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Veterinary aspects of alveolar echinococcosis — a zoonosis of public health significance

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Abstract

Human alveolar echinococcosis (AE), caused by the metacestode stage of *Echinococcus multilocularis*, is a serious zoonosis which caused up to 100% lethality in untreated patients before the 1970s, when modern methods of treatment were not yet established. AE occurs in large areas of the northern hemisphere mostly with low country-wide prevalences, but high prevalences of up to 4% have been reported from small population groups in highly endemic foci, e.g. from China.

AE includes many veterinary aspects which are the topic of this review. Recent studies have shown that *E. multilocularis* has a wider geographic range than previously anticipated. There is evidence for growing populations of red foxes (*Vulpes vulpes*) in some areas, for increasing invasion of cities by foxes and also for establishment of the parasite cycle in urban areas. These and other factors may lead to an increased infection risk for humans. Significant progress has been made in the development of sensitive and specific new techniques for the intra vitam and post mortem diagnosis of intestinal *E. multilocularis* infection in definitive hosts, notably the detection of coproantigen by enzyme-linked immunosorbent assay and of copro-DNA by PCR. Both tests can also be used for the identification of *E. multilocularis* in faecal samples collected in the environment. Recommendations are given for chemotherapy and chemoprophylaxis of the intestinal infection in definitive hosts.

In recent years, infections with the metacestode stage of *E. multilocularis* have not only been diagnosed in humans in several regions, including at least eight countries in central Europe, but also in animal species which do not play a role in the transmission cycle (wild and domestic pigs, dogs etc.). From 1987 to 2000 our group in Zurich has diagnosed 10 cases of AE in dogs and 15 in captive monkeys. In 2 dogs, concurrent infections of the intestine and of the liver with adult and larval stages of *E. multilocularis,* respectively, were observed for the first time. Clinical data are presented, and methods of diagnosis and treatment (surgery, chemotherapy) are described. Furthermore, small liver lesions caused by *E. multilocularis* were diagnosed in 10% of 90 slaughter pigs, and 2.9% of 522 breeding sows had specific serum antibodies against parasite antigens.

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In view of the unpredictable epidemiological situation, all possible measures for preventing *E. multilocularis* infections in humans and in domestic animals should be initiated by the veterinary and health authorities.

Keywords: Echinococcus multilocularis; Alveolar echinococcosis; Humans; Animals; Carnivores; Epidemiology; Pathology; Diagnosis; Treatment

1. Introduction

Alveolar echinococcosis (AE) in humans, caused by the metacestode stage of *Echinococcus multilocularis*, is a zoonosis of public health significance as the parasite in the human host behaves like a malignant tumour, predominantly of the liver. Lethality rates were approximately 100% in certain groups of untreated patients before the 1970s when modern methods of treatment were not yet established (Ammann and Eckert, 1996; Eckert, 1998). Treatment is now possible by surgery and chemotherapy but is costly, and complete cure is rarely achieved. Therefore, concern about the current epidemiological situation is justified, but the public awareness of the potential public health risk posed by AE is still low and countermeasures have not yet been established in most of the endemic countries.

AE as a zoonosis has many veterinary aspects, as wild and domestic carnivores are definitive hosts of *E. multilocularis*. Veterinarians are concerned with epidemiology, diagnosis, treatment and control of the infection in definitive hosts. Furthermore, the infection of certain animal species (pigs, dogs, monkeys, etc.) with the metacestode stage has recently been identified as a new problem in veterinary medicine. These veterinary aspects are the topic of this review.

2. Life cycle and biology of the parasite

The adult stage of *E. multilocularis* that inhabits the small intestine of carnivores is a cestode of only 1.2–4.5 mm length. The parasite is typically perpetuated in a wildlife (=sylvatic) cycle including foxes (genera *Vulpes* and *Alopex*) as definitive hosts and small mammals, predominantly rodents, as intermediate hosts. In some endemic areas, other species of wild carnivores, such as coyote, wolf, and raccoon dog, are involved as definitive hosts. In addition, a synanthropic cycle is known to exist in various epidemiological situations with domestic dogs and cats as definitive hosts, which acquire the infection from rodents originating from the wildlife cycle (Thompson, 1995; Eckert, 1998).

Surprisingly, little detailed information is available on the development and reproduction of *E. multilocularis* in definitive hosts, probably because of the biohazard risks associated with experimental work. In his classical studies, Vogel (1957) in Germany experimentally infected 11 dogs, four red foxes (*V. vulpes*) and six cats with fertile metacestodes (protoscoleces not quantified) of *E. multilocularis*. Egg excretion in the faeces was detected in seven dogs from day 35 post infection (p.i.) onwards, lasting for 1–14 days in six dogs and for 55 days (i.e. 90 days p.i.) in one dog. Three of the four foxes excreted eggs from day 36 onwards for 1–19 days, but only one cat excreted eggs from day 36 p.i. during 14 days.

In a later study in Switzerland, adults of *E. multilocularis* with mature eggs were found as early as 28 days p.i. in experimentally infected dogs (Thompson and Eckert, 1983). More recently Japanese workers (Nonaka et al., 1996) infected four red foxes with approximately 150,000 protoscoleces of *E. multilocularis.* After the first detection of parasite eggs 29–33 days p.i. in faecal samples, egg excretion was rather regular within the first 2–4 weeks of patency, but thereafter, became irregular and lasted only for about 4–7 weeks. According to another report from Japan (Yagi et al., 1996), based on data from 10 experimentally infected dogs and four red foxes, the prepatent period of *E. multilocularis* may be as short as 26 days and the patency period may last for about 1.5–4 months. The survival time of the parasite in definitive hosts is estimated to be about 5 months (Eckert, 1998).

