (Craig *et al*., 2000; Vuitton *et al.*, 2003). In certain years, 350 human cases were reported just in the region of Gansu. Between 1991 and 1997, a wide screening campaign, involving ultrasonography and serological testing, was launched in this region and found a prevalence of 4%. This number signifies a prevalence of 200 for a population of 100,000, taking into account the density of the population in this rural zone (Craig, 1997). **China is thus the first country seriously affected by AE, and the situation there is alarming.** Factors that can explain such rates include activities permitting the proliferation of hosts (deforestation and reforestation) and the proximity of domestic dogs (Craig *et al.*, 1992, Giraudoux *et al.*, 2006) Genetic predisposition in human populations can also be considered.

In Central Asia, *E. multilocularis* was identified in Kazakhstan with a human infection rate ranging from 1 to 10 for every 100,000 persons (Vuitton *et al.*, 2003)

Countries contiguous to Russia have also been affected, including Iran, Iraq, northern Afghanistan, northern India, and Turkey (Yao, 1983). In the Turkish endemic hotbed, AE cohabits with hydatid echinococcosis (Vuitton *et al.*, 2003)

Echinococcus multilocularis cohabits in China with *E. granulosus* and *E. shiquicus*, a new species identified on the Tibetan plain. The study of its mitochondrial DNA permitted the identification of an altogether different species. The species, *E shiquicus,* has not yet been demonstrated to be responsible for echinococcosis in humans (Xiao *et al.*, 2006)

The Russian Federation

The entire territory of the former USSR harbors the parasite (Lukashenko, 1971), and 33 regions are enzootic (Bessonov, 2002). In Siberia, the principal definitive hosts are the polar fox (with prevalence between 25.6%-76.2% according to studies), the red fox (prevalence between 22%-29.3%), and dogs (prevalence between 14%-39%). In the region of Yakutia or the Sakha Republic (in eastern Russia), the prevalence obtained for rural dogs is 18% (Schantz *et al.*, 1995). The prevalence in polar foxes can go as high as 72% in Siberia, where the rate of prevalence in voles exceeds 46%; this number is even greater than that of the bank vole (*Clethriononmys glareolus*) (Bessonov, 2002). Higher rates of infection were also noted in the tundra vole (*Micortus oeconomus*) at 52% and in the brown lemming (*Lemmus sibiricus*) at 21% (Bessonov, 1998).

Infection rates in humans, varying between 0.2%-4.7%, were recorded in the various regions (Bessonov, 2002). Data collected on 20 and 30 year olds demonstrated very high prevalence rates of AE in the far eastern and western regions of Russia (10 sick out of 100,000 inhabitants). Between 1955 and 1986, more than 900 human cases were found in the Russian Federation (Bessonov, 2002). Elevated rates were also discovered in the adjacent regions of Yakutia and certain parts of Kazakhstan (1 to 10 sick out of 100,000 inhabitants). The prevalence was lower in the other regions of the Federation (Bessonov, 1998). However, more recent sets of data are not presently at our disposal.

Japan

The presence of the parasite is well-known in northern Japan, throughout most of the island of Hokkaido, which is recognized as an endemic zone. Sporadic human cases were also reported on other islands (Doi *et al.*, 2003). The species *E. multilocularis* was introduced there in 1924 by the intermediary of parasite-carrying foxes originating from the Kuril Islands (in northeastern Japan). These foxes were imported in order to fight against the proliferation of rodents (Vuitton *et al.*, 2003). From Hokkaido, the parasite spread progressively, covering from 8%-90% of the territory between 1981 and 1991 (Suzuki *et al*, 1996). The presence of the parasite also extended onto the principal island of Honshu (Houin and Liance, 2000), and this phenomenon could worsen due to transportation of infected dogs from the island of Hokkaido (Doi *et al.*, 2003). Disease-control measures led to the elimination of the parasite from the island of Hokkaido, but it was reintroduced with infected definitive hosts (Kamiya *et al.*, 2007). In red foxes and dogs, the prevalence rate rose considerably over several decades and reached a level of 10%-30% (Morishima *et al.*, 2006). Raccoon dogs were also discovered to be infected, and experiments indicate that they are capable of playing a serious epidemiological role. Moreover, this last species has a higher rate of reproduction in comparison to foxes and poses the risk of intensified proliferation under the effects of global warming (Thompson *et al.*, 2006). The rate of infection amongst rodents varies from 4%-22%.

Between 1937 and 1957, 373 human cases were reported (Kimura *et al.*, 1999). In 2000, Oku reported 482 human cases on the island of Hokkaido, which corresponds to an incidence of 48 for a population of 100,000 inhabitants (Oku *et al.*, 2000)

North America

The parasite was first identified in the arctic regions, including Alaska, the Aleutian Islands, and the Canadian Arctic Islands. It was then found in various states in the northern and central part of the U.S., including Minnesota, Nebraska, Illinois, as well as North and South Dakota (Eckert *et al.*, 2000). In the red fox population, the prevalence reached 75% in South Dakota (Hildreth *et al.*, 2000) and over 70% in North Dakota (Rausch and Richards, 1971). In Illinois, the prevalence is between 19%-35%, while Minnesota's was found to be 5% (Leiby *et al.*, 1970). On St. Laurent Island, 12% of dogs were infected (Shantz *et al.*, 1995), and in North Dakota, 1%-5% of farm cats, tested between 1971 and 1976, were found to be infected. In Minnesota, coproantigens were identified in 2.4% of tested farm dogs (Kazacos and Storandt, 1997). The definitive host presenting the highest prevalence is the polar fox (77% on the island of St. Laurent). Prevalence amongst rodents rests between 2%-16%, but can reach up to 80% in certain endemic hotbeds. On St. Laurent, the prevalence ranges between 22%-35% (Shantz *et al.*, 1995)

Between 1937 and 1977, there were only two human cases found on the continent of North America (Eckert *et al.* 2000). Conversely, on the island of St. Laurent, inhabited by only a 1,000 people, 53 cases were diagnosed in a period spanning from 1947 to 1990, which corresponds to an annual incidence of 7 to 98 for 100,000 inhabitants (Shantz *et al.*, 1995).

Between 1990 and 1991, in the endemic regions of South Dakota, blood samples taken from 115 trappers were analyzed by ELISA. While half of these individuals said they had trapped more than 50 foxes in their life, all the results came back negative, (Hildreth *et al.*, 2000)

The *E. multilocularis* species is thus widespread throughout the Northern hemisphere, notably in Central and Western Europe, Central and Northern Eurasia – as far north as Japan - and North America. The expansion of the parasite has been proven in North America and Japan. Even if the situation is rather well-known in certain regions of the world, such as in parts of Europe, northern Japan, the Tibetan plateau in China, and North America, there is a glaring lack of data for other zones.

Euroepan Symposium on Alveolar Echinococcosis, 2010

It is difficult to compare the global situation between the late 1990s and 2010. The maps are indeed mere approximations, and the number of countries where the parasite was researched is not identical on each of the concerned continents (Romig).

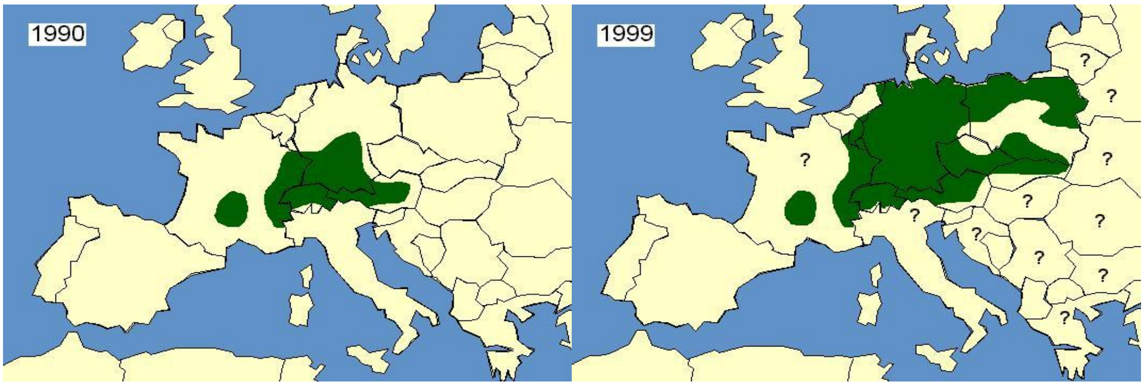
Situation in Europe

Geographic Distribution

The first human cases of AE were diagnosed in Germany in 1852 (Hosemann *et al.*, 1928). The endemic European zones were identified as early as the 1930s. These endemic zones included the southern, northeastern, and western regions of both Germany and Switzerland. Until the end of the 1980s, only four countries were considered endemic: Germany, France, Switzerland, and Austria (Veit *et al.*, 1995). At the end of the 1990s, it became apparent that the parasite had a much more extensive distribution than previously thought, and the list of European countries grew to at least 12: Austria, Belgium, Czech Republic, Netherlands, Switzerland, Denmark, France, Germany, Liechtenstein, Luxembourg, Poland, and Slovakia (Romig *et al.*, 1999; Eckert *et al.*, 2000) (figure 20).

Further discoveries of the parasite in other regions thus appeared highly probable. The endemic areas in the central and eastern parts of Europe, which were previously considered as distinct, were regrouped under a common classification following the detection of *E. multilocularis* in Poland and Slovakia. **It is, however, not possible to determine if the discovery of the parasite in new countries reflects an actual growth and diffusion of the parasite or merely the identification of the parasite's presence in previously unstudied zones.**

Figure 20. Distribution of E. multilocularis in Europe in 1990 and 1999
(according to www.eurechinoreg.org)



Presence of *E. multilocularis*

The assessment of the situation in Europe was drawn up by Eckert *et al.* (2001).

- ✓ Northern Europe

Scandinavia: the parasite was identified for the first time in 1999 in red foxes in Denmark and in rodents on the Norwegian islands of Svalbard in the Barents Sea (Eckert *et al.*, 2001).

- ✓ Western Europe

Belgium: high prevalence rates were recorded among fox populations in the southeastern part of the country; the values are lower in the northwest.

Netherlands: only several foxes tested positive for infection near the German border.

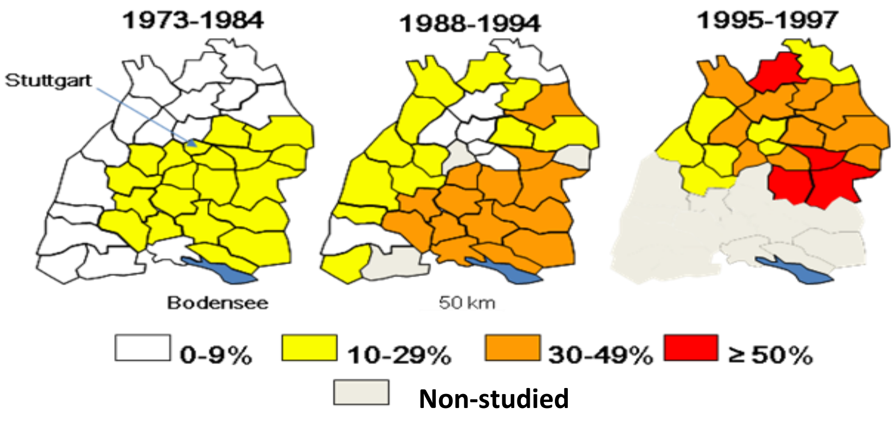
Luxembourg: weak prevalence in foxes.

- ✓ Central and Eastern Europe

Germany: infected foxes were observed in 12 of the 16 federal states. The most elevated rates of prevalence were recorded to the south of the country, as well in several central states. The prevalence of *E. multilocularis* in the red fox in the state of Bade-Württemberg grew between the years 1973 and 1997 (Romig *et al.*, 1997) (figure 21).

Figure 21. Infection Prevalence in the Red Fox by *E. multilocularis* in the State of Bade-Württemberg, Germany

(according to Romig *et al.*, 2002)



Euroepan Symposium on Alveolar Echinococcosis, 2010

In Germany, the prevalence of *E. multilocularis* in foxes has increased since the 1980s in the regions of Bade-Württemberg, Brandenburg, Thuringia, Bavaria, and Saxony (Romig).

In the states of Brandenburg and Thuringia, **the increase in the prevalence since the 1990s has accompanied a spatial extension of the presence of *E. multilocularis***. The prevalence tripled in certain states. In Bavaria, it has reached an average of 52%. **This growth in the prevalence of foxes is accompanied by an increase in human incidence that has passed** from two to three new cases per annum to 10 new cases per annum, as well as by an increase in the prevalence in certain intermediate hosts, notably muskrats.

It must be noted, however, that prevalence is only one necessary element to associate with parasite load and the density of fox populations.

In natural hosts of *E. multilocularis*, the parasite load has multiplied approximately tenfold since the early 1990s.

Several studies have demonstrated a correlation between fox-population density and the prevalence of *E multilocularis*. The 1990s were characterized by a growth in fox population while the 2000s saw a decrease in population and a stable prevalence in certain regions of Germany (Romig).

- Switzerland: *E multilocularis* is present in 21 of 26 cantons with a high average prevalence. The most endemic zones are situated to the north of the Alps.

- Liechtenstein: Foxes carry a high prevalence of infection.

- Czech Republic: the parasite was identified in five regions, each with a relatively high average prevalence.

- Slovak Republic (Slovakia): infected foxes were discovered in the eastern and western regions of the country.

Euroepan Symposium on Alveolar Echinococcosis, 2010

Prevalence can reach upwards of 30% in certain regions (Miterpakova).

- Poland: the average prevalence in foxes is around 2.6%, with prevalence rates higher to the north of the country.

Euroepan Symposium on Alveolar Echinococcosis, 2010

In Poland, for example, the prevalence in red foxes reaches 49.1% in the Carpates region, which borders Slovakia. This prevalence notably grew to the south and east of the country, yet it remained more stable to the west (Karamon).

- Lithuania:

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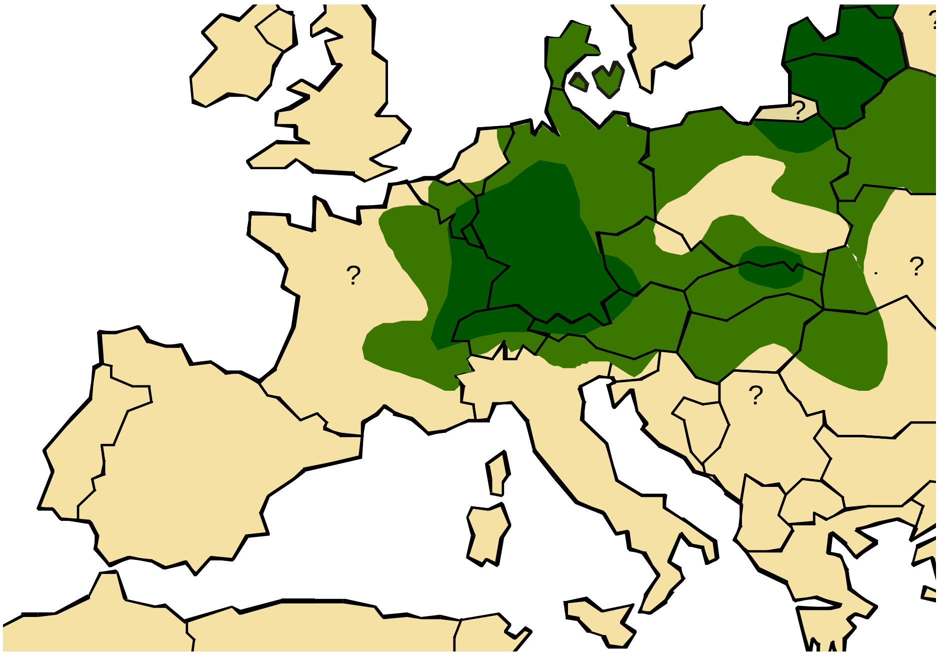
Lithuania is currently part of the endemic zone, with vulpine prevalences able to reach as high as 60% (Sarkunas).

- Assessment of the Present Geographic Distribution of *E. Multilocularis* in Europe

Figure 22. Geographic Distribution of *E. Multilocularis* in Europe in 2010
(according to www.eurechinoreg.org)



Presence of *E. multilocularis* | Hyperendemic Presence of *E. multilocularis*

Based on current knowledge, *E. multilocularis* is present in France (Grenouillet), Germany (Schwarz), Switzerland (Hegglin), Belgium (Vervaeke), the Netherlands (Kortbeek), Austria (Glawishnig), Slovakia (Miterpakova), Slovenia (Soba), Poland (Gawor), Denmark, Belarus, Ukraine, Hungary, Czech Republic, and the Baltic countries (Giraudoux) (figure 22).

During the conference, five European countries were deemed free of *E. multilocularis*. These five include Norway, Sweden, Finland, Malta, and the United Kingdom. It is not possible to be certain of the absence of the parasite in a country. Only a strong probability can be determined following epidemiological studies based on a very large number of samples (Whalstrom).

Data compiled at the end of the 1990s from post-mortem examinations reflect a significant growth in the geographic distribution of the parasite.

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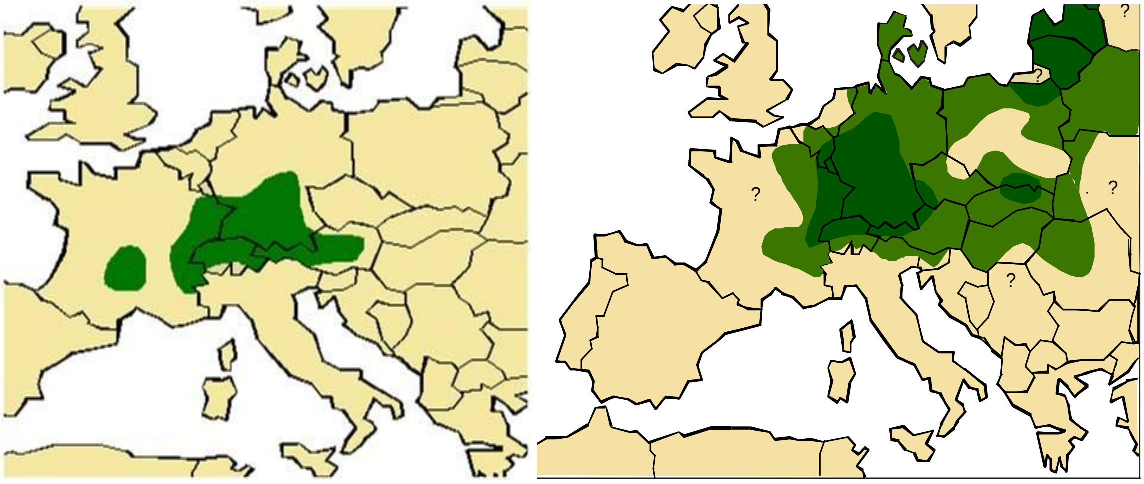
- Evolution of the Geographic Distribution of *E. multilocularis* during the Late 1990s

It is not necessarily clear whether or not the geographic distribution of the parasite has spread during the late 1990s. Indeed, retroactive data do not always exist in the different regions, and the information that has been collected is of a rather variable quality both over time and across countries. The difficulty lies in distinguishing between the newly infected countries and those countries where the parasite was already present, yet had previously been unidentified. Moreover, numerous studies have focused on the existence of human cases in order to identify the presence of *E. multilocularis*, but the recent introduction of the parasite or its minimal presence (low prevalence) could very well be tied to the absence of human cases.

Geographical maps established in 2010 (figure 23) indicate an evolution regarding the distribution of *E. multilocularis* (Romig).

Figure 23. Distribution of *E. multilocularis* in Europe
(according to www.eurechinoreg.org)



1990 2010

Presence of *E. multilocularis* — Hyperendemic Presence of *E. multilocularis*

The parasite is now present in "new" countries, such as Belgium (Vervaeke), Romania (Gottstein), the Baltic countries (Bagrade), Slovakia (Miterpakova), Slovenia (Soba), and Hungary (Gawor).

Echinococcus multilocularis was detected for the first time in 1996 in the Netherlands. The first human case was diagnosed there in 2008 (Takumi). In Belgium, the parasite was observed for the very first time in 1991 in the southeast of the country (Vervaeke).

The parasite was also discovered recently in carnivores from the Baltic countries. It is present in Estonia, Lithuania, and Latvia. In Latvia, it was detected for the first time in foxes, wolves, and raccoon dogs between 2003 and 2005 (Bagrade).