Parasite eggs released from definitive hosts to the environment are immediately infective to intermediate hosts in which the metacestodes develop primarily in the liver. The metacestode stage consists of aggregations of small vesicles (cysts) in which protoscoleces are produced by the germinal layer in natural intermediate hosts. The cyst aggregates form alveolar structures composed of numerous cysts of irregular shapes with dimensions between less than 1 and 10 mm (in some hosts up to 20–30 mm). The cysts contain a highly variable number of protoscoleces or they may be sterile (no protoscolex formation) and partially calcified. In the human host, protoscoleces are rarely produced. Besides rodents, a number of non-rodent mammalian animals and humans may also acquire an *E. multilocularis* infection by egg ingestion. Such hosts, which do not play a role in the transmission cycle, are denominated as aberrant hosts.

In contrast to the hydatid cysts of *E. granulosus,* the metacestode stage of *E. multilocularis* has the capacity to proliferate by external budding and to form root-like protrusions of tissue, enabling the parasite to progressively invade host organs (Thompson, 1995; Eckert, 1998). There is strong evidence that the invading parasite tissue is protected from direct host effector immune mechanisms by the carbohydrate-rich laminated layer containing the immunodominant Em2 antigen (Gottstein et al., 1994; Gottstein and Hemphill, 1997). This layer is formed during in vitro cultivation of activated *E. multilocularis* oncospheres within 13 days of early cyst development (Deplazes and Gottstein, 1991), but is likely to be generated faster in vivo. Metacestode development is rapid in short-lived intermediate hosts with protoscoleces being produced by asexual proliferation of the germinal layer within 40–45 days (Eckert, 1998). In the field situation, the number of protoscoleces per infected *Arvicola terrestris* (N: 25) varied between 14 and 244,400 (mean 38,305, median 4400) as determined in an ongoing study in the city of Zurich (Switzerland) (Stieger and Deplazes, unpublished). However, other rodent species that are more susceptible to *E. multilocularis* than *A. terrestris* may have even a higher capacity for protoscolex formation.

3. Geographical distribution

The geographical distribution of *E. multilocularis* is confined to palaearctic regions of the northern hemisphere extending from central Europe throughout northern and central Eurasia to the Far East, including Japan, and to North America (Alaska, Canada, northern and central USA) (Schantz et al., 1995; Eckert et al., 2000; WHO/OIE, 2001). In central Europe, recent surveys on *E. multilocularis* infection in red foxes have revealed that the parasite has a

much wider geographical distribution than previously reported. Whether new findings of *E. multilocularis* in a given area reflect a recent extension of the parasite's range or just the first identification of a hitherto unknown endemic area cannot be determined, because surveys have not been performed in previous periods. By the end of the 1980s, endemic areas were known to exist in Austria, France, Germany, and Switzerland, but at this time, the documented geographic range of *E. multilocularis* also includes regions of Belgium, the Czech Republic, Denmark (Copenhagen), Liechtenstein, Luxembourg, Poland, the Slovak Republic and The Netherlands (Eckert and Deplazes, 1999; Kolarova, 1999; Romig et al., 1999a; Eckert et al., 2000; WHO/OIE, 2001). To our knowledge, only one endemic focus has been found south of the Alps, in northern Ticino (Switzerland), but additional surveys are currently being carried out in Italy. It is likely that further surveys will also detect *E. multilocularis* in other regions.

4. Epidemiology

Only selected aspects of epidemiology are discussed in this paper, as the present state of knowledge has been reviewed recently (Eckert et al., 2000; WHO/OIE, 2001).

4.1. The E. multilocularis infection in wild red foxes (V. vulpes)

In the central European endemic area, foxes appear to be responsible for most of the environmental contamination with *E. multilocularis* eggs. This was concluded from a study in which the prevalences of *E. multilocularis* in foxes, dogs and cats were related to the estimated population sizes of these definitive hosts (Eckert and Deplazes, 1999). In a given fox population, the distribution of the individual *E. multilocularis* burdens is over-dispersed. Therefore, a few highly infected foxes (carrying thousands of fertile worms) can be responsible for most of the environmental egg contamination in a specified area. For example, 7.5% of a Swiss fox population (10/133) harboured more than 72% of the total worm number with a maximum individual worm burden of about 57,000 (Hofer et al., 2000).