In certain countries, some regions are newly infected. This is the case in Belgium where the parasite spread its reach from the south towards the center of the country. The parasite was discovered for the first time in 2003 in Flanders (Vervaeke).

In the Netherlands, it is expanding towards the northwest (Takumi). In Austria, the entire country is presently concerned (Glawishnig). In France, the expanding zone has spread towards the western part of the country (Combes). In the Netherlands, the geographic distribution of the parasite is spreading 2.7 km per annum towards Limburg and 3.4 km per annum into Groningen (Takumi). In Germany, the geographical distribution of the parasite is extending towards both the north and east (Romig).

Prevalence in E. multilocularis Hosts

- ✓ Red Fox

The percentage of infected foxes is very different when viewed on a European scale (table 8). Indeed, the average prevalence of infection in the red fox, the principal definitive host, varies from 1%-60% (Eckert *et al.*, 2001).

Table 8. Prevalence of Infection in the Red Fox by *E. multilocularis* in Europe
(according to Eckert *et al.*, 2001)

Countries and Regions	Period	Number of infected foxes / foxes examined	Mean Prevalence (%)
Belgium			
Flanders	1996	1/50	2.0
Netherlands	**1996-1997**	**5/272**	**1.8**
Luxemburg	**1990-1992**	**13/255**	**5.1**
France			
Lorraine	1983-1987	112/513	21.8
Doubs	1996	24/39	61.5
Germany			
North and East Brandenburg;	1992-1994	267/6529	4.1
Bade-Württemberg:			
Northwest	1991-1997	706/5365	13.1
Central	1989-1990	47/162	29.0
Southwest and South	1995-1998	2225/6013	37.0
Bavaria	1988-1994	1128/3969	28.4
Switzerland	**1990-1998**	**2217/7457**	**29.7**
Liechtenstein	**1990-1992**	**45/129**	**39.4**
Austria	**1989-1998**	**294/3778**	**7.8**
Czech Republic	**1994_1998**	**214/1528**	**14.0**
Slovakia	**1998-1999**	**6/56**	**1.8**
Poland	**1993-1998**	**76/2951**	**2.6**

Across larger regions, local prevalence rates can reach values higher than the calculated averages. For example, the prevalence rate is 75% in the German endemic hotbed of Bade-Württemberg (Romig *et al.*, 1999), 65% in eastern France, and 35% in west Austria.

Euroepan Symposium on Alveolar Echinococcosis, 2010

The prevalence of *E. multilocularis* is highly variable even within a country's borders (Romig). Very high prevalences can be recorded in small hyper-endemic areas. For example, in Poland, prevalence varies from 0%-41.9% in a mountainous zone (Gawor). Likewise, prevalences vary in Germany between 0%-48.3% across different states. However, the number of foxes tested in each region of the country is quite disparate (Schwarz). In Belgium, prevalence varies from 0% in Flanders to 62% in an endemic hotbed on the French border (Vervaeke). These variations correlate with varying climactic and environmental conditions influencing the ecology of the hosts and the resistance of eggs. For example, in Slovakia, high prevalences found in hyper-endemic hotbeds were compared in relation to the different climatic and ecological conditions in the rest of the country. These hyper-endemic regions are characterized by cold temperatures and high levels of precipitation. Eggs are thus protected here from desiccation (Miterpakova).

Furthermore, data collected up until the end of the 2000s indicate an increase in prevalence in numerous regions where the landscape is dominated by permanent grasslands, such as in Austria, the Netherlands, Poland, and Slovakia (Romig *et al.*, 2005). This was also proven in Germany and France as the result of studies conducted over several years (Romig *et al.*, 1999). High rates of prevalence in foxes were equally discovered in Stuttgart (20%) and Zurich (48%) (Romig *et al.*, 1999; Hegglin *et al.*, 1998).

In many countries, recent information suggests a growth of the prevalence in foxes. This is the case in the Netherlands (Takumi), France (Combes), Austria (Glawishnig), Poland (Karmon), Slovakia (Miterpakova), Lithuania (Sarkunas), and Germany (Scwarz). No study has demonstrated a decrease in prevalence.

Parasite load in foxes varies based on the population concerned. In a 2000 Swiss study that examined 133 foxes, 69% of foxes carried less than 1000 echinococcal worms, the average parasite load was 2997, and only 7.5% of foxes were found to be carriers of more than 10,000 echinococcal worms, with this latter group corresponding to 72% of the total parasite load of *E. multilocularis* (Hofer *et al.*, 2000).

Euroepan Symposium on Alveolar Echinococcosis, 2010

There are many complexities to verifying this elevation in prevalence in Europe, due in large part to the lack of comparability of samples and diagnostic methods. And yet, it is an undeniable fact that all the results are heading in the same direction.

Other regions have shown stable prevalence rates for ten years, notably Austria (Glawishnig), certain parts of Germany (Romig), Slovakia (Miterpakova), Switzerland (Hegglin), certain regions of Poland (Gawor), and eastern France (Giraudoux).

Prevalence allows us to classify countries according to their epidemiological situation as either free of *E. multilocularis*, endemic, emerging, or unknown (Giraudoux).

- ✓ Domestic Carnivores

In infected domestic dogs and cats found in several countries, prevalence is usually less than that which was observed in foxes. In the endemic region of Bade-Württemberg, Germany, there was not a single dog among 145 tested in 1988 that was found to be a carrier of echinococcal worms. In the same region, three studies were led over ten years on 278 cats, with results revealing only a 1.1% rate of infection (EurEchinoReg, 1999). Similar studies carried out in Switzerland reported a prevalence rate of 0.30% in dogs and 0.38% in cats (Deplazes *et al.*, 1999).

- ✓ Intermediate Hosts

At the end of the 1990s, the average prevalence in intermediate hosts was generally estimated as low and inferior to 6% (Eckert, 1998). As in definitive hosts, higher prevalence rates were able to be observed in certain endemic hotbeds. For example, in Switzerland, 11 of 28 examined *A. terrestris* contained the larvae of tapeworms (Schmitt *et al.*, 1997). In the canton of Fribourg, a prevalence of 9% was found in *A. terrestris* (Schmitt *et al.*, 1997).

Several countries discovered high and growing infection rates in muskrats. In the Bade-Württemberg region, prevalence shifted from 0% to 4.1% in 1985 and from 15% to 39% in 1997 (Romig *et al.*, 1999). In the early 2000s, information on the extent to which susceptible species harbor *E. multilocularis* larvae and their importance in the establishment of the parasite life cycle remained limited.

- ✓ Human cases

A network for monitoring and recording human cases, called "*European Echinococcosis Registry*" or "EurEchinoReg," was launched in Europe in 1987. The network groups together 11 Western and Central European countries, along with Turkey. The countries reported **new cases that appeared between 1982 and 2000 and previously diagnosed cases** to this network. They did so on the condition that the cases were confirmed by an adequate method. In total, **559 human cases in Europe were recorded**, 42% of which were French, 24% German, and 21%

Swiss (Kern *et al.*, 2003). Patients were reported in Austria, France, Germany, Liechtenstein, Poland, Switzerland, and Belgium (Eckert and Deplazes, 1999; Kern *et al.*, 2003) (figure 24). Sporadic cases were diagnosed in 2001 in the United Kingdom, Hungary, Greece, and Sweden (Eckert *et al.*, 2001).

Euroepan Symposium on Alveolar Echinococcosis, 2010

In 2010, 1060 human cases of AE were recorded, a figure which represents **a doubling of the number of human cases over ten years in Europe. Today, new countries have been touched by AE, including the Netherlands** (Kortbeek)**, Belgium** (Vervaeke)**, Slovakia** (Miterpakova)**, and Slovenia** (Soba)**.**

In Romania, no human case has yet been confirmed, though it would seem that this is due to problems of methodology. The presence of confirmed human cases in border countries suggests that the probability of the parasite's presence in Romania is relatively high (Gottstein).

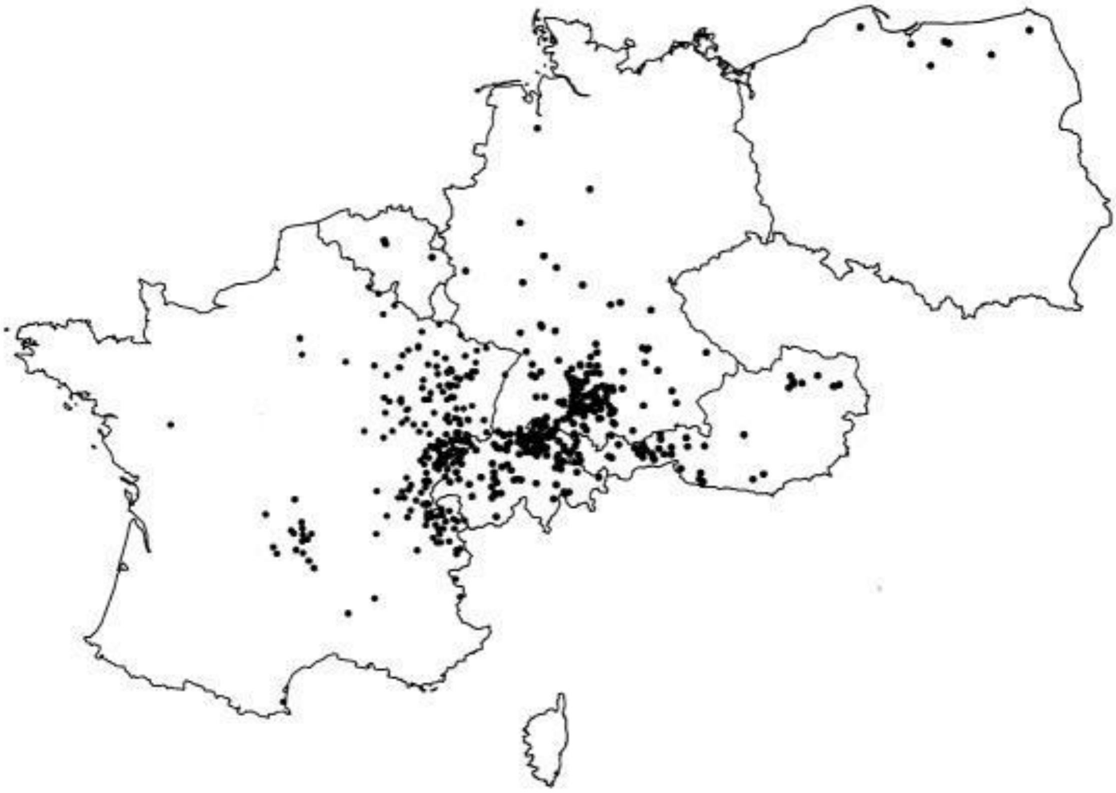
In Belgium, where the parasite was only discovered in foxes in 1991, 11 cases have already been compiled (Vervaeke).

In the Netherlands, three human cases were recently recorded, and only one of these cases constitutes a possible isolated autochthonous case of an immunodepressed individual (Kortbeek).

In Switzerland, the annual recorded incidence until 1997 was 0.10-0.18 for a population of 100,000 (Eckert, 1998). In Austria, between 1985 and 1999, it was around 0.034 per 100,000 persons (Auer and Asprock, 2001). **AE is thus a rare disease in European endemic zones.** However, in certain cantons of Haut-Doubs, the annual incidence can be greater than one per 100,000, and prevalence can reach one per 1000 (Vuitton *et al.*, 2003). Similarly, in a Swiss endemic hotbed, a prevalence of 11.6 cases per 100,000 inhabitants was calculated in 1987 (Gottstein *et al.*, 1987), and prevalence was evaluated at 40 per 100,000 in Bade-Württemberg (Romig *et al.,* 1999)

Once again, average prevalence rates calculated over vast regions are not representative of the actual prevalence in endemic hotbeds. Human cases, which were initially present in only four countries, were listed in seven countries in 2000 (Eckert *et al.*, 2000).

Figure 24. Origin of the 559 Recorded Human Cases by EurEchinoReg
(according to Kern *et al.*, 2003)



The incidence of human cases considerably increased in the 2000s in Germany (Konig), Poland (Gawor), Lithuania (Sarkunas), France (Grenouillet), and Switzerland (Hegglin). In Bavaria, the average incidence is presently 8-10 new cases per annum, as opposed to 2-3 during the 1990s (Konig), and Switzerland saw its incidence increase by a product of 2.5 during the 1980s. **These values can be viewed in relation to the increase in fox population and the increase in the prevalence in definitve hosts.** For 15 years in Switzerland, the increase in human incidence has indeed followed the growth in the vulpine population (Schweiger) (figure 25). In Slovakia, human cases are located in zones of high vulpine prevalence (Miterpakova). However, this elevation in the number of detected cases can also be explained by the implementaiton of screening programs, for example in Poland (Gawor).

In Latvia, since 2006, figures of reported human cases – without distingusing Alveolar or Cystic echinococcosis – have increased (Bagrade).

It is estimated that there are between 20-30 new cases per annum in Germany (Schwarz).

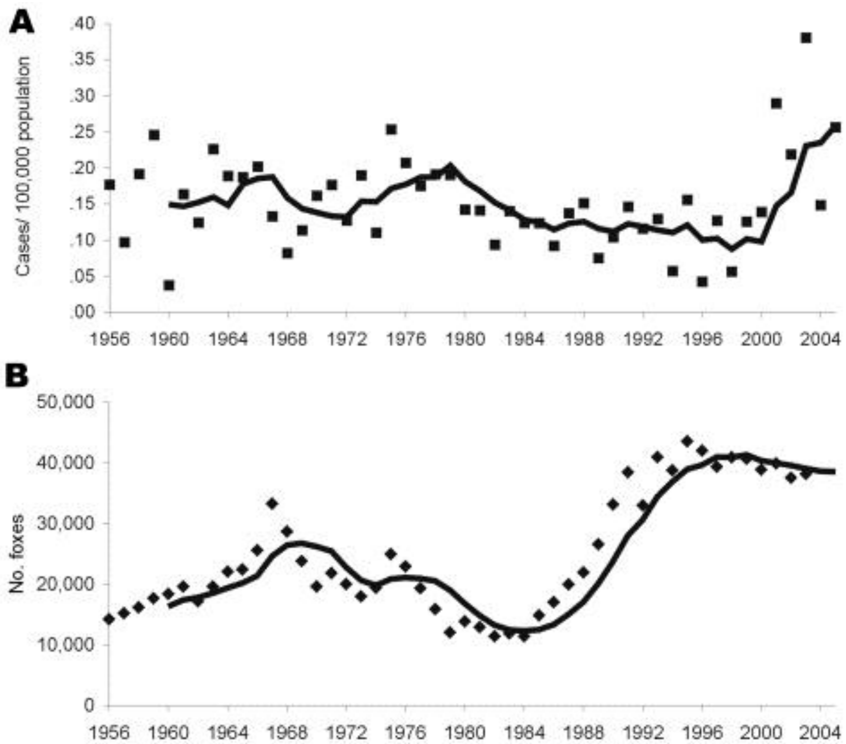
Conversely, incidence is stable in Austria (Glawischnig).

Figure 25.

A. Average Annual Incidence of AE in Switzerland from 1956 to 2004

B. Number of Foxes Killed Annually in Switzerland from 1956 to 2004

(according to Schweiger *et al.*, 2007)



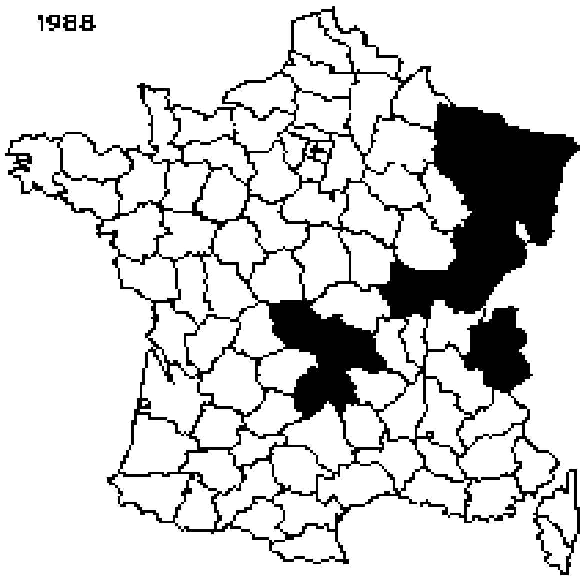
In Germany, the comparison of a number of human cases reported from different sources demonstrated that data sets were not identical and that 67% of cases were not officially reported (Jorgensen *et al.*, 2008).

The increase in prevalence of AE in foxes, the existence of high rates locally, the emergence of human cases outside of known endemic zones, and the extension of the geographic distribution of the parasite in different hosts all suggest that the presence of *E. multilocularis* must be continuously monitored.

France

The distribution of AE in France was first established from inventoried human cases. Starting in the 1930s, 11 human cases have been reported in Haute-Savoie, Auvergne, the northern regions of the country, and above all, the Jura Mountains (eight cases). The endemic hotbed in Lorraine was subsequently added to the list, yet information there changed very little until the 1980s (Petavy *et al.*, 1990). The localization of *E. multilocularis* was thus confined to the northeast quarter of the country and the Massif Central.

Figure 26. Distribution of Red Fox Infection (in black) by *E. multilocularis* in 1988
(according to ERZ, University of Franche-Comté, and ANSES Nancy, BEH 15)



Studies of prevalence in foxes then permitted the establishment of a more precise map. The *E. multilocularis* species was subsequently detected in Savoie (Grivet, 1999), Ain and Doubs (Giraudoux, 1991), Haute-Saône, Vosges (Baudoin and Aubert, 1993), and Ardennes (Depaquit *et al.*, 1998). Therefore, until the 2000s, the known endemic zone was situated in the east, from

Ardennes to Haute-Savoie and along the Belgian, Luxembourg, and German borders – not to mention the endemic hotbed located in Auvergne (figure 26).

- ✓ Fox

Euroepan Symposium on Alveolar Echinococcosis, 2010

ERZ has instituted a program to map *E. multilocularis* in foxes within its 45 member departments in France. This consists of large-scale screening for *E. multilocularis*. In the 2000s, the appearance of human cases in the western part of the country - where the parasite had previously gone unidentified in fox populations - motivated the investigation of *E. multilocularis* in this particular species across the entire territory covered by ERZ. The existence of a link between the extension of human cases and a modification of vulpine contamination was thus the focus of this study.

The success of the project rests in the partnership between ERZ, directing field operations; ANSES-Nancy, which executes laboratory examinations; and the University of Franche-Comté, which handles the spatial analysis of all data. A single standard procedure was developed for the collection of samples and their subsequent analysis. In order to gather the data in the most homogenous manner possible, each department was partitioned into a grid forming one-hundred squares, with a single fox collected for each given square. In five years, 3515 foxes were collected by way of traps and nighttime hunting campaigns over a surface slightly larger than 300,000 km^2 (figure 27). LVD analyzed intestines following the same procedure. The SSCT technique, an optimization of the sedimentation technique, was used in order to evaluate adult tapeworms in the intestines of foxes. Finally, all the results were confirmed by the ANSES laboratory of Nancy (Combes).