Prevalences of *E. multilocularis* in red foxes differ widely within and between endemic areas from about 1% to over 60% (EurEchinoReg, 1999; Lucius and Bilger, 1995; Romig et al., 1999a; Eckert et al., 2000). Furthermore, there are reports on increasing *E. multilocularis* prevalences in some regions (Romig et al., 1999a), but additional information is needed. Variations of parasite prevalences of fox populations have been observed in both rural and urban environments (Tackmann et al., 1998; Hofer et al., 2000), but care is needed when comparing such data (Eckert et al., 2000; Romig et al., 1999a). Seasonal variations with significantly higher *E. multilocularis* prevalences in fox populations during the winter period have been observed, e.g. in southern Germany (Schelling et al., 1991) and in an urban area of Switzerland (Hofer et al., 2000). Information on the relationship between fox age and prevalence of *E. multilocularis* is conflicting. In some studies, prevalences were significantly higher in juvenile foxes as compared to animals exceeding 1 year of age, whereas in other studies no significant age-dependent differences were detected (Tackmann et al., 1998; Hofer et al., 2000). In a high-endemic area of northern Germany, young foxes were significantly more frequently infected with *E. multilocularis* than adults, whereas under

conditions of low endemicity the prevalences tended to be higher in adult foxes (Tackmann et al., 1998). In the 1990s, at least of 71,000 foxes were examined in Europe for *E. multilocularis* (Germany, approximately 57,200; Austria, approximately 3500; Switzerland, approximately 7400; Poland, approximately 2900) (EurEchinoReg, 1999). However, most of these preliminary surveys were restricted to the determination of parasite prevalences in local geographical areas. Only a few studies were focused on the role of rodent populations and geological, climatological and land-use parameters and their potential influence on transmission patterns in the cycle of *E. multilocularis* (e.g. Tackmann et al., 1998; Giraudoux et al., 1996; Viel et al., 1999; WHO/OIE, 2001). In the future, retrospective analyses of collected data, as well as defined prospective ecological analyses, will hopefully elucidate additional key factors in parasite transmission dynamics.

4.2. Ecological changes in fox populations

Ecological changes may have influenced the epidemiological situation of AE in the past in several regions, e.g. China (Craig et al., 2000; Schantz et al., 1995). In parts of Europe, the fox population densities have increased from 1985 onwards (Artois et al., 1997; Breitenmoser et al., 2000), following successful oral vaccination campaigns against rabies in many countries. The invasion of villages and cities by red foxes and the establishment of urban cycles of *E. multilocularis* is another important phenomenon. Red foxes have been reported in urban areas in Great Britain since the 1930s, and during the 1970s and 1980s urban fox populations have been established in several cities with fox densities reaching up to five family groups per km^2 (Harris, 1981; Harris and Rayner, 1986). As these observations were unique world-wide, urban foxes were initially thought to be an isolated British phenomenon (Macdonald and Newdick, 1982). However, a similar development was recently reported from the cities of Oslo (Norway), Arhus (Denmark), Toronto (Canada), Sapporo (Japan), and Zurich (Switzerland) (Gloor et al., 2001). In Switzerland, an inquiry of town officials revealed that foxes are present in 28 out of the 30 largest cities. They are more often observed in larger cities than in smaller towns, and breeding dens have been found in 20 of these cities (Gloor et al., 2001). In the city of Zurich, where hunting and a reporting system are maintained by official game wardens, the number of foxes shot or found dead in the entire city has increased 20-fold from 1985 to 1997 (Gloor et al., 2001). The presence of foxes in urban settings has important implications for the management of fox populations and for control and prevention of zoonoses (e.g. rabies and AE). It may also influence human behaviour and the attitude of people towards urban wildlife (Bontadina et al., 2001).

4.3. Infection pressure in urban areas

In recent years, the occurrence of *E. multilocularis* in urban foxes has been reported from several European cities, e.g. Copenhagen, Geneva, Munich, Stuttgart, Zurich (Romig et al., 1999a; Hofer et al., 2000; Kapel and Saeed, 2000) and from Sapporo (Japan) (Tsukada et al., 2000). A preliminary study on the epidemiological role of red foxes in the urban area of Sapporo was based on the detection of parasite coproantigen in faecal samples collected around active fox dens, most of them located on the urban fringe (Tsukada et al.,

2000). Thirty-three of the 155 samples (21%) which tested positive originated from the surroundings of 11 dens including the one located in the urban area. In this study, *E. multilocularis* was not detected in a small number of 23 rodents at necropsy. A study in Zurich (Switzerland) revealed *E. multilocularis* prevalences of 47% (61/129) in foxes from the city and of 67% (82/123) in foxes from the adjacent rural area during the winter period; prevalences in the summer were lower (Hofer et al., 2000). Furthermore, metacestodes of *E. multilocularis* were found in 14% (19/135) of water voles (*A. terrestris*) in a Zurich city park, and two animals harboured protoscoleces of the parasite (Hofer et al., 2000). Recent investigations on 609 faecal specimens from foxes collected in recreation areas within the city revealed 25% coproantigen-positive samples. Of special interest is the fact that a considerable number of coproantigen-positive samples were found directly on vole ground systems where traces of hunting activities by foxes were observed (Hegglin and Deplazes, unpublished). These data provide strong evidence for the existence of urban wildlife cycles of *E. multilocularis.* Hence, a potential infection risk exists not only for urban residents, but also for urban domestic dogs and cats which may acquire the infection by preying metacestode-infected rodents. These pet animals then may represent an additional infection source for humans.

4.4. E. multilocularis infection in dog and cat populations

In certain epidemiological situations in Alaska and China, high prevalences of *E. multilocularis* have been found in domestic dogs (1–12%) which have apparently played the main role in the transmission of the infection to humans (Schantz et al., 1995; Craig et al., 2000). The epidemiological significance of domestic carnivores is uncertain in other endemic areas like Europe, USA and Japan. Studies performed on dogs and cats at necropsy in France and Germany revealed local *E. multilocularis* prevalences of 0.5–5.6% (Pétavy et al., 2000; WHO/OIE, 2001). These necropsy studies, however, have the disadvantage that the animals represent a selected population and only a low number of animals can be examined. Currently, modern techniques allow surveys of larger populations of living animals. Low *E. multilocularis* prevalences of 0.3 and 0.4%, respectively, were found in Switzerland in 660 randomly selected living dogs and in 263 cats by detection of specific coproantigen and confirmation of positive results by PCR (Deplazes et al., 1999). Higher infection rates of 7% in 86 dogs and of 3.0% in 33 cats were discovered in a rural area of western Switzerland with a high prevalence of *E. multilocularis* in fox and rodent populations (Gottstein et al., 2001). Irrespective of the relative significance of dogs and cats for contaminating the environment with *E. multilocularis* eggs, it should be stressed that in endemic areas all dogs and cats which have access to rodents should be regarded as potential sources of human infection.

5. New epidemiological tools for detecting the intestinal infection in definitive hosts

In the past, parasite detection at necropsy was the only reliable method for diagnosing the *E. multilocularis* infection in definitive hosts. The intestinal scraping technique (IST) has been most commonly used in large surveys of fox populations. This method has a sensitivity of about 80% as compared to the sedimentation and counting technique (SCT)

(Hofer et al., 2000) which should be considered the "gold standard" for test comparisons (Table 1). Detection of serum antibodies is not suitable for estimating the prevalence of intestinal *E. multilocularis* infections in definitive hosts (Deplazes and Eckert, 1996). Two alternative approaches, the detection of *E. multilocularis*-specific coproantigens and of copro-DNA (Table 1), have recently been developed and evaluated, allowing the diagnosis of *E. multilocularis* in both necropsied and living definitive hosts as well as in faecal samples collected in the field.

5.1. Detection of coproantigens

Several groups have described enzyme-linked immunosorbent assays (ELISAs) for the detection of coproantigens released by *Echinococcus granulosus* or *E. multilocularis* in carnivores (Craig et al., 1996; Deplazes and Mathis, 1999). Coproantigens are detectable during the prepatent and the patent periods in dogs, foxes and cats, and disappear within a few days after the elimination of the cestodes from the host. Despite a tendency of higher ELISA absorbance values in heavily infected hosts, quantification of the infection intensity is not possible. Coproantigens remain stable for at least 5 days in fresh faecal samples stored at room temperature. As faecal material may contain *Echinococcus* eggs thereby posing an infection risk for the laboratory personnel, the samples should be decontaminated before further processing either by freezing at −80°C for 4 days or by heat-treatment at 70°C for 12 h (Nonaka et al., 1996; Deplazes et al., 1999).

Two groups have independently developed coproantigen tests using antibodies against *E. multilocularis* antigens (Kohno et al., 1995; Deplazes et al., 1999). In a study with experimentally infected dogs and cats, coproantigens were first detectable 6–17 days p.i. in samples of eight dogs (worm burdens at necropsy: 6330–43,200) and from 11 days p.i. onwards in samples of five cats infected with 20–6833 worms (Deplazes et al., 1999). The sensitivity of this ELISA was 83.6% in 55 foxes infected with 4–60,000 *E. multilocularis,* but reached 93.3% in the 45 foxes harbouring more than 20 worms. This test identified those animals harbouring approximately 99.6% of the total number of adult *E. multilocularis* in a fox population investigated. The specificity of the ELISA with regard to other non-*Echinococcus* helminths was 95.0–99.6% as shown by the examination of faecal samples from 32 foxes, 658 dogs, and 262 cats. The specificity was also surprisingly high (84%) in samples from 32 dogs naturally or experimentally infected with *E. granulosus* (Deplazes et al., 1999).

Two test kits for the detection of *Echinococcus* coproantigen have been developed by companies: *Echinococcus* ELISA® (Genzyme-Virotech GmbH, Rüsselsheim, Germany) and the Chekit® Echinotest (Dr. Bommeli AG, Liebefeld-Bern, Switzerland). Extended evaluations of these tests for detecting *E. multilocularis* are currently being carried out in independent laboratories.

5.2. Detection of copro-DNA

Echinococcus DNA originating from parasite eggs, proglottids or cells can be detected in faecal material of definitive hosts after amplification by PCR. DNA isolation from native

Table 1
Characteristics of test systems for diagnosing *Echinococcus multilocularis* (*E. m.*) infection in definitive hosts (modified after Deplazes and Mathis (1999))

Test system	Test characteristics: sensitivity (S) and specificity (SP) for *E. m.*	Number of animals/samples that can be investigated per person and day
Sedimentation and counting technique (SCT) (Hofer et al., 2000)	S and SP: ~100%; reference method, precise quantification, application at necropsy, laborious for routine screening; polyspecific for intestinal helminths	10 animals (necropsy included)
Intestinal scraping technique (IST) (Hofer et al., 2000)	S: 78% (compared with SCT), SP: ~100%; semi-quantitative, application at necropsy; polyspecific for intestinal helminths; parasitological routine test at necropsy	20 animals (necropsy included)
Coproantigen ELISA (Deplazes et al., 1999)	S: ~80% (compared with SCT), SP: 95–99% (on genus level), coproantigen detectable during prepatent infection, in vivo and post mortem diagnosis possible, can be used for faecal samples collected in the environment; suited for routine mass-screening	200 samples
Coproantigen ELISA (Sakai et al., 1998)	S: ~87% (compared with SCT), SP: ~70% (on genus level); test characteristics see above	200 samples
Combined microscopy/PCR[a] (Mathis et al., 1996)	S: 94% (compared with SCT), SP: 100%; in the first step (microscopy) polyspecific for helminth eggs (except taeniid eggs!), PCR detects *E. m.* eggs only, confirmation test for coproantigen positive results or for identification of *E. m.* eggs	15 samples
PCR[b] (Dinkel et al., 1998)	S: 89% (compared with IST), SP: 100%; total DNA isolation from faeces allows detection of *E. m.* eggs and parasite tissue during prepatency and patency, in vivo and post mortem diagnosis possible, can be used for faecal samples collected in the environment	15 samples