Figure 27. Collection of Foxes in 45 ERZ member departments as part of ERZ's Mapping Project

(according to Combes, European Symposium on AE, 2010)

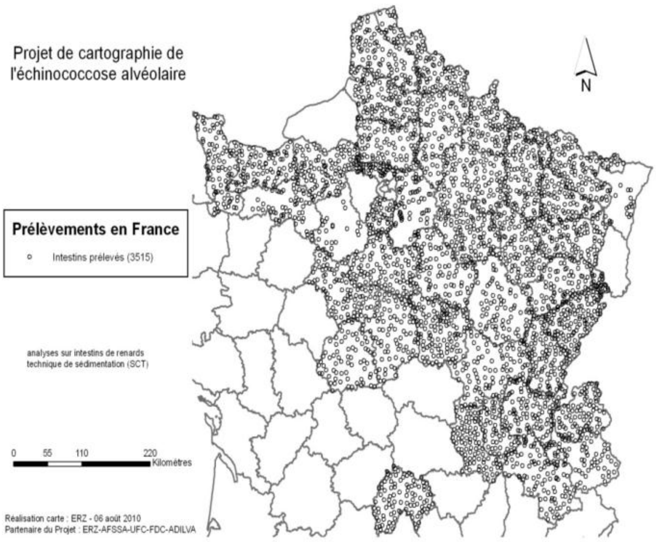
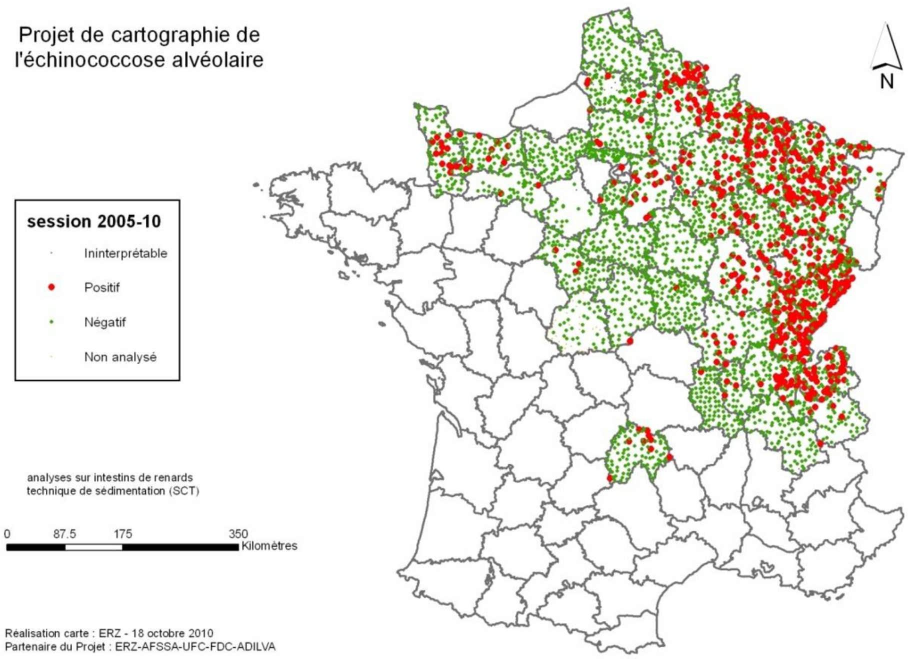


Figure 28. Results of the Investigation of *E. multilocularis* in Foxes, Collected from 45 French Departments during ERZ's Mapping Project
(according to ERZ)



The results indicate that the presence of *E. multilocularis* in foxes is concentrated to the east and northeast of the country, notably in the northern part of the Rhône Alpes, Franche-Comté, Lorraine, and Champagne-Ardenne. The parasite is also present to the west of the Parisian region, in Normandy, and to the south of Auvergne. The endemic zone, historically confined to the eastern half of France and Auvergne, is today much wider, as it has expanded both to the west and to the north (Combes) (figure 28).

The prevalence of infection in red foxes is not uniform across France. Its value has not been stable over the years. Until 1999, the highest prevalence rate, 61.5%, was observed in Doubs (Vuitton, 2006). Between the periods 1884-1986 and 1989-1990, the prevalence rate doubled in Franche-Comté (Grisot, 1990) There is also Haute-Savoie, which recorded a value of 47.3% in 1983 (Contat, 1984).

However, one must prudently take into consideration the increase in observed prevalence. This might indeed be the reflection of an increase in real incidence or instead the increased sensitivity of diagnostic methods to detect AE.

In 2010, the mapping project indicates a prevalence between 0%-65% in foxes in the French departments. The historical endemic zone has a very high prevalence: 51% in Meurthe and Moselle and 49%-65% in Doubs, which shows that the situation was undoubtedly underestimated in France. At the end of the 1980s, this prevalence was 20%-25% in Meurthe and Moselle and 30%-35% in Doubs. Thus, the prevalence seems to have doubled there over the past past twenty years.

Moreover, the former correlation between the presence of the parasite and the existence of permanent grasslands is today under question. Prevalence from 30% to 35% was detected in the countryside of certain particular areas that were not recognized as especially favorable to the presence of rodents or *E. multilocularis*. Said areas are open landscapes devoid of grasslands (Combes).

The increase of prevalence in foxes seems to be strongly tied to the expansion of geographic distribution of the parasite in France and in Germany.

In certain departments in the center of France, very rare foxes were found to be parasitic. For the moment, it is not possible to determine if these areas are emergent zones or if the parasite has been present there for a while, albeit with a low prevalence, which might explain why it was previously undetected. This also applies for departments in western France that have recently seen positive tests.

The same question has been asked in other European countries: is this an actual emergence of a new endemic area for *E. multilocularis* or, instead, evidence of an endemic area that was previously undetected?

- ✓ Domestic Carnivores

The infection of dogs was demonstrated by Contat (1984). At the time, studies over a small number of domestic carnivores (there was an absence of useful tools for large-scale studies) revealed a prevalence of 5.6% among 36 dogs (Contat, 1984) and 3% of 33 cats in Haute-Savoie (Deblock *et al.,* 1989). A recent assay performed using ELISA coprotests revealed an infection rate in canines of 0.3% and in felines, 0.4% (Eckert and Deplazes, 1999).

In France, no infected dog was detected in the highly endemic departments of Meuse and Haute-Saône (Umhang). However, a positive dog was found in Annemasse and Pontarlier (Comte).

In Lithuania, 0.8% of farm dogs were detected positive in certain villages (Sarkunas).

Table 9. Infection Prevalence in Dogs by *E. multilocularis* in Switzerland, Germany, and France

(according to Umhang, European Symposium on *E. multilocularis*, 2010)

Country	Method of Analysis	Number of Dogs Tested	Infection Prevalence by *E. multilocularis* (%)	References
Switzerland	ELISA/PCR	41	12	Gottstein *et al*. 1997
Switzerland	ELISA	660	0.30	Deplazes *et al*. 1999
Switzerland	Flotation/PCR	505	0.20	Sager *et al*. 2005
Germany	Flotation/PCR	17,894	0.24	Dyachenko *et al*. 2008
France	Flotation/PCR	980	0	Dyachenko *et al*. 2008

The observed prevalence in dogs in Germany and in Switzerland is generally low, between 0.1%-0.3%. Nevertheless, it reached 12% in hyperendemic zones (Hegglin) (table 9).

- ✓ Intermediate Hosts

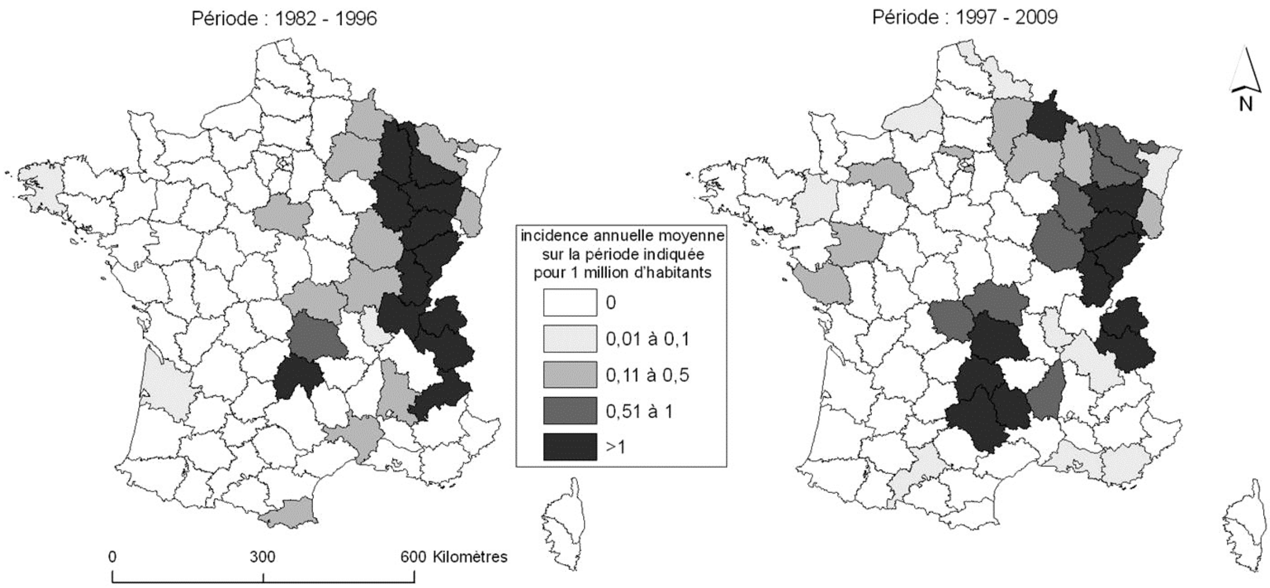
Global prevalence in rodents was inferior to 1% in 1991 (Delattre *et al.*, 1991). However, local values are very heterogeneous, with rates able to reach 15%-20% around the borders of a ploughed field (Gottstein *et al.*, 1996).

Euroepan Symposium on Alveolar Echinococcosis, 2010

Prevalence in intermediate hosts is generally low, but data here can be rather lacking. In this field, research seems to focus only on certain targeted species, responding to precise questions in regions where the prevalence in definitive hosts is high (Giraudoux).

✓ Human Cases

Figure 29. Changes in Spatial Distribution of Human Cases of AE in France since 1980
(according to Bresson-Hadni *et al.*, 2011)



In France, the France Echino network, the collaborative center of OMS, reported 100 cases between 1975 and 1983, which represents a mean annual incidence of 0.5 cases per 100,000 inhabitants (Bresson-Hadni *et al.*, 1994). In total, 258 French cases were recorded between 1982 and 2000 by EurEchinoReg.

French cases recorded between 1982 and 2000 were principally located in Franche-Comté, Lorraine, and Rhône-Alpes (figure 29). Thus, a significant concentration has been identified in the eastern part of the country. Between 2002 and 2005, seventy new cases were declared. In the mid-1980s, a substantial number of patients were found to be positive, yet this number subsequently fell. These values can be explained by the mass-screening efforts carried out during this time period by the Social Agricultural Mutual Fund of Franche-Comté.

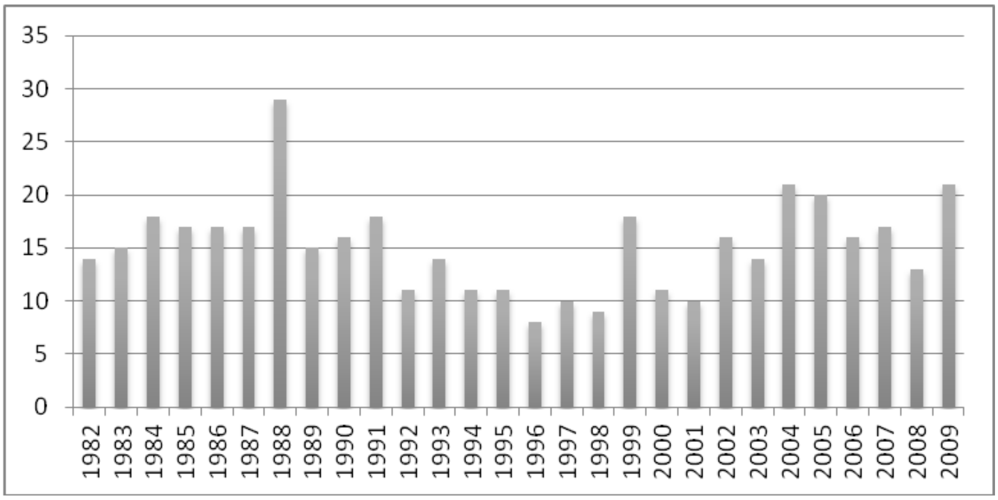
The national annual incidence is on average approximately 14 new cases per annum. Eighty-five percent of patients originated from endemic zones in the center and east of the country; however, the localization of new patients indicates **that endemic hotbeds of transmission are extending towards the south, west, and north** (Piarroux *et al.*, 2006). The erratic nature of foxes can be an explanation for the geographic extension of AE endemic zones and the appearance of cases outside endemic hotbeds. The majority of foxes can travel at least 5-10 km; however, some walk up to 50-70 km. The role of domestic carnivores in dispersing the parasite seems limited, as the rates of prevalence in these definitive hosts are rather low. Regarding rodents, they only displace themselves in small distances (Tackmann *et al.,* 1998). Birds could, as is described for *Taenia hydatigena*, transport eggs over distances greater than 60 km (Torgerson *et al.*, 1995).

Since 2003, the FrancEchino network has been responsible for collecting human cases in France. In 2005, data that had been gathered by the FrancEchino network were analyzed, representing the first long-term data analysis on the subject.

In total, 417 cases were recorded in France up until 2009 with an annual median equal to 15 new diagnosed cases per annum. A concentration of cases has been observed in the departments of Doubs, Haute-Saône, Jura, Vosges, Haute-Savoie, Cantal, and Haute-Marne. These seven departments represent two-thirds of all cases. **Half of these cases were spread across only four departments** (Doubs, Haute-Saône, Jura, and Vosges). **The emergence of a new endemic hotbed in the Massif Central** appears clear.

A mass-screening campaign was led in Doubs between 1987 and 1994, which explains a peak in 1988 (figure 30).

Figure 30. Incidence of AE in France between 1982 and 2009
(according to Bresson-Hadni *et al.*, 2011)



Mean incidence in France is approximately 0.26 cases per annum per 1,000,000 inhabitants. The incidence reaches a maximum of three per 1,000,000 in Doubs.

An increase of the number of reported human cases has been observed since 2009: 29 cases in 2009 and 50 cases expected in 2010. This can be explained by retrospective serological analyses conducted by hospitals.

It is presently difficult to distinguish a stability of incidence in France from an eventual emergence.

An autochthonous case was recently recorded on the island of La Réunion, despite the absence of foxes on the island. The patient was 13 years old and had never travelled before. The hypothesis of contamination from an imported dog from China was formulated in this particular case (Grenouillet).

Euroepan Symposium on Alveolar Echinococcosis, 2010

New cases in western departments of France, where the parasite had never before been detected in foxes, have been added to the historically stable endemic hotbeds to the east of the country. **The distribution of cases, concentrated in the eastern part of the country during the 1990s, has consequently spread westward**. The appearance of these cases outside the historically endemic hotbeds can be attributed to increased human mobility and the extension of the distribution area of infected foxes, as evidenced by ERZ (Combes).

Euroepan Symposium on Alveolar Echinococcosis, 2010

Franche-Comté

Though the mean incidence remains stable at 5.5 new cases per annum in this region, the prevalence rates of the department have increased since the 1980s.

Dividing our period of study into three decades, a decrease in prevalence was detected in Doubs, whereas an increase in prevalence occurred in the Haute-Saône (Giraudoux).

Over short periods of time, prevalence fluctuated from year to year. In Slovakia, the variations in prevalence and parasite load in the red fox were attributed to climactic conditions (Miterpakova).

Franche-Comté, along with Lorraine, represents the first region in France where *E. multilocularis* was studied. In the 1980s, a grouping of human cases was shown to exist in these endemic hotbeds.

Statistical analysis uncovered a modification in the spatial distribution of the parasite. The grouping of cases in an identified cluster, as seen in the 1980s, no longer holds true, as the cluster has presently dissipated, and the disease has spread evenly in all directions. This expansion first followed a path characterized by abundant grasslands, but presently, it is not clearly tied to this geographic characteristic. **These changes indicate a modification of the epidemiological situation on a regional scale (Giraudoux).**

The geographic distribution of the parasite is increasingly extended, and its prevalence has increased in most countries within the endemic zone. The current state of knowledge provides no indication that these developments are going to come to an end.

In the Netherlands, a modeling of the extension of *E. multilocularis* distribution reveals that the parasite's front is advancing approximately three kilometers per annum on average. At this speed, models predict a cumulative rate equal to 15 human cases by the year 2030 in Limburg, where the first case was diagnosed in 2008 (Takumi).

In Belgium, a country situated on the border of the European endemic zone, a spatial analysis indicated decreasing prevalence in foxes from the southeast towards the northwest of the country. The existence of a gradient of prevalence in the northern direction indicates that the parasite continues to extend its geographic distribution. However, the speed of this extension is still unknown. The existence of this gradient is tied to the migration of infected foxes into new regions, which enables the establishment of a sylvatic cycle. No infected fox, however, has yet been found in Flanders or Brussels, both of which are situated in the center of the country (Vervaeke).

Fox populations continue to grow in Europe, and the discovery of new host species of the parasite is favorable to its continued expansion, as well as to the establishment of a life cycle in new regions.

Therefore, the conglomeration of necessary conditions for an *E. multilocularis* life cycle to occur is present in Europe, and it is probable that the parasite will continue to spread in the years to come.

The Importance of Alveolar Echinococcosis

In Animals

Definitive hosts are blood carriers of the disease. Even in highly endemic zones, foxes do not develop clinical signs and function very well despite elevated parasite loads. *E. multilocularis* thus circulates silently in its natural definitive hosts without disrupting their way of life. In natural intermediate hosts, the development of protoscolices is rapid and leads to the weakening of rodents, which, due to their weakened state, become easy prey for foxes. The perpetuation of the life cycle is thus assured.

In Humans

AE is considered to be one of the most serious zoonoses in temperate and arctic regions of the northern hemisphere. AE behaves as a slowly evolving liver cancer. In Europe, it affects men and women in equal proportion. The disease is more often diagnosed late, up to 10-15 years after infection, and the average age of diagnosis is around 52.5 years (Kern *et al.*, 2003). The number of human cases in France remains undoubtedly undervalued, which results from diagnostic difficulties and the frequent confusion of AE with liver cancer. In France, diseases reside principally in the east, though some cases were diagnosed in Auvergne, the northern part of the country, and the Midi-Pyrénées (Vuitton *et al.*, 2009).

- ✓ Clinical Expression

The first symptoms correspond to pain in the hypochondria or in the epigastrium. Cholestatic jaundice is frequent, as well as constant hepatomegaly.

Parasites in their larval form settle in the hepatic parenchyma and then invade the bile ducts and the vascular network. Metastases cause the virus to spread into the bones, the brain, the lungs, the adrenal glands, the large intestine, and the right atrium (Vuittion *et al.*, 2009). Though exceptional, there also exist purely extrahepatic ganglionic forms of the disease, caused by an extradigestive contamination from the bite of an infected carnivore (Bresson-Hadni *et al.*, 2005).

- ✓ Diagnosis

As a first measure, diagnosis begins with ultrasonography (Bresson-Hadni *et al.*, 2005). Computed tomography (CT) scans and magnetic resonance imaging (MRI) allow for the assessment and follow-up therapy of patients. Confirmation takes place by specific serological testing (Vuitton *et al.*, 2009).

The disease is sometimes only discovered through liver function tests or following an ultrasound that has been given to a patient complaining of abdominal pain. The latter is the most frequent way the disease is diagnosed; such diagnoses allow for the discovery of asymptomatic forms of the disease, which have a greater chance of being effectively treated, as opposed to symptomatic forms (Vuitton *et al.*, 2009) The fortuitous detection of hepatic lesions during surgery is also possible (Bresson-Hadni *et al.*, 2004).

- ✓ Treatment

While incidence may be low, the disease is fatal in the absence of treatment. Treatment is intensive and expensive, and a complete recovery is rarely achieved. It consists of mass reduction surgery and chemotherapy. The lesion must be entirely excised, at which point the patient must undergo chemotherapy for at least two years. This is only possible when the diagnosis is early. The molecule most often implemented is albendazole, which is parasitostatic in the majority of cases. It thus stops the evolution of the parasite without destroying it (Bresson-Hadni *et al.*, 2005). At this point in time, there is no chemotherapy that is fully satisfactory in treating for *E. multilocularis* (Vuitton, *et al.*, 2009)

In patients diagnosed late, radiology and interventionist endoscopy are implemented, and patients then undergo long-term chemotherapy using albendazole or mebendazole – in some cases for life. Hepatic transplantation is carried out exceptionally for those sick individuals with very symptomatic forms of the disease. Between 1983 and 1993, 24% of patients could benefit from a curative ablation compared to 3% between the years 1973 and 1983 (Vuitton *et al.*, 2009). Without appropriate treatment, patients die within fifteen years in almost all cases (Charbonnet *et al.*, 2004). There have been some cases of self-recovery by calcification and encapsulation of the early-stage lesion (Gottstein *et al*., 2001).

Over the past 20 years, the progress of medicine has considerably improved the survival rate and quality of life of patients. Indeed, in 1970, the life-expectancy of a patient of 54 years of age was reduced by 18.2 years on average; in 2005, this reduction was limited to 3.5 years (Torgerson *et al.*, 2008).