[a] Target gene: U1 sn RNA gene (Bretagne et al., 1993).
[b] Target gene: mt12S rRNS gene (Dinkel et al., 1998).

faeces is either achieved by alkaline lysis (Bretagne et al., 1993) or by boiling the samples in 0.5% SDS followed by proteinase K digestion (Van der Giessen et al., 1999). Due to the presence of substances that are inhibitory for DNA amplification, extensive purification of the DNA is indispensable (Deplazes and Mathis, 1999). To overcome such limitations, an initial step of concentrating helminth eggs by a combination of sequential sieving with an in-between step of flotation in zinc chloride solution has been implemented (Mathis et al., 1996).

Two different genes coding for U1 snRNA gene (Bretagne et al., 1993) and the mt12S rRNA gene (Dinkel et al., 1998) have been used in the diagnostic PCR for detecting *E. multilocularis* DNA in faecal samples of foxes. In an initial study using other tapeworm species for comparison, the single primer pairs described by Bretagne and co-workers were shown to be species-specific for *E. multilocularis.* However, in another laboratory, this strict species-specificity of these primers could not be confirmed, as a product of the expected size was also obtained with a horse strain of *E. granulosus* (Van der Giessen et al., 1999). The species-specificity for *E. multilocularis* of the nested PCR described by Dinkel et al. (1998) targeting the mt12S rRNA gene was confirmed with three isolates of *E. granulosus,* several *Taenia* spp. isolates and nematode species of fox origin. This test showed a high diagnostic specificity and sensitivity (Table 1) and the capacity to detect prepatent infections.

5.3. New diagnostic strategies

PCR has proven its value for diagnosis of *E. multilocularis* in definitive hosts, however, DNA isolation from faecal specimens remains very laborious and this method is unsuitable for large-scale studies (Table 1). The method of choice for this purpose is coproantigen detection by ELISA, especially in animal populations with a low prevalence of *E. multilocularis*, resulting in a very high negative predictive value of this test. As the positive predictive value of the ELISA is relatively low for such populations, positive ELISA results need further confirmation with the more laborious PCR (Deplazes and Mathis, 1999).

An important advantage of the alternative methods is that they can also be applied to faecal samples collected in the field, thereby avoiding direct physical contact of the investigator with the carnivore hosts. *E. multilocularis* eggs are highly resistant in the environment, and the stability of coproantigens in faecal samples collected in the field was found to be sufficiently high for this approach (Deplazes and Mathis, 1999). Nonaka et al. (1998) and Tsukada et al. (2000) used coproantigen detection in fox faeces collected in the field for monitoring the infection pressure in Hokkaido (Japan). Parasite detection at necropsy in foxes originating from two areas of France with significantly different prevalences of *E. multilocularis* (14.7 and 65.3%) was compared with coproantigen detection in faecal samples collected in the field using two types of ELISAs (Deplazes et al., 1999; Kohno et al., 1995). The results of both coproantigen tests were in the range expected from the necropsy data, and it was concluded that this new strategy can be applied in epidemiological studies and fundamental research on transmission ecology (Raoul et al., 2000). Ongoing field studies in our laboratory aimed at the control of the *E. multilocularis* infection in urban fox populations by distributing praziquantel-containing baits (see below) have shown that detection of coproantigen and copro-DNA in fox faecal samples collected from the environment appear to be excellent tools for monitoring the progress of control and for

estimating the degree of egg excretion by definitive hosts to the environment (Hegglin and Deplazes, unpublished).

6. Control

Control of *E. multilocularis* is difficult because its main life cycle involves wild intermediate and definitive hosts. A control strategy by treatment of rural foxes with baits containing 50 mg praziquantel (20 baits per km^2 distributed by aircraft) is currently under evaluation. In two large scale studies in southern and northern Germany (Romig et al., 1999a; Tackmann et al., 1997), it has been shown that by regular praziquantel baiting the prevalence of the parasite can be significantly reduced. However, the long-term effects and the cost-effectiveness remain to be determined. Also, the application of this control option to restricted foci of disease transmission, e.g. urban areas, is currently being investigated (Deplazes, unpublished). In synanthropic cycles, dogs (or cats) may play a significant epidemiological role (see Section 4.4). In such situations, mass-treatment of dogs over prolonged periods may be considered (Schantz et al., 1995). In epidemiological situations with low endemicity in domestic carnivores, treatment of only those animals that have access to infected intermediate hosts may be an option, but the practical value of such measures remains to be determined (see also Section 7.3). Spreading of *E. multilocularis* by transporting dogs, cats, foxes or other definitive hosts from endemic to non-endemic areas should be prevented by treating these animals before shipment in a quarantine unit on two consecutive days with therapeutic doses of praziquantel (WHO/OIE, 2001).