In Germany, the cost of life-long treatment was estimated at $300,000 per patient per annum (Romig *et al.*, 1999) In France, the cost for taking care of a patient is roughly €108,800 (Vuitton *et al.*, 2009)

In Doubs, a study was conducted to compare the cost of screening an endemic region with the cost of treating the disease at its symptomatic stage (Bresson-Hadni *et al.*, 1994). The total cost, including diagnosis and treatment, was shown to be lower for patients who had been tested.

- ✓ Prevention

It would be unrealistic to foresee the implementation of a vaccine against human AE due to the rarity of the disease. In addition, none of the vaccination trials were conclusive.

Given this particular impasse, preventive efforts are absolutely necessary. This requires information of populations concerning risk factors and systematic screening of the most exposed individuals, such as agricultural workers. A prolonged stay in an endemic zone must be taken into consideration in an epidemiological diagnosis. Several Japanese studies demonstrated that mass-screening programs enable the detection of the sickness in the nascent stages of its development, which increases the chances of successful curative treatment (Suzuki *et al.*, 1996).

In summary, since the beginning of the 1990s, significant changes appeared in global epidemiological data, notably with the discovery of a Chinese endemic hotbed and the extension of the European hotbed towards the east and north. Some cases were reported in Northern Africa, but they were never verified (Vuitton *et al.*, 2009). In France, in the beginning of the 2000s, it appeared as if the geographic distribution of *E. multilocularis* was in expansion and was not restricted to the east of the country (Boucher *et al.*, 2001). Regarding the prevalence of the disease, it grew in Central Europe. These trends were confirmed by recent data brought to the European Symposium in December of 2010.

4.2. Analytic Epidemiology

Sources and Modes of Transmission

- ✓ In Definitive Hosts

The infection of carnivores is directly linked to their dietary behavior, for they become infected **by ingesting contaminated intermediate hosts** whose liver contains hydatid larvae. In order to become infected, the larvae must be fertile. Fertility takes place after 45-50 days (Contat, 1984). Micromammals constitute nearly a quarter of the red fox's diet. Hunting dogs, dogs who are occasionally allowed to roam free, and cats can regularly consume these micromammals.

- ✓ In Natural Intermediate Hosts

Micromammals become infected **orally through ingestion of free-living *E. multilocularis* eggs** in the outside environment. Ingestion of five embryophores suffices for the development of lesions (Contat, 1984). Eggs can be found on leaves or roots of vegetables. In this case, intermediate hosts ingest eggs at the same time as they ingest vegetables. Burrowing animals can also be contaminated during the construction of tunnels while using contaminated soil.

- ✓ <u>In Humans</u>

The existence of a long asymptomatic period, 5-15 years, makes it difficult to determine the origin and conditions of contamination. Given that humans are not the best host for *E. multilocularis*, it appears as if repeated contacts over long periods of time would be necessary for the development of larvae.

Like rodents, humans are contaminated **by oral ingestion of *E. multilocularis* eggs**. They may become infected through food **consumption**, such as from plants gathered from ground that has been contaminated by feces (dandelions, berries, mushrooms, lettuce, vegetables), or through consumption of contaminated water (Vuitton *et al.*, 2006). **Oral contamination can also occur due to a lack of hygiene, such as not washing hands** after handling pets whose fur may contain eggs. Indeed, infected dogs or cats can deposit eggs on their fur after having licked their perianal region. Additionally, certain dogs have the habit of rolling in the feces of foxes. The handling of foxes, particularly by hunters or trappers, also represents a risk. The same applies with handling soil, droppings, or contaminated objects (such as a plate licked by a domestic carnivore). Human infection translates into a higher contamination of the environment (Deblock *et al.*, 1986)

Predisposing Causes

High resistance of the eggs in the outside environment and their **directly infective** nature are two factors favorable to the infection of intermediate hosts.

Larvae of *E. multilocularis* can also survive several days after the death of the intermediate host. In fact, the protoscolex maintains its viability for several weeks to several months at temperatures between 4°C and 15°C (Euzeby, 1971). Therefore, carnivores can likewise become infected by consuming dead rodents.

The spreading of eggs by water run-off; trampling of cattle; and earthworm, slug, and bird activity promotes the contamination of rodents, which are both vegetarian and non-coprophagous (Henry, 1984).

Non-fenced gardens that are accessible to carnivores facilitate their ability to defecate thereon and to hunt rodents therein (Prost *et al.*, 1989). The risk of contamination of vegetables is higher in vegetable gardens near a path or road and in periods of high-vole density (Giraudoux, 1991). Thus, non-fenced crop cultivation presents a non-negligible risk for humans, specifically as it relates to **the raw consumption of wild plants**.

The **proximity of humans to domestic carnivores** represents another non-negligible predisposing cause. Patients reported by EurEchinoReg were for the most part (70.5%) owners of domestic carnivores or were in regular contact with them. The absence of deworming efforts for carnivores, the use of ineffective molecules against *E. multilocularis*, or a prolonged delay between successive dewormings all represent additional predisposing causes. Hunting, free-ranging, or country dogs are those that are most at risk. Higher prevalences are observed in domestic carnivores that have regular access to infected rodents, notably in endemic zones. Furthermore, in a Swiss endemic hotbed, a prevalence of 12% was recorded among 41 dogs. **In Germany, owning a free-ranging dog or one with access to rodents was identified as a risk factor** (Kern *et al.*, 2004).

For a long time, AE was considered **a rural disease in relatively mountainous areas** (Vuitton *et al.*, 2009). Agricultural workers represent the most exposed category of humans, constituting 40% of all cases. There are also tradesmen, merchants, and other professionals, notably veterinarians, taxidermists, and pet groomers, which represent 22% of all cases. Inhabitants of cities are, however, more frequently affected than they were in the past, in large part due to their increased participation in “nature activities” (Kern *et al.*, 2003). An investigation on patients’ professions in Franche-Comté revealed that members of all professions could become infected. In this investigation, no forest ranger or game warden was sick, which confirms that this is more of a disease along the fringes of woods, as opposed to one strictly of the forest (Bresson-Haddni *et al.*, 1997). **In more than 60% of cases, patients had**

some connection with agricultural activity, such as logging, gardening, or hunting (Kern *et al.,* 2003).

Unlike Cystic echinococcosis (CE), there is generally only one case in a given family. An epidemiological constant applies to all patients of AE: a prolonged stay in an endemic zone.

Foxes have the tendency of defecating on headlands, such as earth mounds, so as to mark their territory. This encourages contact with burrowing animals as they rework the ground. Additionally, foxes defecate in proximity to hunting grounds, which initially increases the probability of rodent infection and subsequently the risk of contamination of other foxes (Herrenschmidt, 1984). High densities of feces are found near the edges of forests, the place with the highest observed prevalence amongst rodents (Delattre *et al.*, 1991)

Micromammals with parasites have the tendency to develop a behavioral pattern of fleeing the burrow, which heightens both the vulnerability of said mammals, along with the risk of transmitting the parasite (Delattre *et al.*, 1988).

Zones with high-vole density can temporarily attract large numbers of carnivores and, in so doing, increase the intensity of parasitic flux in certain areas (Boucher *et al.*, 2001). It was demonstrated in the Doubs department that high densities of *A. terrestris* population constituted a risk factor for human infection (Viel *et al.*, 1999). The prevalence of disease in humans coincides with areas of rodent outbreak, as these areas are themselves influenced by environmental characteristics (Giraudoux *et al.*, 2003). For example, the conversion of cultivated land into permanent grasslands and the deforestation of mountains are factors capable of increasing the population of micromammals, thus strengthening the intensity of the disease's transmission (Wang *et al.*, 2006)

In France, the oral rabies vaccination of foxes, combined with an outbreak of rodents, led to **a significant growth in fox populations** in the 2000s (Artois, 1997). No method permits the exact determination of fox population density. Estimations are calculated from the number of foxes killed annually on roads or from hunting (Lloyd *et al.*, 1976). Furthermore, ERZ noticed a tenfold increase in the Kilometric Abundance Index (KAI) of foxes between 1986 and 1996 in the department of Ardennes. Between 1980 and 2004, KAI went from 0.7 to 2.4 after a study was conducted on 30 French departments (ERZ, 2005). Therefore, due to the intensive disease-control campaigns conducted upon foxes, this species has reoccupied an ecological niche that had been empty in the 1980s. In Switzerland, a negative correlation was shown between the number of foxes killed from hunting and the prevalence of *E. multilocularis* in definitive hosts (Ewald, 1993).

This increase in fox population was also accompanied by a behavioral modification. **Foxes are increasingly moving closer to cities** and find themselves in close proximity to urban centers. They can therefore contaminate public gardens and playgrounds. This proximity to humans considerably increases the risk of infection, and cohabitation between wild and domestic hosts poses the risk of leading to the establishment of a local parasite life cycle (Deplazes *et al.*, 2004). This phenomenon, which manifested itself in the United Kingdom during the 1940s, has been observed in continental Europe (Hofer *et al.*, 2000) and in Japan (Tsukada *et al.*, 2000). Foxes are also increasingly observed in the daytime (Chautan *et al.*, 2000). Infected foxes were listed and classified in Zurich, Stuttgart, Geneva, Copenhagen, Pontarlier, Annemasse, and Annecy (Hofer *et al.*, 2000; Robardet *et al.*, 2008). The gradient of prevalence is shifting from the periphery to the center of cities, where prevalence can reach 10%-20%. The limited amount of habitats for rodents at the center of towns explains this gradient. Fox populations can reach greater densities there than in rural zones due the abundance of anthropogenic food (Contesse *et al.*, 2004). The zone possessing the highest amount of risk is on the peripheries of cities since foxes and rodents are both present there in high densities (Robardet *et al.,* 2008)

In a park in the city of Zurich, *E. multilocularis* larvae were found in 47% of rodents of the *A terrestris* spp. (Hofer *et al.*, 2000). This indicates that an urban cycle exists and that contamination by way of *E. multilocularis* is not simply a danger among rural populations. A retrospective study conducted in Switzerland over the course of 50 years showed that changes in the incidence of human cases were preceded by a parallel evolution of fox-population density ten years earlier. Despite the establishment of the parasite in an urban zone in Germany, the infection risk remains nevertheless correlated to rural life (Schweiger *et al.*, 2007)

Receptivity and Sensitivity Factors

Intrinsic Factors

- ✓ In Definitive Hosts

Species type represents a major factor concerning receptivity. Foxes and dogs are highly receptive to *E. multilocularis*, whereas cats are much less so (Thompson and Eckert, 1983). Red foxes constitute by far the most receptive species. They can hold up to 200,000 echinococcal worms (Bourdeau and Beugnet, 1993). Dogs allow for, under equal infection conditions, the development of eight times as many echinococcal worms as cats.

Receptivity is higher in young animals (Contat, 1984).

Diet also represents an important factor of receptivity. The fox exhibits a preference for consuming rodents living in open areas.

- ✓ In Natural Intermediate Hosts

Different species of micromammals do not share the same level of receptivity. *A terrestris* is a poor epidemiological host for *E. multilocularis*. In fact, 89% of parasitic livers in *A.*

terrestris harbor larvae without protoscolices, thus rendering them infertile. With regard to fertile larvae, they only possess a small amount of protoscolices, which can only lead to very low infection rates (Petavy and Deblock, 1983).

M. arvalis and *C. glareolus* are better intermediate hosts. In definitive hosts, they lead to infections by several hundred to several thousand of echinococcal worms (Petavy and Deblock, 1990). *M. arvalis* is receptive up to 100% and enables a rapid maturation of the parasite in six to eight weeks (Vogel, 1960).

The muskrat is an especially receptive intermediate host (Euzeby, 1996). It is, however, very rarely hunted by carnivorous animals due to its aquatic lifestyle, its corpulence, and its ability to defend itself. This species represents a sentinel since its infection acts as a bioindicator to the presence of parasitic eggs in the environment. The same may be said of the Alpine marmot.

The receptivity of rodents depends upon their age; maximum receptivity occurs in animals of less than two months old.

Ecology and behavior also constitute non-negligible factors influencing receptivity. Certain rodents live near borders, and others inhabit open spaces, the latter of which represent very accessible prey. *A. terrestris*, for example, has the habit of using its teeth to arrange molehills, soil on which foxes may have defecated, which thus increases its risk of infection.

✓ In Humans

The incubation period is 5-15 years, and the disease becomes manifest in humans between the ages of 30 and 70; the predominant age class affected is between 60-70 years old (Grisot, 1990). The median age of first diagnosis was around 56 years old between 1982 and 2000 (Kern *et al.*, 2003).

The disease affects both genders equally. Between 1982 and 2000, patients included 46.2% men and 53.8% women (Kern *et al.*, 2003)

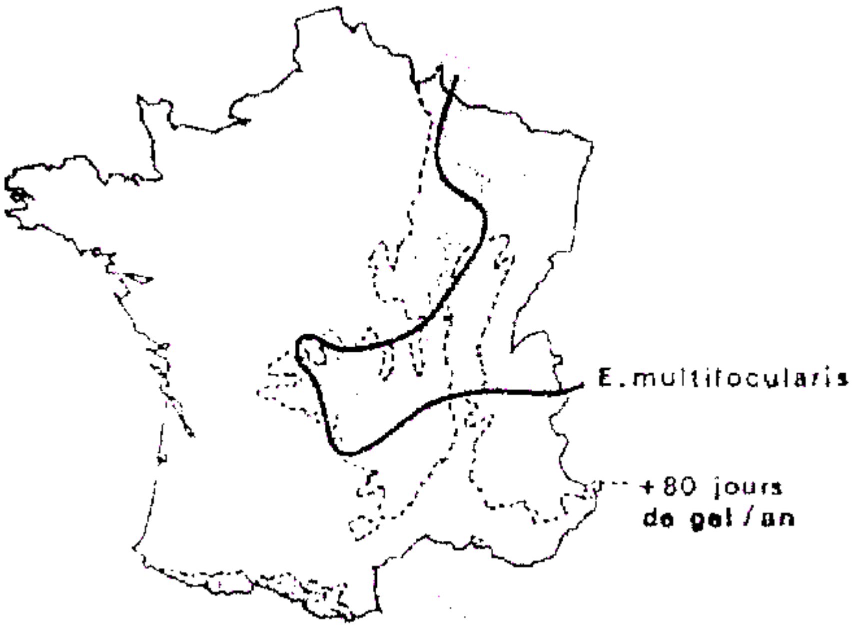
Manifestations of the disease can be accelerated following an immunodeficiency, such as an infection by HIV (human immunodeficiency virus) or by immunosuppressive therapy after transplantation (Bresson-Hadni *et al.*, 1999; Sailer *et al.*, 1997).

The contrast between, on one hand, the wide distribution of *E. multilocularis,* combined with high prevalence rates in foxes in certain endemic hotbeds, and, on the other hand, the low incidence of human cases suggests that there are certain factors limiting the risk of infection. This could very well be an immunogenetic predisposition to resistance (Gottstein *et al.*, 1995)

Extrinsic Factors

Geomorphological and climactic factors influence the survival and transmission of *E. multilocularis* (Viel *et al.*, 1999). The distribution of *E. multilocularis* corresponds to geographic areas with a cold, rainy climate (Aubert *et al.*, 1987; Pesson and Carbiener, 1989), such as Haute-Savoie, Massif Central, and Ain (figure 31). The parasite, however, disappears above a certain altitude (Depierre, 1999; Aubert *et al.*, 1987), which proves that there are factors other than temperature and precipitation at play. For example, in the Alps, Vosges, Jura, and Massif Central, fox carriers were found on calcareous massifs, but not on crystalline massifs (Depierre, 1999).

Figure 31. *E. multilocularis* Portage and Hibernal Severity in France
(according to Boucher, 2001)



Vegetation represents another important factor. Studies conducted in Alsace (Pesson and Carbiener, 1989), in Doubs (Grisot, 1990) and in Haute-Savoie (Prost, 1988) revealed that infected foxes live in oak-hornbeam forests or in beech-fir forests, which are typically humid, as well as hilly and mountainous (Boucher, 2001).

The transmission of the parasite takes place neither in forests nor in vast cultivated plains, but rather, in **areas with copious permanent grasslands** (Depierre, 1999; Pesson and Carbiener, 1989) with relatively significant isolated wooded areas (covering 35%-40% of the total surface). Spaces where the ground's surface has undergone modifications create conditions in which eggs are buried underground, in such a way that borders of ploughed fields, stretches of grass used as passageways for bovine (cattle-rearing regions), and zones contiguous to wooded areas all become linked with high-infection rates among rodents (Giraudoux, 1991). Conversely, the transformation of grasslands into developed zones in lowland regions is unfavorable to the stability of the parasite (Vuitton *et al.*, 1990)

In summary, regions harboring *E. multilocularis* have the following characteristics: elevated, rich in permanent grasslands, cold and humid in climate, and low-mountainous or hilly-mountainous, with oak-hornbeam or beech-fir forests.

Euroepan Symposium on Alveolar Echinococcosis, 2010

- Human Infection Risk Factors

The EchinoRisk project has clarified the risk factors of AE in Europe, along with updating data.

The majority of patients exhibit three to four common risk factors, but the long duration of time from contamination to the manifestation of clinical signs poses difficulties in determining the mode of infection (Grenouillet).

- ✓ Age

Euroepan Symposium on Alveolar Echinococcosis, 2010

The median age of diagnosis is 59.4 years old (figure 32).

The incidence of human cases increases with age up to approximately 60 years old (Grenouillet).

✓ Gender

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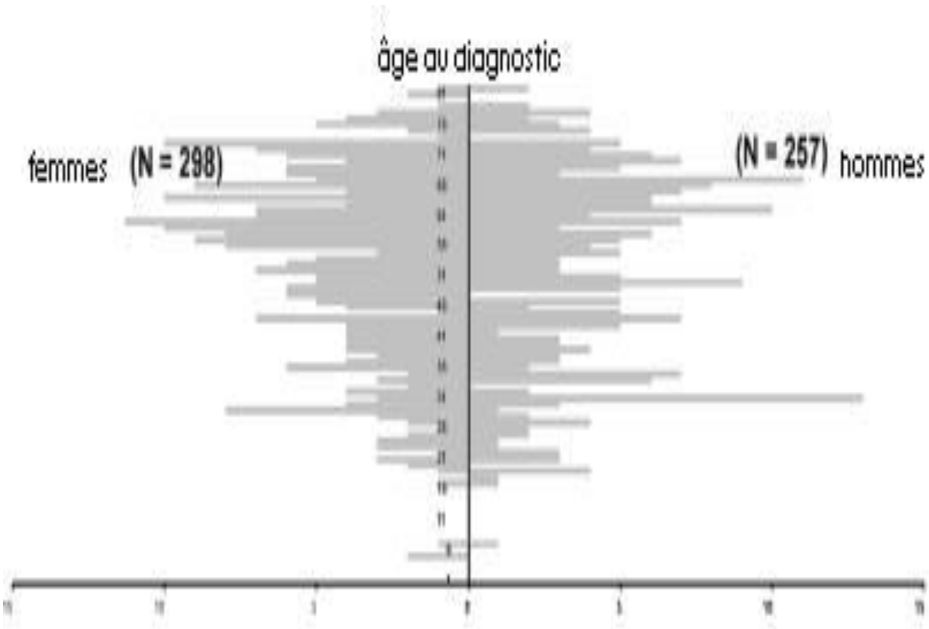
At present, women in Europe seem to be generally more affected than men (figure 32). There are five women for every four men (Gottstein). Nevertheless, this predisposition in women has only been verified unequally across different countries.

In Switzerland, women are presently more at risk (Schweiger). Similarly in China, more than two out of every three cases concern women, and in Lithuania, 60% of cases were of women. This could be explained notably by behavioral differences, such as gardening practice or the care provided to dogs (Boufana).

Conversely in France, gender does not constitute a risk factor, as the gender ratio is roughly one to one (Grenouillet). In Franche-Comté, the proportion of men and women has been constant since the 1980s: 52% of men compared to 48% of women (Giraudoux)

Finally, the inclusion of gender as a factor of risk has not been fully clarified, and it would appear instead to be due to behavioral differences (Grenouillet)

Figure 32. Age of Diagnosis and Distribution by Gender of Patients Infected by AE Recorded in the European Registry
(according to Kern *et al.*, 2003)



- ✓ Profession

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Even if agricultural workers still represent the most affected social-professional category of humans, they are becoming less and less numerous. On the other hand, other professions, such as the merchant and retail profession, are becoming more and more of concern. This can be viewed in relation to the behavioral modifications of foxes as they increasingly approach cities.

In France, a third of the patients consist of farmers and loggers. Ninety-five percent of patients ate berries or raw vegetables gathered in nature. This percentage must nonetheless be considered with a certain degree of reservation since this practice is widespread, and it does not necessarily constitute a risk factor. It would be necessary to control for this set of data with a case-control study or by comparing it with the standard population. Twenty-two percent of patients had been in contact with a fox - either dead or alive - and 16% were hunters (Grenouillet).

- ✓ Location

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Inhabiting an endemic zone of the parasite constitutes a significant risk factor.

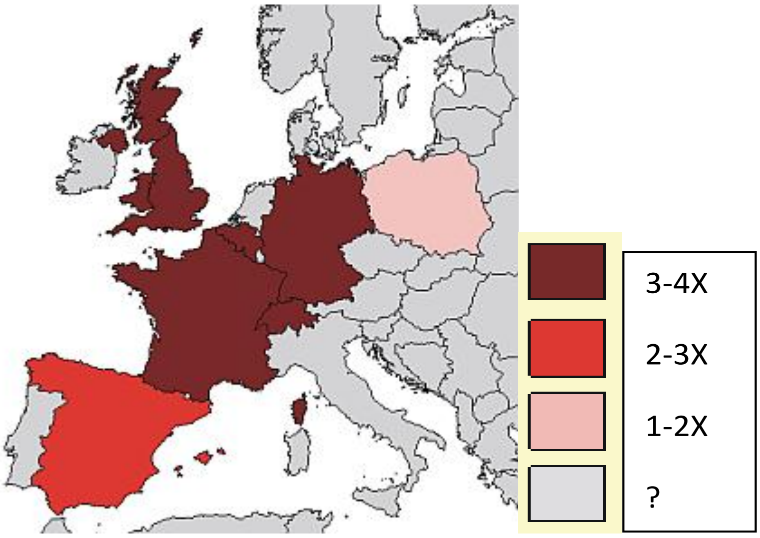
In France, among the twenty patients living outside the endemic zone, all those who provided epidemiological information had previously resided in an endemic zone. Nevertheless, it is still not possible to pinpoint the exact location of their contamination.

A short stay in an endemic zone would not be sufficient to contract the disease. Repeated exposure to *E. multilocularis* would be necessary in order for the parasite to proliferate in the liver (Grenouillet).

- Increase in Fox Population

Throughout Europe in the 1980s, there was an increase in fox populations correlated with anti-rabies vaccination campaigns. This increase was observed in France, Slovakia, Belgium, Germany, Netherlands, Poland, Lithuania, and Switzerland (figure 33). **It would seem that the density of foxes is a significant risk factor connected to the level of endemicity.** This has been indicated most notably by the observed correlation in Germany between population density and prevalence of the parasite. However, the geographic range of foxes is appreciably more extended than that of the parasite, and the relation between fox density and prevalence has not been confirmed in urban areas. Indeed, the largest density of foxes has been observed in the center of cities, yet the prevalence of *E. multilocularis* there is lower than it is in the surrounding countryside (figure 34) (Deplazes *et al*., 2004). Thus, the risk caused by fox population density has not been fully clarified and is dependent upon location (Romig).

Figure 33. Growth in Red Fox Populations in Europe between 1985 and 1999
(according to Chautan *et al*., 1999).



In France, ERZ's mapping project did not allow for a relationship to be established between *E. multilocularis* distribution and fox-population density. Indeed, there are no statistical results explaining the increase in populations. Rather, the project provided more general observations. For example, in certain departments, foxes multiplied tenfold since the disappearance of rabies (Combes).

In Belgium, foxes were eradicated in Flanders in the 1980s out of fear of rabies. Today, the combination of hunting restrictions, conservation efforts, and the adaption of foxes to urban areas explain the reappearance of this species in the northern part of the country. There, the parasite has not yet been detected, but its range is extending in that direction (Vervaeke).

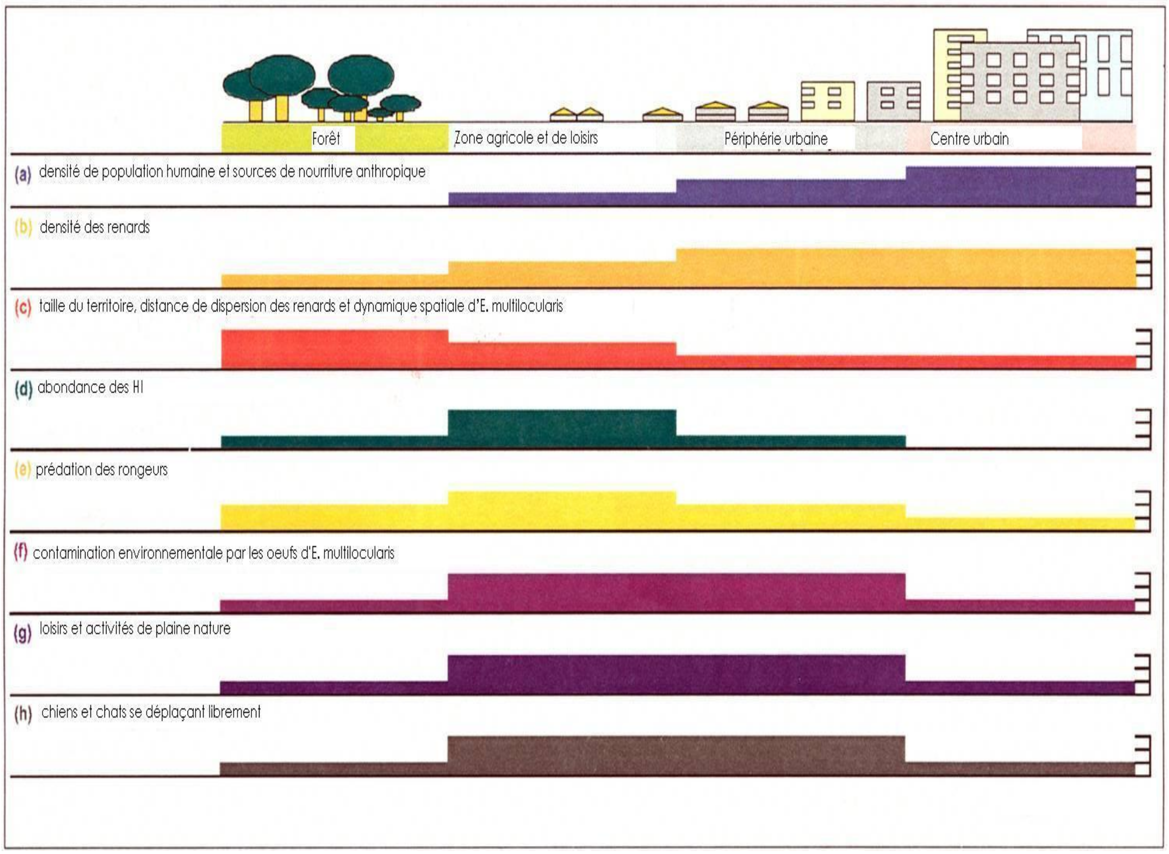
In Switzerland, the increase of human cases in the 2000s seems to follow the increase in vulpine population observed in the mid-1980s (Hegglin).

- Urban Foxes and the Establishment of an Urban Cycle

Foxes are regularly observed in several European cities, where their presence has become more and more abundant over time, notably in Zurich (Hegglin), Maastricht (Romig), Nancy (Raton), Berlin (Romig), and Brussels (Vervaeke).

Figure 34. Distribution of Intermediate Hosts of *E. multilocularis* based on Degree of Urbanization

(according to Deplazes *et al*., 2004)



Fox density is higher in urban centers where resources are in abundance, and it diminishes towards the periphery. With respect to intermediate hosts, they are more abundant on the periphery (figure 34), and it is here where predation is most frequent and where humans are most at risk (Hegglin).

Studies on the prevalence of vulpine infection in European cities provided contrasting results from 1% in Vienna (Duscher *et al*., 2005) to 44% in Zurich (Deplazes *et al*., 2004). It can be supposed that ecological conditions, especially as they concern intermediate hosts, can explain these differences (Romig).

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In Switzerland, where more than 75% of the population resides in an urban environment, incidence cases amongst city dwellers have been rising since the 2000s, while they had previously been stable since the 1970s. Today, the number of cases in urban areas is higher than in rural areas, yet incidence does remain higher in the countryside.

A concentration of human cases is especially high around Zurich and other Swiss cities (Schweiger).

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Foxes are equally numerous and active in both small cities and towns. In Germany, Janko *et al.* studied the behavior of foxes in a rural setting. Tracking foxes by telemetry monitoring demonstrated that the territory occupied by foxes, 75 hectares on average, was principally located in towns and prairies. While foxes frequented towns regularly at night, they preferred by and large to rest in more natural habitats, such as forests. They are attracted to the periphery of cities because they can easily find an abundance of alimentary resources in gardens. In total, they spend 40% of their time within cities. In comparing the prevalence of the parasite in rural foxes to foxes dwelling in towns or small villages, no significant difference was discovered. Both sets of foxes travelled across rural and urban zones. Therefore, strictly urban foxes do not exist, since they simultaneously inhabit towns and the surrounding areas. **This way of life represents a major risk for humans because foxes become infected in permanent grasslands, in which there are an abundance of intermediate hosts, then transport this parasite within zones populated by humans** (for example by defecating in gardens). Due to the proximity of foxes, inhabitants of towns or small villages find themselves increasingly exposed relative to those inhabitants living in the countryside (Janko).

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Just like vulpine populations, high densities of *A. terrestris* were identified as a risk factor for human infection (Giraudoux *et al*., 1999).

In short, data compiled at the symposium confirmed the increase in the populations of foxes and their observed urbanization in the latter part of the 2000s.

The presence of foxes and rodents in an urban environment creates an environment in which an *E. multilocularis* life cycle may be established. Humans can thus be directly infected by foxes or by intermediaries, specifically domestic carnivores.

The proximity between these vectors of *E. multilocularis* and humans poses a potential major risk for human health in urban areas. This risk is even greater given the fact that humans generally view the presence of these animals in their environment as favorable.

Thus, human populations in urban and peri-urban zones seem to represent a new at-risk category.

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- Environment

A study led by the department of Doubs in the 2000s revealed a correlation between the number of human cases and the proportion of permanent grasslands in the countryside. This has to do with the abundance of *M. arvalis* and *A. terrestris* rodents in these permanent grasslands. Their presence was demonstrated to be a contributing factor to the incidence of the parasite (Giraudoux *et al*., 2003). However, **the link between the distribution of human cases and geographic setting is still not entirely clear for the time being** (Giraudoux).

✓ Ownership of Domestic Carnivore

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Even if the general population of dogs was presently recognized as only rarely infected, they nonetheless represent a key element given their proximity to humans (Hegglin).

In Tibet, it was demonstrated that dogs were strongly implicated in human contamination (Boufana).

Average prevalence of infections in dogs is 0.3 % in Germany and Switzerland (Hegglin); in France, it has reached 1% in highly endemic regions, such as Annemasse and Pontarlier (Raton). These non-negligible frequencies underline the risk of contamination via domestic pets.

- ✓ Immunosuppression

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Immunosuppression provides favorable conditions for the multiplication of the parasite, the development of lesions, and metastases. These conditions can derive from natural immunodepression (for example, following HIV infection) or from immunosuppressive treatments, such as in the transplantation of solid organs, autoimmune diseases, or cancers. Between 1982 and 2007, among recorded patients with a complete medical history (all of which was compiled by the EchinoREg registry), **23% of cases showed a certain degree of immunosuppression**. These patients increasingly manifest unusual clinical profiles and negative serological results, even though PCR assays detect them as positive.

Furthermore, it is difficult to pinpoint the infection date of patients. The relatively rapid development of hepatic lesions can be attributed to a recent or former infection, the latter of which occurs due a reactivation of the parasite following a prolonged period of latency.

Given the prevalence of cancers and autoimmune or inflammatory diseases, 29 million individuals in Europe received immunosuppressive treatment and are therefore at risk for AE. This risk is particularly high in endemic regions characterized by high population density, particularly with a concentration of urban inhabitants. **A future outbreak of the disease in urban centers amongst immunodepressed patients should be feared. It thus appears abundantly clear that the at-risk population should be reconsidered given that inhabitants in urban centers will be increasingly more vulnerable to the disease.**

The gender-ratio observed in Europe might be explained by the fact that women are more often affected by chronic inflammatory diseases than men.

A growth in the parasite's development during pregnancy was also noted (Vuitton).

✓ Individual Factors of Resistance

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It is important to distinguish between the risk of infection and the risk of becoming sick, since not all seropositive persons develop symptoms. **In endemic zones, studies indicate the existence of around one patient per 200 seropositive individuals**. Due to an effective response from the immune system, not all humans who were in contact with the parasite develop AE. **Reasons for this resistance remain unclear**, though the amount of contaminated eggs could be an element explaining this response.

Alternatively, there could exist an immunogenic predisposition in humans linked to the severity of the disease (Eiermann *et al*., 1998; Vuitton, 2002). It would thus be interesting to compare genes contained by those who are sick and by those with abortive lesions. However, because of the protracted delay between contamination and the manifestation of clinical signs of the disease, patients are identified only after lesions have already developed, which is too late for a proteomic study (Gottstein).

In conclusion, data obtained from the latter part of the 1990s suggest a modification of the epidemiological situation of *E. multilocularis*. **The geographic distribution of the parasite is expanding**. New countries and regions, previously considered unaffected, have been added to the endemic zone. In France, ERZ's mapping project showed the extension of the endemic zone. Human cases appeared to the west of the country, well beyond the historically endemic zone. New hosts, whose role in maintaining the cycle remains uncertain, were discovered throughout Europe.

The prevalence of infection in foxes is equally on the rise. Emerging zones are characterized by the rapid growth in the prevalence of the parasite. With an especially heterogeneous distribution, prevalence can reach near 50% in hyperendemic hotbeds, such as France and Poland. The number of detected human cases has doubled in 10 years.

The absence of retrospective data in certain regions and the development of detection techniques make it difficult to distinguish between emerging zones and historically endemic zones that had not previously been identified or researched.

New countries, including Belgium, are now part of the endemic zone, with prevalence undergoing a rapid increase and reaching elevated values similar to those detected in highly endemic regions.

A similar sequence of events was able to be observed in all countries: the introduction of the parasite, its establishment, its extension, and finally its emergence as a human disease.

The profile of patients is currently being modified. The concentration of inhabitants in cities, combined with the colonization of urban centers by foxes, makes it such that citizens are now more exposed than ever before. Also, immunodepressed patients constitute a new at-risk category.

5. Disease-Control Measures against *Echinococcus multilocularis*

5.1. Regulating Fox Populations

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It can be assumed that by decreasing fox-population density, the total size of the area in which foxes reside would increase thereafter. Consequently, the proximity between foxes and rodents would be altered, leading to the extinction of *E. multilocularis*. This hypothesis was partially verified in Doubs where poisoning foxes as part of consecutive chemical disease-control campaigns against vole outbreaks led to a reduction in the quantity of eggs in the feces of foxes (Quintaine).

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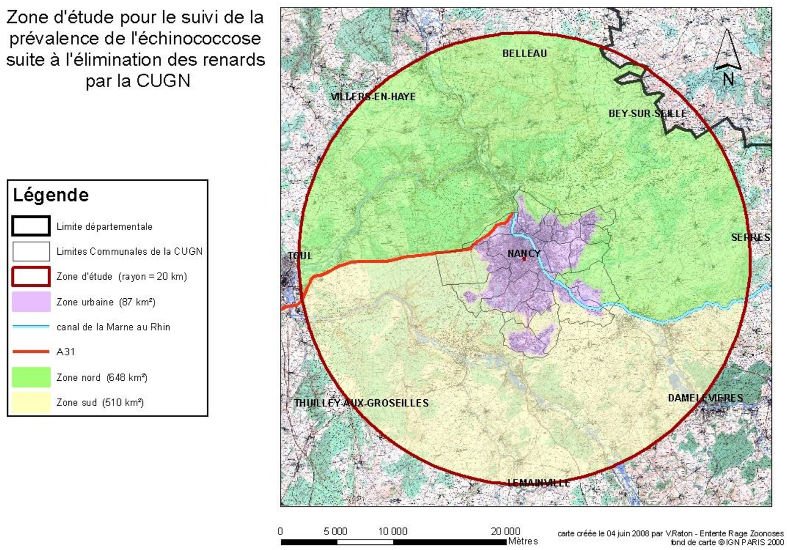
Studies have demonstrated that it is possible to regulate fox populations through culling. However, only **very intensive campaigns, conducted in small zones over a long period of time**, produce significant results. Regulation is difficult to effectuate over larger zones. For example, in the city of Zurich, there was no effect from the culling of fox populations.

This can be explained by the existence by self-regulating mechanisms within vulpine populations. The number of pups is variable, and high mortality occurs within the first year of life. Consequently, among foxes that were culled, many of them would not have reached an adequate age to reproduce. When populations attain high densities, certain adults do not reproduce at all. This flexible social structure makes it such that a culled fox can be easily replaced by another fox. **These compensatory mechanisms make it especially difficult to regulate vulpine population by the mere physical elimination of individual foxes (Hegglin).**

Figure 35. Zone of Study of AE Prevalence after Fox Culling in the Metropolitan Area of Nancy
(according to http://www.ententeragezoonoses.com/JeuCadreProfessionnels)



In 2006, following a presentation that demonstrated the presence of *E. multilocularis* in foxes living in Nancy, the authorities of the city decided to allocate finances for a vulpine culling campaign. ERZ and the ANSES laboratory of Nancy then set up a protocol for evaluating this practice. Foxes were killed by hunters, trappers, as well as during night-hunting campaigns. The culling zone encompassed a radius of 20 km, a figure corresponding to the maximum distance of displacement for foxes per annum (figure 35). The study is still ongoing, and only intermediate results are available. For the time being, analysis of the intestines of foxes by SSCT at ANSES has not shown a significant decrease in the prevalence of the parasite. Additionally, KAI figures obtained by counting foxes at night have not significantly changed over the first year of the culling campaign.

Thus, **the vulpine culling campaign does not seem, at least for the moment, to be sufficiently effective around the city of Nancy**. This method is also very time-consuming. **Nonetheless, it is necessary to wait for definitive results in order to draw final conclusions**.

The increase of AE receptivity in fox populations following fox population reduction campaigns is also a cause for concern. It is certainly possibly to envision young, more parasitic foxes taking the place of culled foxes (Comte, Raton).

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The University of Reims performed modeling on the effectiveness of fox culling. Their results indicated that in zones where rodent-consumption was high, that is to say rural areas, the growth in home-range size did not have an effect on the perpetuation of the parasite, and so on this front, culling would seem to be ineffective. **Conversely, this disease-control method should be considered in urban areas where rodent-consumption is low**. Other parameters, however, should be taken into account, such as the perpetuation of eggs in the environment. **The widespread availability of *Microtus spp*. to foxes, which was observed in the city of Nancy, could indeed explain these discouraging results.**

Despite the unpromising results of this culling campaign in a rural zone, it does seem as if culling could be worth pursuing in urban areas where intermediate hosts are less abundant (Quintaine).

5.2. Deworming of Foxes

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At first, a comparison with the oral rabies vaccination campaigns for foxes seems worthy of consideration. Here, the situation appears rather different and more complex though. In the case of a parasitic infection, vaccination cannot be considered, and the difficulty resides in the long perpetuation of the parasite in the environment in free-living stage and within intermediate hosts. **Long-term, repeated treatments thus appear necessary** (Hegglin).

Deworming campaigns were led in Switzerland (Hegglin), Germany (Konig), and France (Comte).

✓ Deworming Area

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The treatment area must be determined judiciously so as to guarantee its effectiveness at minimal cost. Foxes live in high densities within the center of urban areas, where resources are in abundance.

As far as rodents are concerned, they are concentrated in the periphery of cities, specifically permanent grasslands, to which they have successfully adapted. The transition area between rural and urban zones constitutes the prime habitat for *E. multilocularis* since two of its hosts reside there (figure 34).