7. Infection in definitive hosts

7.1. Pathology, symptoms and immunity

In the intestine of dogs, *E. multilocularis* attaches between the villi, and worms extend the apical region of the rostellum deeply into the crypts of Lieberkühn (Thompson and Eckert, 1983; Thompson, 1995). Although the epithelium of parasitised crypts is commonly flattened, no evidence of breakdown of the integrity of the crypts was seen in prepatent infections. However, the available data are limited. Natural and experimental intestinal infections of carnivores (foxes, dogs and cats) are typically asymptomatic, even in animals with very high individual worm burdens.

In dogs infected with *E. granulosus,* immunological responses to protoscolex, adult worm and oncospheral antigens occur, leading to the production of specific serum antibodies (Gasser et al., 1993) and lymphocyte cell proliferation in Payer's patches and regional lymph nodes (Deplazes et al., 1994). Attempts to stimulate protective immune responses by repeated infections with infective stages of *E. granulosus* or other taeniid cestodes or by vaccination have so far been unsuccessful or only partially successful (Heath, 1995; Ligthowlers and Gottstein, 1995).

There are several indications that some degree of acquired immunity to *E. multilocularis* may develop in foxes, but detailed knowledge is lacking. Field observations revealed that

young red foxes (<1 year) had significantly higher infection intensities of *E. multilocularis* than older foxes (Hofer et al., 2000). However, it is unclear whether these differences are due to the development of immunity in older animals or a higher exposure in young foxes.

7.2. In vivo diagnosis of the intestinal infection in dogs and cats

The diagnostic techniques available have been discussed above (Section 5). For routine diagnosis of the intestinal *E. multilocularis* infection in domestic dogs and cats, a coproantigen ELISA should be applied as a primary test. In positive cases, the PCR can be used as a secondary test. Furthermore, in carnivores in which taeniid eggs have been detected by routine coproscopy, PCR may be applied for the identification or exclusion of *E. multilocularis* eggs. With this strategy we detected 15 patent *E. multilocularis* infections in domestic dogs at our diagnostic centre in the last 5 years. It is of special interest to note that *E. multilocularis* is the most frequently diagnosed taeniid species in dogs in our laboratory while *T. hydatigena* and other *Taenia* species have become very rare in recent years. We recommended treatment or euthanasia of all dogs or cats with positive coproantigen and/or PCR results for *E. multilocularis* (see Section 7.3). Owners of positive dogs are supplied with an information sheet advising persons who have been possibly exposed to *E. multilocularis* eggs to contact a physician for serological testing within several weeks and to repeat serology 6 and 12 months later.

7.3. Therapy of the intestinal infection

Because domestic dogs or cats with intestinal *E. multilocularis* infections represent a potential risk for humans, they should be immediately euthanised or treated. For the treatment procedure, strict safety precautions are mandatory (WHO/OIE, 2001). Drugs with high efficacy against intestinal *E. multilocularis* infections are the isoquinoline derivatives praziquantel (Droncit® and other trade names) and epsiprantel (Cestex® and other trade names). Praziquantel is given to dogs and cats at doses of 5.0 mg/kg body weight (b.w.) per os or of 5.7 mg/kg b.w. intramuscularly. For cats, a formulation of the drug for topical application is now available (8.0 mg/kg b.w.) (Jenkins and Romig, 2000). A single dose of praziquantel eliminates virtually 100% of the worm burden (=high intensity effect) and eliminates the parasites from almost all animals (high extensity effect) (Eckert et al., 2001). Epsiprantel is applied per os at a dose of 5.5 mg/kg b.w. to dogs and of 2.75 mg/b.w. to cats. This drug showed a high intensity effect of 99.6 and 99.9% against *E. multilocularis* in two heavily infected groups of dogs and of 100% in cats. After a single treatment, all 10 cats were free of the parasite (extensity effect 100%), but in four of eight dogs relatively small residual burdens persisted (extensity effect 50%) (Eckert et al., 2001). In two studies, a single treatment with 5.0 mg/kg b.w. epsiprantel eliminated 99.9% of the *E. granulosus* burden from dogs, but in one of the studies, only two of five animals were parasite-free after treatment (Arru et al., 1990; Thompson et al., 1991).

In order to reduce the potential risk of residual worm burdens, we recommend in accordance with international expert groups (WHO/OIE, 2001) to treat dogs and cats with proven *E. multilocularis* infection on two consecutive days with a therapeutic dose of praziquantel.

The result of the treatment can be assessed 4–5 days after treatment by examinations for parasite coproantigen.

7.4. Prevention of infection in domestic definitive hosts

The new findings of *E. multilocularis* infected voles and dogs in urban and peri-urban areas underline the well-known fact that dogs and cats having access to infected rodents are at special risk (Schantz et al., 1995; Hofer et al., 2000). Considering the short prepatent period of *E. multilocularis,* monthly treatment with praziquantel or epsiprantel is recommended for such dogs or cats. Dogs used professionally as guide dogs, companion animals for children, police dogs etc. could be included into such a scheme in order to avoid any discussion about their potential role as sources of human infections. Regular treatment of all dogs and cats in endemic areas is difficult from the practical point of view, costly and has not been assessed regarding its epidemiological and prophylactic value.

8. Alveolar echinococcosis in humans

8.1. Incidence and prevalence of AE and risk of infection

The country-wide average annual incidences of new cases of human AE are not well documented in many regions. In some of the endemic regions, e.g. in central Europe and Japan, they varied between 0.03 and 1.2 per 100,000 population (Eckert and Deplazes, 1999; WHO/OIE, 2001). However, incidences up to 98 per 100,000 have been extrapolated from small population groups living in highly endemic foci, e.g. in Alaska (Schantz et al., 1995). In Switzerland, the country-wide average annual incidences of human AE did not vary markedly (0.10–0.18) during 36 years (1956–1992) suggesting a stable epidemiological situation (Eckert and Deplazes, 1999). The prevalence values are also of interest in assessing the current situation. Recently, a very high prevalence of 4% was recorded from a population group of 3331 persons in a focus in China (Craig et al., 2000), and a lower prevalence of 0.04% was found in a rural community of 2560 persons in southern Germany (Romig et al., 1999b).

Humans acquire *E. multilocularis* infection via the oral route, and it is assumed that egg transmission may occur (a) via contaminated hands after handling infected definitive hosts and after contact with contaminated soil or plants without taking proper hygienic precautions, and (b) by ingestion of food contaminated with eggs. Some reports suggest that egg transmission may also occur by waterborne routes (WHO/OIE, 2001). However, the relative significance of the various potential transmission routes has not yet been studied in detail.

8.2. Course of the infection

The tumour-like proliferation of the *E. multilocularis* metacestode in the human host may lead to severe disease which is often fatal if not diagnosed in the early stages and correctly treated. However, self-cure with encapsulation and calcification of the “died-out”

metacestode lesions has been observed in a few cases (Gottstein and Hemphill, 1997). It is important to note that AE has a long incubation period of about 5–15 years and clinical symptoms often occur in a rather late phase of the infection when large parts of the liver are already infiltrated by the parasite. The symptoms are vague and include *inter alia* cholestatic jaundice, epigastric pain, hepatomegaly and weight loss. Following primary infection, the liver is the predominantly affected organ (about 99%), but later on the parasite may spread to other organs by local or distant metastasis formation. Fatality rates in groups of untreated or inadequately treated patients have reached 94–100% within 10–15 years following diagnosis (Eckert, 1998).

8.3. Diagnosis and treatment

The diagnosis of human AE is based principally on the identification of parasite lesions by imaging procedures (e.g. ultrasound (US) examination, computer tomography) and detection of specific serum antibodies (Ligthowlers and Gottstein, 1995; WHO, 1996; Craig et al., 1996; WHO/OIE, 2001). Treatment still is difficult, and complete cure has only been achieved when AE was diagnosed early and the entire parasite lesion could be removed by surgery. Even in such cases post-operative chemotherapy for at least 2 years is recommended by WHO (WHO, 1996; WHO/OIE, 2001). Total or partial excision of parasite lesions (or organ segments) is also recommended for more progressed operable cases. In such cases and in inoperable patients (up to 80%; Ammann and Eckert, 1996), long-term chemotherapy with high daily doses of mebendazole or albendazole is mandatory for many years or even for life. From 1979 to1999, a total of 110 AE patients were included in a long-term chemotherapy study at a clinical centre in Zurich (Ammann et al., 1999). In this group the average duration of chemotherapy was 9.4 years. The cost of chemotherapy alone in Germany ranges from US $5500 to $17,800 per patient per year and can total $300,000 per patient for lifelong treatment (Romig et al., 1999b). Fortunately, chemotherapy is generally well tolerated, but in most cases it appears to be parasitostatic rather than parasitocidal. The 10-year survival rate of 110 inoperable or non-radically operated AE patients (including severe forms) on long-term chemotherapy increased to 80% as compared to 6% of untreated historical control patients (Ammann et al., 1999). In view of the malignancy of the disease, this should be considered a significant success. Currently, a total of about 390 patients are under continuous chemotherapy and medical supervision in France (190), Germany (110) and Switzerland (90) (EurEchinoReg, 1999, Petra Kern, pers. comm., 2001; Ammann, pers. comm., 2001).

9. Alveolar echinococcosis in animals

In recent years, a variety of mammalian animal species have been described from European countries and Japan as aberrant hosts of the metacestode stage of *E. multilocularis*, including domestic dogs, domestic and wild pigs, horses, monkeys, and the nutria (*Myocastor coypus*) (Eckert, 1996; Ohbayashi, 1996; Losson and Coignoul, 1997). Surprisingly, this list not only contains herbivorous or omnivorous animals, but also the dog that, as a carnivore, normally acts as definitive host. As in humans, the metacestode of *E. multilocularis* develops primarily in the liver of the aberrant hosts and may cause severe

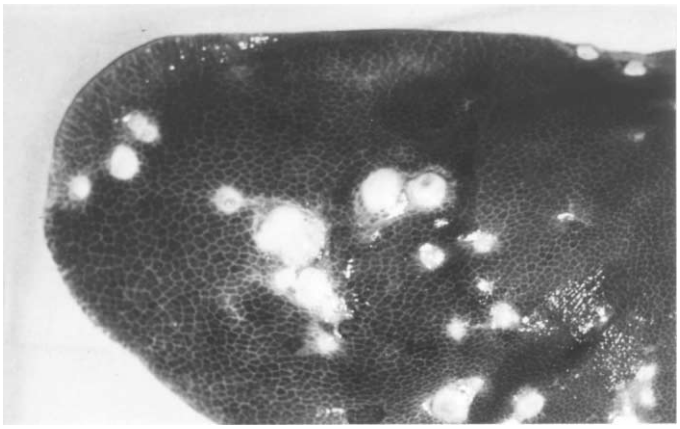

Fig. 1. AE in a domestic pig: liver lesions (3–10 mm in diameter) caused by *E. multilocularis* metacestodes.

forms of AE with fatal outcome. Such cases are not only of clinical interest, but also of epidemiological importance as they are indicators of environmental contamination with *E. multilocularis* eggs and the potential infection risk for humans. Some examples of AE in aberrant hosts are described here in more detail.