This distribution corresponds with that of the human population. In Switzerland, a large portion of the population is concentrated in a condensed space, and only a quarter of the population lives in the countryside. Among citizens, the inhabitants of large urban centers are not at risk with regard to AE because intermediate hosts do not populate these areas. By contrast, 60% of the remaining inhabitants are concentrated in suburbs and small towns, both of which correspond to transition zones, whose environment is particularly contaminated by *E. multilocularis* eggs. **In short, small transition zones between urban and rural areas are particularly at risk and should be the focus and priority of deworming campaigns.** This high-risk zone represents 40% of the city of Zurich encompassing 37 km^2.

In Germany, Janko *et al.* demonstrated in their recent study that foxes' high frequentation of towns and small cities, where they import the parasite, justifies the implementation of disease-control measures in these high-risk areas (Hegglin).

✓ Frequency of Deworming

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Studies conducted in Munich reveal that trimestral deworming does not result in effective regulation of vulpine infection. Instead, **a monthly administering produces drastic decreases in prevalence, accompanied by a long-term stabilization** after the completion of bait delivery. The prevalence of *E. multilocularis* in *A. terrestris* is equally reduced by fox deworming campaigns (Konig).

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The National Institute of Public Health and the Environment (RIVM) in the Netherlands performed modeling showing that fox deworming produced diminished quantities of the following: tapeworms in the intestines of foxes, eggs in the environment, and (after a long period of time) larvae in intermediate hosts. This confirms the necessity of **a long period of regulation. Three years would represent the minimum duration necessary to achieve the elimination of all stages (Takumi).**

In France, deworming campaigns were led by ERZ in Annemasse and Pontarlier (figure 36), communes (municipalities) exhibiting high prevalence of *E. multilocularis* in foxes, reaching 49% and 51%, respectively. From 2006 to 2009, forty baits per km^2 were delivered at the rate of **five treatments per year**. Significantly different results were obtained in the two cities (figure 37). In Annemasse, the comparison of *E. multilocularis* prevalence in foxes by CoproElisa, in relation to the control area, indicates a significant diminution in the first year of treatment, followed by its stabilization at low rates during the rest of the campaign. The prevalence fell from 13.3% to 2.1% in the treated zone. By contrast, in Pontarlier, the delivery of baits did not have significant results on the prevalence of vulpine infection, which remained unchanged from 2006 to 2009, with no significant difference in the control area. This can be explained by environmental differences between these two urban areas: in Annemasse (figure 38), infection predominantly resides in the suburbs, whereas the metropolitan area of Pontarlier contains copious amounts of permanent grasslands that are heavily populated by rodents. As a result, foxes in the latter region undoubtedly become re-contaminated despite the treatment. **Such an ecological zone, with an abundance of permanent grasslands, clearly necessitates more intense treatment (Comte).**

Figure 36. Deworming Zone of Foxes in the Metropolitan Municipality of Pontarlier
(according to http://www.ententeragezoonoses.com/JeuCadreProfessionnels.htm)

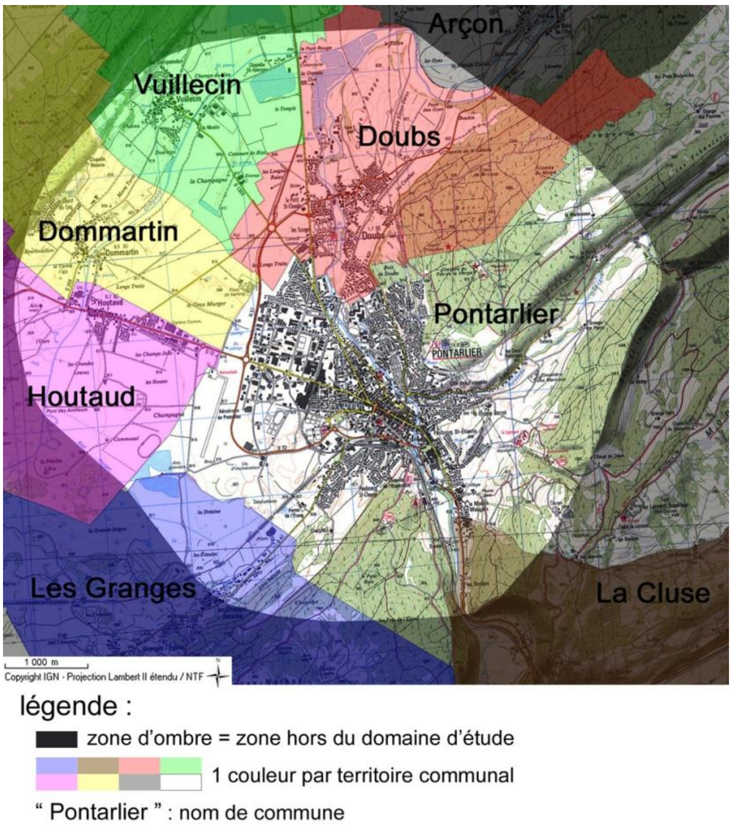
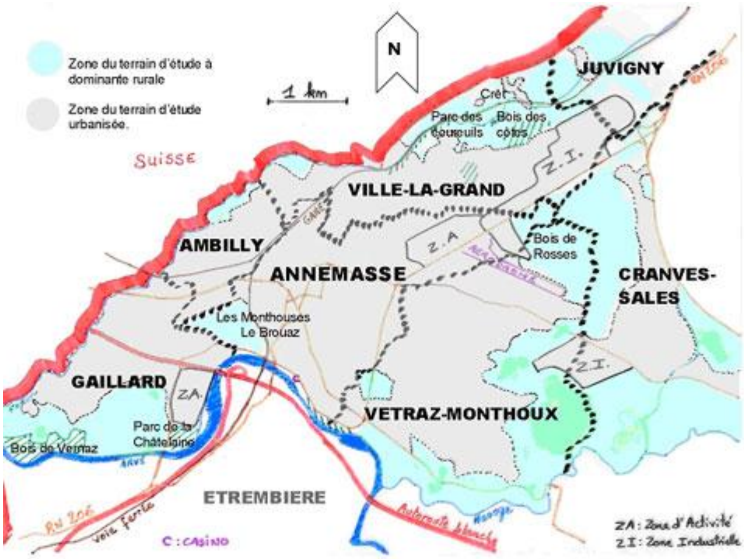


Figure 37. Results of Fox Deworming Campaigns in Annemasse and Pontarlier
(according to ERZ, Synopsis 2009)

Figure 38. Distribution between Urban Zones and Predominantly Rural Zones in the Metropolitan Municipality of Annemasse

(according to http://www.ententeragezoonoses.com/JeuCadreProfessionnels.htm)



Consequently, the frequency of deworming must be adapted to suit the environmental and epidemiological situation.

✓ Season

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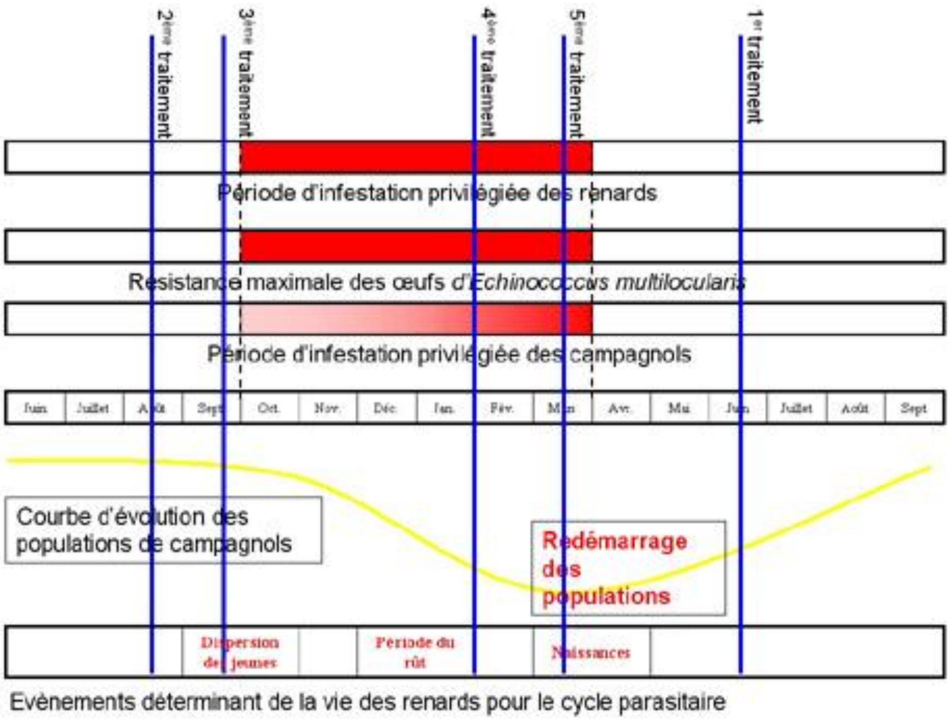
A series of French and Swiss studies suggest an increase in the populations and prevalence of infection of both *M. arvalis* and *A. terrestris* at the end of winter (figure 39). **Fox deworming conducted in autumn and winter**, periods corresponding to the strongest infection pressure on intermediate hosts, allows for maximum effectiveness to be attained (Giraudoux).

However, no seasonal influence was demonstrated in Annemasse and Pontarlier (Comte), contrary to what had been predicted in Zurich (Hegglin).

Figure 39. Infection of Different *E. multilocularis* Hosts over the Course of a Year
(According to ERZ, 2006)



✓ Method of Bait Delivery

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In Annemasse and Pontarlier, baits were delivered manually by car when it was possible, but oftentimes on foot (Comte).

In Bavaria, bait delivery was carried out primarily by hand, but also by plane. This manual bait delivery method ends up being very time-consuming (Konig).

✓ Distribution Area of Delivered Baits

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In Germany, following the distribution of 50 baits per km^2 in a rural zone, Janko *et al.* observed that 75% of baits were disappearing in the first three nights, 90% were disappearing within a week, and 40% were being consumed by foxes. Dogs are competing over their consumption. Baits are consumed more rapidly in the periphery of inhabited areas than in urban centers.

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In Annemasse and Pontarlier, the monitoring of bait consumption was performed by camera, which revealed that the fox was the species most attracted to the baits, consuming 40% of the total baits. This confirms results obtained in Switzerland and in Germany. Consumption is dependent on the city and on the degree of urbanization. Baits were most often consumed in peri-urban areas and in Pontarlier. On average, 15% of baits remained after 14 days, which indicates that the dispersion of 40 baits per km^2 is adequate since it avoids both the saturation and shortage of baits in the environment (Comte).

✓ Results

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In Germany, The University of Munich led a program in a highly endemic zone within Bavaria. Monthly deworming was performed, followed by weekly deworming every six weeks. Baits were provided by Bayer laboratory. They weighed 13.5 g containing 50 mg of the anthelmintic drug praziquantel and used an identical matrix to the one used during the rabies vaccination of foxes. The prevalence in foxes, initially equal to 53%, fell drastically and stabilized at 2% in treated zones and 26% in control areas. In the non-treated zones surrounding the treated ones, foxes were able to consume baits, which explains the subsequent decrease of prevalence in the control area. **Deworming can also diminish the presence of parasites in gravid proglottid form in treated foxes, as well as the probability of human contact with *E. multilocularis* eggs by 90%.**

In order to conduct an effective deworming campaign, it is imperative to attain a long-term decrease in prevalence (Konig).

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Similar campaigns are planned in other European cities, including the city of Limburg in the Netherlands, where infection is increasing at an alarming rate (Takumi).

Fox deworming campaigns thus represent a control mechanism for infection that is concretely achievable and produces satisfactory results.

- **Financial Cost of Deworming Campaigns**

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In Zurich, Switzerland, the cost of an intense fox deworming campaign was estimated at €43,000 per annum, whereas **the care for one case of AE costs around €100,000** (Torgerson *et al*., 2008). Therefore, the cost of such a campaign is theoretically justified in the long term if it precludes one case of AE per annum. For Zurich, with an incidence of 0.94 per annum, the cost of running long-term deworming campaigns appears justified. It must be noted, however, that the results of such efforts on the number of human cases cannot be determined for at least a decade due to the long incubation period before clinical signs of AE become manifest (Hegglin).

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In Bavaria, Germany, total costs were evaluated at €2.5 per inhabitant of a treated zone per annum, which is relatively small over the long term. This low value can be explained by high-population densities in the zones tested, including notably the southern suburbs of Munich. In other less populated German areas, the cost is roughly €3-€4 per person per annum (Konig).

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In France, the regulation of fox infection costs more, estimated at €235,000. Bait distribution constitutes a significant portion of the total cost. The cost could be reduced by 42% with the participation of local technicians. Labor costs would then cost €66 per km^2 as opposed to €114 in the study. **The conclusion drawn in Zurich is the same: deworming campaign costs could be justified on the condition that they prevent infection in at least one human patient per annum** (Comte).

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In the future, a more precise determination of zones requiring treatment could also enhance efforts to economize money and bring down costs.

In addition, long-term effects following deworming campaigns further justify these costs (Hegglin).

The cost of deworming campaigns conducted in high-risk zones is thus inexpensive and perfectly **justified over the long term** if they can reduce human incidence. The cost, variable from country to country, becomes even lower **in treated zones with high population densities** of humans.

5.3. Deworming of Dogs

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Dogs represent ideal definitive hosts for *E. multilocularis*. They can play a key role in human contamination due to their close proximity to humans. Humans can become infected after ingesting plants carrying eggs that were excreted in dog feces or by a lack of hygiene, such as not washing hands after handling a dog whose fur contains eggs (Hegglin).

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In order to become contaminated, dogs must be predators of infected rodents, and they must also lack proper deworming. A suitable deworming requires the use of an **efficient molecule** against E. multilocularis and the **correct frequency** of application (Deplazes).

- ✓ Lack of Information and Hazardous Deworming Practices

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From 2008 to 2010, the ANSES laboratory of Nancy, in collaboration with ERZ and veterinarians from the highly endemic departments of the Meuse and Haute-Saône, gathered 493 and 367 fecal specimens from each respective department after deworming. A questionnaire completed by dog owners shed light on **the hazardous practice of deworming, as well as the lack of public information**. Sixty-three percent of dog owners did not know if the molecule employed in deworming was effective against E. multilocularis. Most owners do not know the risk of AE and are content in placing confidence in the deworming advised by their veterinarian (Umhang).

✓ Dogs Particularly at Risk

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A little over one third of dogs are recognized as having consumed rodents by their owners. However, these dogs are not as frequently dewormed as other dogs. Among at-risk dogs, there is the same proportion of pets, farm dogs, and hunting dogs. This information must be viewed carefully, however, since dogs that were not identified as rodent-predators were found infected in other regions of Europe. Only five of the 860 dogs included in the study were dewormed once per month, of which one was dewormed without praziquantel.

Finally, in properly considering the rodent-predator dogs that were not dewormed, it was found that **35% of the dogs considered were at risk for *E. multilocularis*** in Haute-Saône, **regardless of the dog's breed** (Umhang).

✓ Effectiveness of Deworming

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Analyses revealed that in the Meuse the frequency of deworming, when it was administered more than two times per annum, corresponds to lower parasitic infection in the aggregate (Umhang).

✓ Difficulties in the Implementation of Systematic Deworming

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Firstly, monthly deworming represents a non-negligible cost for owners, especially when they own several dogs.

Additionally, it can be complicated to convince owners to deworm their pets when no dog tested positive for the parasite in the Meuse and Haute-Saône, which are considered to be two highly endemic departments. Nevertheless, infected dogs were identified in other epidemiologically similar areas, proving that there is in fact a risk.

The prevalence of *E. multilocularis* in dogs was evaluated at 0.3% in Switzerland and Germany by the Institute of Parasitology at the University of Zurich. By comparing this percentage to the total dog population, it appears that **there are 1000 infected dogs in Switzerland and 10,000 infected dogs in Germany**. This prevalence thus appears to be non-negligible since the numerous animals carrying and excreting parasites are in close contact with humans (Hegglin).

In Pontarlier and Annemasse, ERZ's collection of more than 1000 fecal specimens of dogs from 2006 to 2008 revealed **one positive dog in each city** through PCR assays (Comte). This corresponds to a prevalence of 0.2% in highly endemic hotbeds, a figure approaching the 0.3% prevalence estimated in Switzerland and Germany. This alarming set of data proves that domestic carnivores must be dewormed more regularly given this epidemiological context. In addition, it is interesting to note that the fecal analyses were collected thanks to veterinarians. Therefore, the dogs in the study represented regularly treated dogs, which are not necessarily representative of the general canine population. It can be assumed that the dogs taken to a veterinarian are better dewormed than others. As a result, the prevalence of infection amongst dogs is underestimated (Umhang).

✓ Recommendations

Dog owners' lack of information highlights the importance of communication concerning this particular subject. This must also be brought to veterinarians' attention. The majority of veterinarians generally recommend deworming twice per year. Given the prepatent period of the parasite **in an endemic zone, it is recommended that dogs with ready access to rodents be dewormed once per month with an anthelmintic drug containing praziquantel. This recommendation was clearly emphasized by the group of European experts at ESCAAP (www.esccap.org and www.esccap.fr).**

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It must also be noted that even if available treatments are effective against adult tapeworms, none of them have ovicidal activity. Also, a 24-hour timetable is necessary before the elimination of parasitic forms within animals, and highly infected animals must be administered the treatment twice. Nevertheless, the administering of one anti-parasitic treatment does not eliminate the danger (Deplazes).

- Risks Related to Deworming Domestic Dogs

- ✓ Excretion of Eggs into the Environment

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E. multilocularis eggs are discharged into the environment in 24 to 48 hours following the administering of praziquantel. It can thus be questioned whether this massive excretion, by releasing a significant concentration of eggs into the environment, induces an increased risk of contamination for humans, as well as for other intermediate hosts. This must, however, be kept in perspective since excreted eggs following the deworming process are not all mature, and thus, not all of them are infective. Only roughly 20% of the eggs excreted after treatment are mature. No data have indicated if these eggs can continue their development in the environment. These eggs would have been released in the three months following full maturation anyway (thus, they would all have been infective). The most prudent recommendation consists in gathering the first feces with precaution and disposing of them so as not to contaminate the environment. Nonetheless, such advice runs the risk of frightening owners. Instructions must be provided in such a way to avoid inciting the fear of dog owners, which would only serve to discourage them from taking action.

Even if the information is not presently sufficient, it is commonly agreed that deworming leads to the decrease in the quantity of discharged eggs (Deplazes).

It is of interest to calculate the probability of human contact with these eggs following deworming. This probability of contact is diminished by 90% (Konig).

✓ Effects on Other Intestinal Parasites and Vulpine Microflora

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In Bavaria, monthly deworming of foxes resulted in a decrease in the presence of other tapeworms, though nematodes of *Uncinaria* were more abundant in the intestines of foxes in the treated area (Konig).

These observations prove that in attempting to eliminate *E. multilocularis*, the development of other digestive parasites is also exacerbated. **It thus appears legitimate to speculate if such deworming campaigns may in fact disturb biodiversity and even lead to the resistance of praziquantel** (Giraudoux).

Secondary effects are even more a cause for concern since campaigns are conducted on a large scale and over a long period of time. There is very little information on this subject and nothing for the time being to prove that there are eventual detrimental effects. The quantity of distributed praziquantel in the environment (50 mg per km^2) appears too weak to be able to contaminate a large zone. Consequently, the impact seems to be minimal (Romig).

Modification of vulpine microflora following repeated deworming efforts can also be feared, since this might result in the appearance of other types of zoonosis (Vuitton).

The appearance of similar effects can be foreseen in domestic carnivores that frequently travel and in those animals that have not yet received weekly treatment, for example.

Nevertheless, these results must be considered with prudence since the prevalence of parasites is strongly tied to the age of foxes. *Uncinaria* nematodes have a significantly higher prevalence in animals that are older than one year. In order to interpret data, it would thus be necessary to consider age as a factor that might cause confusion. However, at Stuttgart, the stratification of infection rates based on age indicated higher levels of infection by nematodes after deworming with praziquantel (Deplazes).

Presently, the amount of data is lacking with regard to the emergence of eventual resistances to praziquantel or the disorder of intestinal microflora in carnivores.

5.4. Other Disease-Control Measures

✓ Regulating Vulpine Reproduction

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Strategies other than culling or poisoning can be implemented to manage fox populations, such as the regulation of vulpine reproduction. On this front, promising results were observed in Australia (Hegglin).

The delivery of baits could be used to accomplish this end (Boué). It does not seem easy though to establish specific protocol for foxes without influencing other species (Hegglin). To date, there has been no study led in Europe on this subject, but **it represents an intriguing area of research**.

✓ Regulation of Intermediate Host Populations

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The regulation of intermediate host populations could also be an avenue worth pursuing in certain cities such as Nancy, for example, where *M. arvalis* is abundant and could create the conditions for an urban cycle.

Agricultural research, however, demonstrates the complexities inherent in limiting the population of rodents. It is, generally speaking, extremely difficult to regulate host populations (Giraudoux).

✓ Implementation of Private Initiatives

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In the city of Zurich, only 10% of the population has a negative opinion with regard to the presence of foxes near their homes. Seventy percent of the population expresses a positive or wholly ambivalent attitude. Generally speaking, inhabitants of cities perceive the presence of foxes as favorable and view culling campaigns negatively. This attachment of citizens to the presence of wild animals could be employed to organize deworming campaigns. Inhabitants, however, believe that it is not their responsibility to take care of wild animals and that the implementation of campaigns should be in the hands of public authorities (Hegglin).

✓ Prevention and Public Information

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All disease-control strategies must first include recommendations concerning the prevention of infection. The public must be informed of expected risks and personal hygiene measures in order to protect themselves. This information needs to underline the importance of washing and cooking food, washing hands, and the risk associated with contacting foxes. Citizens must equally be made aware of the importance of deworming domestic carnivores and the danger inherent in the consumption of rodents by their pets. In the United States, where there are also infected foxes, no human case has been recorded following information campaigns. Better adapted human behavior undoubtedly explains this difference with respect to Europe.

It is necessary to provide uniform recommendations, especially regarding deworming in Europe.

ESCCAP (*European Scientific Counsel Companion Animal Parasites*) plays a crucial role in these information campaigns. ESCCAP was founded with the objective of providing veterinarians and domestic animal owners alike with the best information possible to manage the infection and zoonoses of their animals. They provide independent advice for the optimal treatment of animals, all the while stressing the various elements of risk.

ESCCAP is a powerful communication device due to the accessibility of available information on its websites (www.esccap.org and www.esccap.fr) and its brochures, which nearly 25% of owners of carnivores in Switzerland have received.

Public health communication is not in the hands of the EFSA, which is in charge of animal health, but rather the ECDC (*European Centre for Disease Prevention and Control*). Therefore, ECDC **also has a role in educating the public and disseminating information on a European scale**.

Even if certain recommendations need to be harmonized across Europe, it is also vital that messages be tailored to local populations. Risk factors can indeed be very different from country to country, including each country's economic situation and their citizens' respective lifestyles.

The increase in human cases in certain countries - such as France and Switzerland - these past few years, despite the advice that has been given, highlights how difficult it is to alert and heighten the awareness of the public (Hegglin).

✓ Usefulness of Modeling

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The use of modeling can generate responses to precise questions. For example, a model allows for a culling strategy to be tested in different epidemiological contexts. This could also be adapted to deworming. With preliminary models, the best strategies can be employed, and money can be saved (Quintaine).

In Germany, modeling is used in order to limit sampling bias. As a result, it is possible to estimate the actual prevalence of *E. multilocularis* and to analyze its distribution in space and over time (Schwarz).

Assuming that there exists a direct relationship between contamination of the environment by eggs excreted from foxes and the number of human cases, it is possible to create a model of expected risks for humans and to evaluate the number of cases in the future. In the Netherlands, the National Institute of Public Health demonstrated that modeling enables the effectuation of a risk analysis and the adaption of future disease-control measures (Takumi).

Also, models represent a useful tool for the implementation of effective prevention methods.

6. Risk Management

6.1. Characterization of Risk

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Before claiming a risk-free status, countries must supply ample demonstration of the absence of the parasite in their territory. Having said that, it is impossible to test every single animal, so only **a probability of the absence of the parasite** can be issued by a method of risk analysis.

Five countries are considered free of *E. multilocularis* in Europe: Sweden, Norway, Finland, Malta, and the United Kingdom.

The National Veterinary Institute of Sweden (SVA) provided a demonstration of the parasite-free status of the following countries: Sweden, Norway, and Finland (Whalstrom).

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In order to estimate the probability of introduction and establishment of *E. multilocularis* in a country, it is necessary to aggregate the results of several monitoring systems, all of whose sensitivities have been taken into account. Monitoring systems concern parasitic hosts: foxes, rodents, and raccoon dogs. In Europe, the presence of the parasite was first detected in foxes in six countries, humans in five countries, and rodents in four countries. It is thus necessary to monitor several species. Some data are approximations, including probability of the absence of the parasite at the study's inception and eventual prevalence in hosts. The choice of expected prevalence in hosts is critical, albeit very difficult. The present absence of human cases indicates that the parasite was undoubtedly absent in the territory fifteen years prior to the study.

By testing the model with decreasing vulpine prevalence, ranging from 1% to 0.05%, the following can be observed: for the same risk of introduction, the lower the prevalence, the more the sensitivity of the system decreases and the greater probability that the parasite-free status is weak.

The probability of a parasite-free status is higher and more stable for Sweden, Norway, and Finland. In 2008, it was around 98% (Whalstrom).

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EFSA issued advice regarding the evaluation of the risk of *E. multilocularis* introduction by pets in parasite-free countries. This risk depends on the prevalence of the parasite in the hosts of its country of origin, the number of infected pets introduced, and the effectiveness of deworming protocol at the time of introduction. When *E. multilocularis* is present in wild animals or in pets of a country of origin, there is a risk of introduction (Whalstrom).

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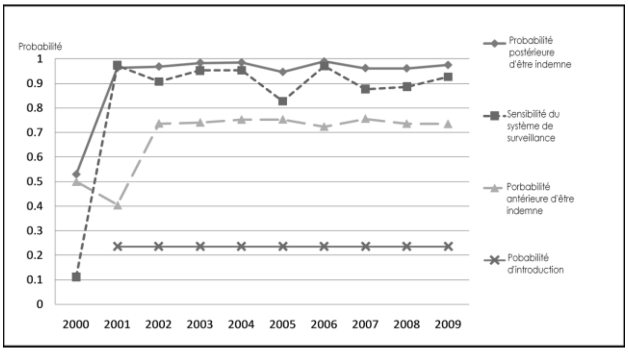
In Sweden, the risk of losing parasite-free status is tied to the eventual introduction of parasitic dogs, more so than the migration of foxes because its contiguous countries are similarly parasite-free.

Despite the high probability of the parasite's absence, there still exists a risk of introduction and establishment of *E. multilocularis* (figure 40).

From the moment when the risk is non-negligible, EFSA recommends the employment of protection measures, such as preliminary deworming of animals. To quantify this risk, the probability of *E. multilocularis* introduction is calculated every year (Whalstrom).

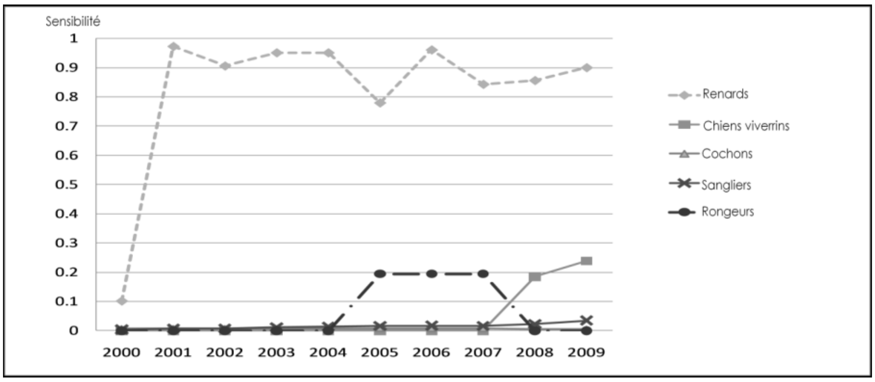
Figure 40. Probability of Parasite-Free Status in Sweden
(according to Whalstrom *et al.*, 2011)



The sensitivity of detection systems in different species is a key element for monitoring. In parasite-free countries, it is between 80%-90%. Sensitivity represents the probability of detecting the parasite if it is present in the country at the estimated prevalence of the model. The greater number of animals examined, the higher the sensitivity. In Sweden, the monitoring system with the highest sensitivity concerns foxes (figure 41). **Thus, in this country, surveillance of the parasite's introduction focuses on foxes** (Whalstrom).

Figure 41. Sensitivity of Detection Systems for the Presence of E. Multilocularis in Sweden Based on Monitored Species

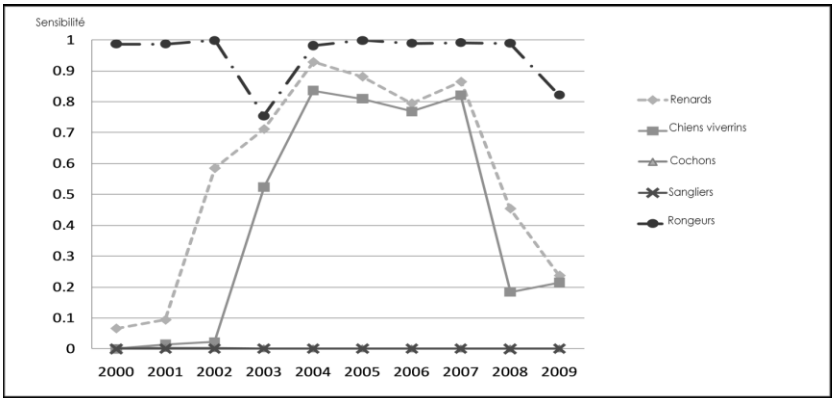
(according to Whalstrom *et al.*, 2011)



In Finland, where the sensitivity is greater for other species, **surveillance covers foxes, rodents, and raccoon dogs** (Whalstrom) (figure 42).

Figure 42. Sensitivity of Detection Systems for the Presence of E. Multilocularis in Finland Based on Monitored Species

(according to Whalstrom *et al.*, 2011)



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In Sweden in 2009, the probability of being parasite-free was higher, greater than 95%. **The probability of the introduction and establishment of the parasite was estimated at 24%, which is non-negligible.**

The probability of the establishment of *E. multilocularis* in the country upon introduction is estimated at 55%. This means that if two infected dogs are introduced, one of the two will be the source establishing the cycle.

All dogs and cats introduced in Sweden that come from a country other than Sweden, Finland, Malta, or the United Kingdom must be dewormed by a veterinarian who then affixes a tampon on the passport. However, this is not systematically verified upon introduction. Moreover, it is necessary to be ensured that the molecule employed in deworming is effective and that the treatment has no ovicidal activity.

The sensitivity of detection systems is not 100%, and no monitoring system is infallible (Whalstrom).

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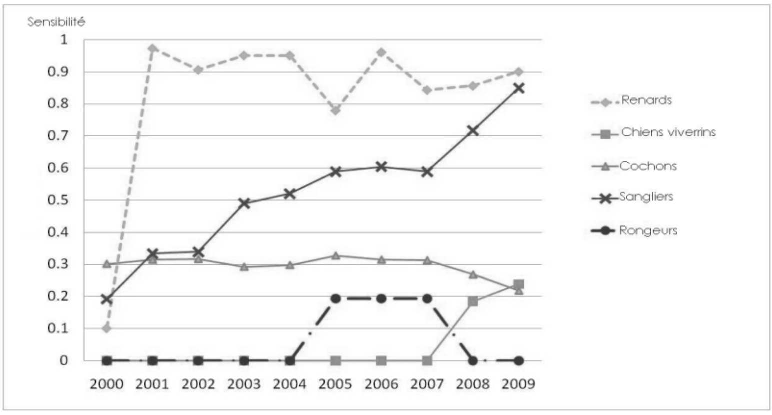
Wild boars are abundant in Sweden, and the eventual prevalence used in the model is 0.02%. The present sensitivity of detection is very weak.

Figure 43. Sensitivity of Detection Systems in Sweden after Educating Hunters to Search for Lesions

(according to Whalstrom *et al.*, 2011)



It is possible to improve the detection system by educating hunters to recognize lesions and to send in specimens for analysis. Sensitivity could then be increased (figure 43), and there would be over an 80% chance of detecting *E. multilocularis* if it were present. This assumes that all culled wild boars are inspected (Whalstrom).

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It would be of particular interest to know the risk of contact between imported dogs and intermediate hosts, as well as the number of imported dogs and the type of dogs, since the risk is different among companion and hunting dogs. Furthermore, infection rates of companion dogs are rarely known. In spite of these uncertainties, risk analysis remains a useful tool (Whalstrom).

The risk of introduction and establishment of *E. multilocularis* in parasite-free countries is thus rather high. As the principal risk is linked to the introduction of infected dogs, particular attention must be paid to proper deworming of imported dogs. Monitoring of wild animals is essential in order to be promptly alerted in case of parasitic presence.

6.2. Epidemiosurveillance

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Recent epidemiological data indicate an increase in the prevalence of *E. multilocularis* and its distribution in Europe. As a result, EFSA recommends the rapid implementation of monitoring systems of *E. multilocularis* in pets and wild animals in order to observe epidemiological changes. This is necessary to understand at-risk zones, temporal developments, and the development of disease-control methods (Have).

Standardized surveillance, along with common sampling strategies, is thus necessary. These are the required conditions in order to be able to compare results. A comprehensive European approach is absolutely essential. Since countries share borders where prevalence is identical, each country cannot individually monitor its own territory (Romig).

Switzerland has designed a "Swissgrid," dividing the country into squares of 100 km^2. The number of human cases and their location were reported on the grid (Scheiger). This rigorous system enables the acquisition of a complete, detailed perspective of the area. This highlights the **importance of designing a systematic method of collecting cases based on a standard European scheme**. Such a system should be initiated in every European country.

The valuable results obtained by ERZ's mapping project in France underline the importance of producing **an active epidemiosurveillance** of *E. multilocularis*. In effect, the knowledge of the geographic distribution and the prevalence of vulpine infection create the framework on which the risks of human disease may be predicted. **This type of project must be extended onto a European scale and conducted regularly** with a frequency that remains to be determined.

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Nevertheless, it is worth nothing that surveillance programs are of no interest if they are purely descriptive. They must be conducted for example in response to specific questions. It is not useful to investigate all host species, but instead, only those species capable of providing information of interest (Giraudoux). Following prevalence in peripheral zones of endemic areas allows for gradients to be set up and enables the monitoring of the direction of parasitic expansion (Vervaeke).

Information that is obtained by monitoring systems must be compiled and recorded.

In order to conduct effective epidemiological monitoring, it is generally agreed that parasitologists, clinicians, pharmacists, laboratory directors, veterinarians, epidemiologists, and biologists must work together and communicate the results of their work to one another. Information has to be shared between researchers and clinicians in the field.

Scientists must continue to work together following the conference, and work groups could in particular be formed (Vuitton).

It is equally essential to pursue research on issues of importance, and supplemental epidemiological research is required as well.

Data provided by scientific studies must subsequently be **disseminated widely** and serve to support the implementation of disease-control measures in order to stop the expansion of the parasite.

It is necessary to increase the number of public lectures and to share information between different types of human and animal epidemiology.

Local authorities have to inform their inhabitants of the risks of AE, as well as prevention methods. Additionally, recommendations need to be provided. This information must be disseminated amongst clinicians, veterinarians, hunting associations, and individuals. To this end, 100,000 posters concerning prevention methods were distributed in the 45 participating departments of ERZ (Combes).

European institutions play a major role in recording data. Every year, EFSA collects figures concerning zoonoses in order to evaluate human exposure. Each country has data collection centers that transmit the data to EFSA. EFSA produces a report on its website. In France, for example, it is the DGAL that gathers data concerning animal health (Boué). The National Institute of Health Surveillance (L'Institut National de Veille Sanitaire) sends human cases to the ECDC. The system of data collection has certain flaws since **there are discrepancies in figures reported by EFSA and scientists.** In Europe, only five cases per year are reported, which underestimates the reality of the situation. Different organizations involved in the process must be informed of this anomaly so that they can remedy it. It is equally the responsibility of scientists to verify how data collection systems function in each country.

This difference between acquired data on the ground and figures reported by EFSA can be explained by a lack of harmonization in data reporting formats - which may not ask for enough information - and by a wide diversity of sampling and detection methods. It is necessary to agree upon a similar framework to be provided by all countries.

Certain countries, notably those that are parasite-free, do not consider the official reporting of human cases to be obligatory (Grenouillet). This makes it difficult for the FrancEchnio network to acquire a comprehensive collection of data. Official reporting appears to be a necessary condition in order to be able to claim parasite-free status. In addition, based upon European directive 2003-99, member states are obligated to report data concerning zoonoses inscribed on list A to EFSA. A lack of coordination with regard to official reporting of AE cases currently exists within the scientific community.

Effective epidemiosurveillance necessitates the use of standardized methods combined with the pursuit of research in areas of particular interest. In Europe, authorities have a role to play in coordinating action.

Another problem identified over the last several years concerns the distinction of Echinococcus spp. **during the transmission of numerous human cases**. Thus, it is critical to distinguish human cases of AE from those of Cystic echinococcosis (CE) in order to better evaluate the data (Vuitton).

To guarantee the effectiveness of monitoring systems and the implementation of suitable disease-control measures, a collection of data, one that separates information for *E. multilocularis* and *E. granulosus*, must be prescribed.

In conclusion, disease-control measures for fighting *E. multilocularis* are based upon the knowledge of the parasite's cycle and the ecology of its definitive and intermediate hosts. All strategies rest firstly on informing the population on the subject of general personal hygiene best practices, as well as the deworming of pets. Managing fox populations by way of culling seems very difficult; the regulation of their reproduction thus appears to be the best avenue worth studying in the future. Intensive deworming of foxes in zones that are labeled as at-risk is an excellent, relatively low-cost strategy over the long term. It is crucial, however, to remain vigilant concerning the eventual appearance of resistances to praziquantel and to other modifications in the balance of nature.

Hazardous deworming practices underline the importance of heightening awareness amongst veterinarians and owners of domestic carnivores by associations such as ESCCAP. It is necessary to give uniform recommendations with regard to deworming of domestic carnivores across all of Europe. Specifically, deworming of dogs must take place once per month in endemic zones. Deworming programs must be adapted to the local epidemiological situation.

In the future, a homogenized and standard monitoring system must be established on a European scale. Information then needs to be gathered and disseminated. European institutions have a key role to play here as well. Finally, research must also be carried out on points of interest.

CONCLUSION

Though Alveolar echinococcosis is asymptomatic in animals, it represents an extremely serious zoonosis. In light of this, AE was declared a “priority” disease by the National Institute of Health Surveillance and is considered one of the most serious zoonoses in the temperate zone of the Northern Hemisphere (Capek *et al.*, 2006).

Since the epidemiological situation of *E. multilocularis* in Europe is evolving, and studies are being carried out independently within different countries, the organization of a conference is required in order to consolidate and compare information. However, the lack of retrospective data and the use of varying diagnostic methods from country to country render the comparison of the results from studies rather difficult. Additionally, the fact that investigations are by and large carried out on a small scale poses complications when attempting to extrapolate on a national and indeed continental scale. Therefore, the methodological diversity employed to find evidence of the parasite’s presence in hosts should be abandoned in favor of a uniform and standard strategy across all of Europe.

The exchange of information between different European countries was nevertheless incredibly enriching and allowed us to update our knowledge on the subject. The recent epidemiological data that was shared during the European conference confirmed the extension of the endemic zone of *E. multilocularis*, which supposedly took place at the end of the 1990s. The heightened rate of prevalence can clearly be observed at the local level. Therefore, it is clear that the endemic zone of *E. multilocularis* is spreading, and given the current state of knowledge, nothing can be foreseen regarding the stability of the situation.

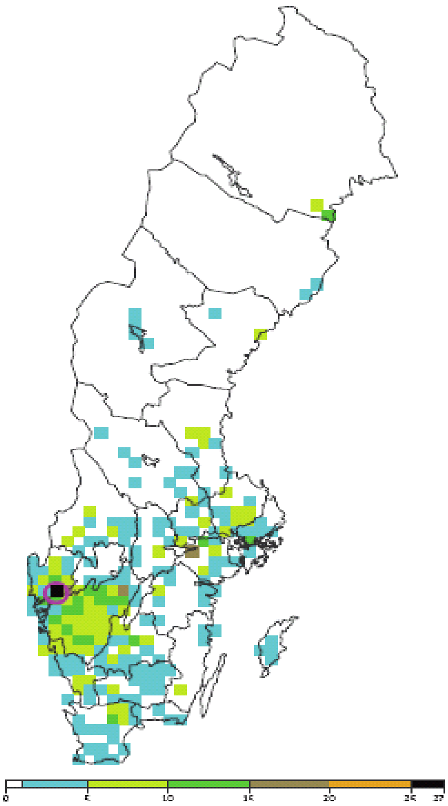
The rapidity with which the epidemiological situation is evolving in emerging zones and the risk of introduction in parasite-free countries indicate the need for regular updates. In Sweden, a country recognized as parasite-free at the time of the conference, two infected foxes were discovered in February and March of 2011. The two infected foxes were adult females inhabiting the southwestern area of the country that were tested under a national surveillance program. The first fox, killed in December, 2010, was analyzed in February, 2011. The diagnosis was made by real-time PCR analysis of DNA extracted from the feces and confirmed by both a conventional PCR and SCT. This fox was the only one that tested positive out of 304 examined in 2010. After this positive result, the sample number of foxes was then intensified. Up until March 31, 2011, 1140 foxes were analyzed by SSCT, and a second infected fox was identified within this group (Osterman *et al.*, 1011) (figure 44).

During the conference, the potential consequences stemming from the introduction of *E. multilocularis* in Sweden, which had previously been discussed, were presented in the form of a poster, entitled “Potential Consequences of Introduction of *E. multilocularis* into Sweden” (European Symposium on Alveolar echinococcosis, Wahlstrom, 2010). The loss of parasite-free status immediately generated consequences in terms of public health, but it also presented heavy economic impacts. Indeed, an increased surveillance of both foxes and intermediate hosts was thus effectuated in order to follow the spread of the disease. In addition, prevention campaigns must now be conducted in at-risk populations, and future treatment costs for human patients must be considered as well.

The cause of the introduction of *E. multilocularis* in Sweden was not clearly determined. However, the hypothesis of dogs having imported the parasites, despite the obligatory deworming efforts, was strongly incriminated. It must be noted that since January of 2012, the treatment against *E. multilocularis* is no longer necessary when importing dogs into Sweden (European Regulation n° 1152/2011, Official Journal of the European Union, November 15, 2011). The geographic distribution of *E. multilocularis* in Sweden remains unknown. The common geographical location of the two infected foxes indicates that intermediate hosts there were most likely contaminated as well. However, the prevalence of the parasite is nonetheless low (Osterman *et al.*, 1011).

Climatic conditions in Sweden and the existence of potential intermediate hosts provide favorable conditions to the establishment of *E. multilocularis*. It is unquestionably important to pursue screening efforts so as to clarify the epidemiological situation and to decide upon necessary measures to take (Osterman *et al.*, 2011).

Figure 44. Geographic Distribution of Foxes Collected and Analyzed in Search of *E. multilocularis* in Sweden between January and March of 2011
(according to Osterman *et al.*, 2011)



Number of Foxes per 400 km^2

The circle represents the locations where the two foxes infected with *E. multilocularis* were killed.

The fact that there has been a constant annual incidence rate in humans indicates that prevention efforts have not been sufficiently effective. The discovery of new host species of the parasite, the increase in the red-fox population, their newfound infiltration in cities, and the establishment of an urban cycle are likely to increase the risk of human infection and to modify risk factors. The inhabitants of rural areas are thus not the only individuals of concern. These elements, combined with increasing urbanization and the future appearance of a new category of at-risk humans (namely immunodepressed individuals), also evoke fear with regard to the risk of the disease's future emergence. Consequently, AE is part of a class of emerging diseases. Thus, an epidemiosurveillance is required now more so than ever, and *E. multilocularis* still represents a parasite of interest for public health.

Foreseeable disease-control measures include intensive deworming efforts of foxes in zones deemed to be high-risk, the regulation of their reproduction, and finally information for the general population, particularly on the subject of general hygiene and pet-deworming.

Finally, a consensus was reached on several points. It is necessary to work on a European level - rather than independently in each respective country. The detection techniques for *E. multilocularis* must be homogenized in order to obtain uniform epidemiosurveillance. Information exchanged during the conference must now be widely disseminated to other member countries of the European Union, and clear recommendations must be sent to the public. European institutions seem to represent an essential body for the collection of data, the homogenization of methods, and the communication of recommendations.

At the conclusion of this European symposium, it appeared as if the objectives prompting the conference's formation were met. The conference indeed enabled the comparison of information hailing from twenty European countries and provided a general understanding with respect to the extent of the endemic zone of *E. multilocularis*. Discussions were rich in ideas. An agreement was reached on certain points while others necessitate the pursuit of additional research.

This European symposium paved the way for the pursuit of concerted investigations in the European Union. A future meeting could be envisioned in order to agree on the details of parasite detection.

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LIST OF PRESENTATIONS AT THE EUROPEAN SYMPOSIUM ON DECEMBER 8-9, 2010

BAGRADE G. *Echinococcus* spp. found in carnivores in Latvia. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

BOUÉ F. and WORONOFF N. Segmental Sedimentation and Counting Technique: an adaptable method for qualitative diagnosis of *E. multilocularis* in fox intestines. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

BOUFANA B. Optimization of PCR assays for the specific detection of *E. granulosus*, *E. multilocularis*, *E. shiquicus* DNA extracted from tissues and canid faecal samples. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

CASULLI A. Genetic diversity of *E. multilocularis* in Hungary inferred by multi-locus microsatellite analysis. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

COMBES B. Large scale screening of red fox intestines in search of *E. multilocularis* in France. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

COMTE S. and RATON V. Urban control of *E. multilocularis* in France. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

GAWOR J. *Echinococcus multilocularis* in final hosts in Poland – results of ten years survey. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

GIRAUDOUX P. Epidemiological trends of human alveolar echinococcosis in Franche-Comté from 1980 to 2010. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

GLAWISHNIG W. Epidemiological human situation and studies in foxes in Austria. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

GOTTSTEIN B. Current facts and trend in human alveolar echinococcosis. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

GRENOUILLET F. Human alveolar echinococcosis in France, update 2010. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

HAVE P. Scientific opinion of the AHAW, 18th of January 2007. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

HEGGLIN D. Control of *E. multilocularis*: epidemiological and strategic considerations. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

HEMPHILL A. *In vitro* screening for new compounds against *E. multilocularis* metacestodes identifies anti-echinococcal activity of mefloquine. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

JANKO C. Rural settlements – a habitat for foxes and the fox tapeworm? *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

KARAMON J. Monitoring of the prevalence of *E. multilocularis* in red foxes in Poland. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

KNAPP J. Genetics and methodology introduction – Phylogeny and genotyping studies in Cestodes to trace back their molecular evolution history. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

KÖNIG A. Succesful long time baiting campaing against the fox tapeworm in Southern Bavaria. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

KORTBEEK T. *Echinococcus multilocularis* in the Netherlands: what about the human situation?. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

MITERPAKOVA M. Ten-year of *E. multilocularis* occurrence in Slovakia. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

QUINTAINE T. Capability of *Echinococcus multilocularis* to persist within fox home ranges of increasing sizes depending on vole distribution and fox behavior. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

ROBARDET E. Fox defecation behavior in relation to spatial distribution of volves in an urbanized area: an increasing risk of transmission of *E. multilocularis*. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

ROMIG T. *Echinococcus multilocularis* in animals: geographical distribution an host species. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

SARKUNAS M. Helminths of red foxes and raccoon dogs in Lithuania. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

SCHWARZ S. *Echinococcus multilocularis* in Germany. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

SCHWEIGER A. Human alveolar echinococcosis in Switzerland 1956-2006. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

ŠOBA B. Serological evidence for human alveolar echinococcosis in Slovenia (2006-2010). *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

TAKUMI K. Increasing risk of human alveolar echinococcosis in the Netherlands and possible control options. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

UMHANG G. Domestic dog situation for *E. multilocularis* in different endemic areas in France. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

VERVAEKE M. *Echinococcus multilocularis* epidemiology in flemish Belgium. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

VUITTON D.A. Alveolar echinococcosis emergence in patients with immune deficiency: what is the epidemiological impact?. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

WHALSTROM H. Demonstrating freedom from *Echinococcus multilocularis* in Sweden, Norway mainland and Finland. *In*: Symposium européen sur l'échinococcose alvéolaire. Nancy, 8-9 décembre 2010

Appendix 1. Poster Produced by ERZ on Alveolar Echinococcosis Prevention

Appendix 2. Leaflet Produced by ERZ on Alveolar Echinococcosis Prevention

Appendix 3. Abstracts of European Symposium on Alevolar Echinococcosis, December 8-9, 2010

ECHINOCOCCUS MULTILOCULARIS IN ANIMALS: GEOGRAPHICAL DISTRIBUTION AND HOST SPECIES

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Abstract

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

ECHINOCOCCUS SPP. **FOUND IN CARNIVORES IN LATVIA.**

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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.

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Abstract

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

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

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Abstract

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

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

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

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Abstract

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

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

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

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

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

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Abstract

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

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

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

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

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

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

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

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

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

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Abstract

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

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

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

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

ECHINOCOCCUS MULTILOCULARIS IN GERMANY.

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Abstract

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

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

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Abstract

Alveolar echinococcosis is one of today major zoonoses. Its infective 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 250,000km^2) by night shooting and trapping. Average prevalence in each Department shows great disparities going from 0% to 52%.

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

ECHINOCOCCUS MULTILOCULARIS IN DOGS OF TWO ENDEMIC AREAS IN FRANCE.

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Abstract

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

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

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

CURRENT FACTS AND TRENDS IN HUMAN ALVEOLAR ECHINOCOCCOSIS.

B. Gottstein

Abstract

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

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

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

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Abstract

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

HUMAN ALVEOLAR ECHINOCOCCOSIS IN FRANCE, UPDATE 2010.

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Abstract

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

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

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

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

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Abstract

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

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

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

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Abstract

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

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

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

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

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

HUMAN ALVEOLAR ECHINOCOCCOSIS IN SWITZERLAND FROM 1956 TO 2008.

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Abstract

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

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

We will also present the first geographical analysis of AE cases in Switzerland.

In conclusion we communicate the changing trends in epidemiology of human AE in Switzerland over the last 53 years.

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

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Abstract

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

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

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

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

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

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Abstract

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

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

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

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Abstract

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

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

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

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Abstract

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

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

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

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Abstract

Cystic and alveolar echinococcoses are co-endemic on the Qinghai-Tibetan plateau of western China (Qiu *et al.*, 1995; Li *et al.*, 2007; 2009). The causative organisms, *Echinococcus granulosus* (*sensu stricto*) and *E. multilocularis* occur sympatrically with *E. shiquicus.* The infectivity of this latter species to humans is unknown (Xiao *et al.*, 2005; Li *et al.*, 2008). The species-specific optimization of PCR assays (van der Giessen *et al.*, 1999; Abbasi *et al.*, 2003; Štefanić *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 $MgCl_2$ and PCR buffers. Specificity of the assay was assessed using a panel of closely related DNA from tapeworm tissue and infected canid faeces and was found to be 100% specific. The three sets of primers detected at least 1.22×10^{-3}ng/ul of target DNA. In addition, *E. granulosus* primers were able to detect at least 1 egg from faeces. The *E. granulosus* and *E. multilocularis* primers were validated independently (AFSSA laboratories, France). A new approach was applied for maximizing the detection of low copy numbers of faecal target DNA.

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

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Abstract

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

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

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

CONTROL OF *ECHINOCOCCUS MULTILOCULARIS*: EPIDEMIOLOGICAL AND STRATEGIC CONSIDERATIONS

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Abstract

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

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

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

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Abstract

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

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

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Abstract

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

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

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Abstract

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

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

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

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

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

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

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

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

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

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Abstract

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

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

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

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

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

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Abstract

A baiting strategy against E. multilocularis in foxes was conducted in the Starnberg district including the city of Starnberg. Air distribution of baits in agricultural and recreational areas was combined with the distribution of baits by hand in the district's towns and villages in order to cover the entire fox population (Baiting area: 213 km^2). Within the first year, baits were put down monthly and within the following years in six weeks intervals. Bait distribution density was 50 pieces/km^2. The pre-baiting prevalence was 51% (45-57% CI 95%, N=286). During a one-year period following the first 4 months of bait distribution, only one positive fox was found (prevalence 1%; 0-7% CI 95%). Prevalence rates declined to 2% (2007), 3% (2008) and 2% (2009)/and could be manifested under a 3% level. In contrast, no significant change had occurred in the untreated control area.

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

URBAN CONTROL OF *ECHINOCOCCUS MULTILOCULARIS* IN FRANCE.

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Abstract

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

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

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

Appendix 4. Program of European Symposium on Alveolar Echinococcosis, December 8-9, 2010

Wednesday 8th December 2010

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

Thursday 9th December 2010

09:00-09:35	**A**	**GENETICS AND METHODOLOGY INTRODUCTION** Phylogeny and genotyping studies in Cestodes (*Echinococcus* and *Taenia*) to trace back their molecular evolution history	Knapp J.	France
09:35-09:50	A	Genetic diversity of *Em* in Hungary inferred by multi-locus microsatellite analysis	Casulli A.	Hungary, Italia
09:50-10:00	QUESTIONS			
10:00-10:15	A	Optimization of PCR assays for the specific detection of *E. granulosus* (G1 Genotype), *E. multilocularis*, *E. shiquicus* DNA extracted from tissue and canid faecal samples	Boufana B.	United-Kingdom
10:15-10:30	A	Segmental Sedimentation and Counting Technique (SSCT): an adaptable method for qualitative diagnosis of *Echinococcus multilocularis* in fox intestines	Woronoff N. Boue F.	France
10:30-10:40	QUESTIONS			
10:40-11:00	***COFFEE BREAK***			
11:00-11:30	**C**	**CONTROL OF *ECHINOCOCCUS MULTILOCULARIS*: EPIDEMIOLOGICAL AND STRATEGIC CONSIDERATIONS**	**Hegglin D.**	**Switzerland**
11:40-11:55	C	Scientific Opinion of the AHAW, 18th of January 2007	Have P.	EFSA
12:00-12:15	C	Rural settlements – A habitat for foxes and the fox tapeworm?	Janko C.	Germany
12:20-12:35	C	Fox defecation behaviour in relation to spatial distribution of voles in an urbanized area: An increasing risk of transmission of *Echinococcus multilocularis*	Robardet E.	France
12:40-12:55	C	Capability of *Echinococcus multilocularis* to persist within fox home ranges of increasing sizes depending on vole distribution and fox behaviour	Quintaine T.	France
13:00-13:15	C	Demonstrating freedom from *Echinococcus multilocularis* in Sweden, Norway mainland and Finland	Whalstrom H.	Sweden
13:30-14:45	***LUNCH***			
14:45-15:00	C	Increasing risk of human alveolar echinococcosis in the Netherlands and possible control options	Takumi K.	Netherlands
15:05-15:20	C	Successful long time baiting campaign against the fox tapeworm (*Echinococcus multilocularis*) in Southern Bavaria	Konig A.	Germany
15:25-15:40	C	Urban control of *Echinococcus multilocularis* in France	Comte S. Raton V.	France
15:45-16:15	***TEA BREAK***			
16:20-16:40	**WORKSHOP A CONCLUSIONS**		Pozio E.	
16:50-17:10	**WORKSHOP B CONCLUSIONS**		Bresson-Hadni S.	
17:20-17:40	**WORKSHOP C CONCLUSIONS**		Boue F.	

Appendix 5. Protocol for Handling Foxes as part of ERZ's Night-Hunting Operations

Appendix 6. Protocol for Gathering Feces Published by ERZ

MÉTHODES DE DÉTECTION ET BILAN D'EXTENSION DE L'ÉCHINOCOCCOSE VULPINE EN EUROPE DEPUIS 2000

Synthèse bibliographique et restitution des informations échangées au cours du symposium européen des 8 et 9 décembre 2010

NOM et Prénom: DESCLOIX Anne-Laure

Résumé:

L'échinococcose alvéolaire est une zoonose due au cestode *Echinococcus multilocularis* dont l'hôte définitif principal est le renard roux (*Vulpes vulpes*). Depuis la fin des années 1990, les études menées indépendamment au sein des pays européens, indiquent une évolution de la situation épidémiologique du parasite. L'Entente Rage Zoonoses (ERZ) a organisé, les 8 et 9 décembre 2010, un symposium européen afin de confronter les données européennes et d'actualiser les connaissances. Ce travail de thèse propose une synthèse des données bibliographiques sur le cestode *E. multilocularis*, mises à jour par les informations du congrès.

Tout d'abord, des éléments généraux concernant *E. multilocularis* sont présentés notamment la découverte de nouveaux hôtes intermédiaires. Au sein des hôtes définitifs, le renard roux se caractérise par une augmentation de la densité des populations et une urbanisation. Les méthodes de détection du parasite sont ensuite décrites. La diversité des techniques utilisées à l'échelle européenne nécessite une uniformisation de manière à pouvoir comparer les résultats. Les données épidémiologiques sont quant à elles dominées par l'extension de l'aire de répartition géographique du parasite. La prévalence peut atteindre des valeurs élevées à l'échelle locale et l'échinococcose alvéolaire n'est plus aujourd'hui une maladie propre aux zones rurales. Enfin, les méthodes de lutte incluent la prévention des catégories humaines à risque, le contrôle des populations de renards et la vermifugation des hôtes définitifs.

La perte récente du statut indemne de la Suède est le témoin de la dynamique actuelle de l'infestation par *E. multilocularis* et indique l'importance à accorder à la surveillance épidémiologique dans les pays indemnes. A l'issue du symposium, certaines interrogations restent en suspens alors que d'autres ont abouti à un consensus. Cette rencontre ouvre la voie à la réalisation d'études à l'échelle européenne.

Mots clés:
ECHINOCOCCUS MULTILOCULARIS, ECHINOCOCCOSE ALVEOLAIRE, ZOONOSE, SURVEILLANCE EPIDEMIOLOGIQUE, DETECTION, RENARD, RENARD ROUX, *VULPES VULPES*

Jury:
Président: Pr.
Directeur: Pr. Jacques GUILLOT
Assesseur: Dr. Pascal ARNE
Invité: M. Benoit COMBES

DETECTION TECHNIQUES AND EVALUATION OF EXTENSION IN EUROPE OF VULPINE ECHINOCOCCOSIS SINCE 2000

Review of the literature and of new data presented at a European symposium held on December 8 and 9, 2010

SURNAME: DESCLOIX

Given name: Anne-Laure

Summary:

Alveolar echinococcosis is a zoonosis caused by *Echinococcus multilocularis,* a tapeworm whose major final host is the red fox (*Vulpes vulpes).* Studies conducted since the early 1990’s in several European countries demonstrated a change in the epidemiology of the parasite. The ERZ organized, in December 2010, a European symposium designed to review and update current data on the subject. This thesis is a review of the literature completed by new data presented at that congress.

In the first part, general information about the biology of *E. multilocularis* is reviewed. Among definitive hosts, the red fox is characterized by an increase in population density and urbanization. A variety of techniques are being used in Europe to detect the parasite. Some standardization appears necessary to make results comparable from country to country. The major feature in the epidemiology of the parasite is the extension of its geographic distribution. Its prevalence is high in certain regions so that alveolar echinococcosis can no longer be considered as an exclusively rural disease. Control strategies should include the prevention of human contamination by control of fox populations and by routine deworming of final hosts, including dogs.

Reports on the recent introduction of *E. multilocularis* in Sweden demonstrate the current dynamics of the parasite population and points out the importance of epidemiological monitoring across Europe. At the end of the symposium, some aspects remained controversial while other scientific questions led to a consensus. This meeting paved the way to the initiation of cross-European studies.

Keywords:
ECHINOCOCCUS MULTILOCULARIS, ALVEOLAR ECHINOCOCCOSIS, ZOONOSIS, EPIDEMIOLOGICAL MONITORING, DETECTION, FOX, RED FOX, *VULPES VULPES*

Jury:
President: Pr.
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Guest: Mr. Benoit COMBES