9.1. Alveolar echinococcosis in pigs

In Japan, small, nodular (1–20 mm) liver lesions with suppressed development of the metacestode of *E. multilocularis* have been described in pigs (Sakui et al., 1984; Ohbayashi, 1996). Similar observations were made in European wild boars infected with viable *E. multilocularis* metacestodes without development of protoscoleces (Pfister et al., 1993). In a recent study in Switzerland (Sydler et al., 1998), 10% of 90 slaughter pigs kept outdoors were found to have liver lesions in the form of sharply demarcated, dense white spots of about 0.2–1.5 cm in diameter (Fig. 1). Histological and molecular examinations have identified metacestodes of *E. multilocularis* as cause of these lesions, which could easily be differentiated from "milk spots" caused by larvae of *Ascaris suum* or *Toxocara* spp. In eastern Switzerland, 2.9% of 522 breeding sows (from 146 farms, 3–5 animals per farm) which had been fed with grass, had serum antibodies against the highly specific *E. multilocularis* metacestode antigen EmG11 (Deplazes and Gottstein, 1991), and animals from seven farms were seropositive (Deplazes, unpublished data). Furthermore, three sows were necropsied and multiple *E. multilocularis* metacestode lesions were diagnosed in all livers (Fig. 1) (Deplazes, unpublished). These infections in pigs are indicators for the environmental egg contamination which is also likely to be relevant for humans.

9.2. Alveolar echinococcosis in monkeys

Our laboratory in Zurich (in cooperation with other institutes) has diagnosed at least 15 cases of AE in monkeys post mortem during a period of 13 years (1987–1999) (Table 2).

Table 2
Aveolar echinococcosis in captive monkeys: cases diagnosed post mortem between 1987 and 1999 at the Veterinary Faculty Zürich in cooperation with the Institute Galli-Valerio, Lausanne (preliminary data) (PS: protoscoleces, +: present, L: living, –: not detected, ?: no information)

Case No.	Year of diagnosis	Animal species	Age (years)/ sex	Main organs affected and selected other findings	PS
1	1987	*Macaca fascicularis* — crab-eating macaque	5/m	Liver	–
2	1989	*Lemur catta* — ring-tailed lemur	?/f	Liver and perirenal tissue	+
3	1990	*Miopithecus talapoin* — talapoin monkey	12/m	Liver, lung	+ (L)
4	1990	*Miopithecus talapoin*	8/w	Liver, lung, mesenterium	+
5	1992	*Macaca nigra* — celebes crested macaque	12/m	Liver, lung, lymph nodes, abdominal cavity	+ (L)
6	1992	*Macaca fascicularis*	?/w	Liver	+
7	1992	*Macaca nigra*	6/m	Liver, lung, lymph nodes, spleen, pancreas, kidney	?
8	1992	*Macaca fascicularis*	8/m	Liver, lung, abdominal cavity, ascites	?
9	1994	*Hylobates* spp. — gibbon	8/?	Liver	+
10	1995	*Macaca fascicularis*	15/?	Liver, abdominal serosa	?
11	1996	*Macaca fascicularis*	1.5/m	Liver	?
12	1996	*Macaca fascicularis*	5/m	Liver and abdominal serosa	+ (L)
13	1996	*Macaca fascicularis*	1.5/m	Liver, ascites, pleuritis	?
14	1999	*Macaca fascicularis*	3/m	Liver, abdominal cavity, ascites	+ (L)
15	1999	*Macaca fascicularis*	4/f	Liver, lung	+

The animals had been maintained in zoos or other institutions; 14 of them originated from eastern or western Switzerland, and one (No. 9) from Germany. As shown in the table, one lemur and several other species of primates suffered from AE and died from the disease or had to be euthanised. The liver was affected in all cases, but other organs were also involved. The metacestode in the liver showed a similar structure as in other aberrant hosts (Fig. 2), including cases with formation of a central necrotic cavity but, in contrast to the situation in humans, protoscoleces were found in at least nine of the 15 cases (60%) (information was lacking in five cases). Another case of fatal AE was found in a gorilla from a zoo in Switzerland (Gottstein, personal communication). These cases, as well as reports from Germany (*Macaca fasciularis*; Rietschel and Kimmig, 1994) and Japan (orang-utan, gorilla and a ring-tailed lemur, Kondo et al. (1996), Taniyama et al. (1996)), show that AE should be considered in the differential diagnosis of internal diseases of captive monkeys in endemic regions. In some monkey colonies, where animals died from AE, we have used serological examinations alone or in combination with US examinations for screening the surviving animals.

The infection route in captive monkey colonies is unclear. In our situation, carnivores living in zoos could be excluded with high probability as direct infection sources. However, in one case, wild red foxes had access to a fence enclosing a macaque colony. The monkeys could grasp through the fence allowing them to pick up grass or faeces contaminated with

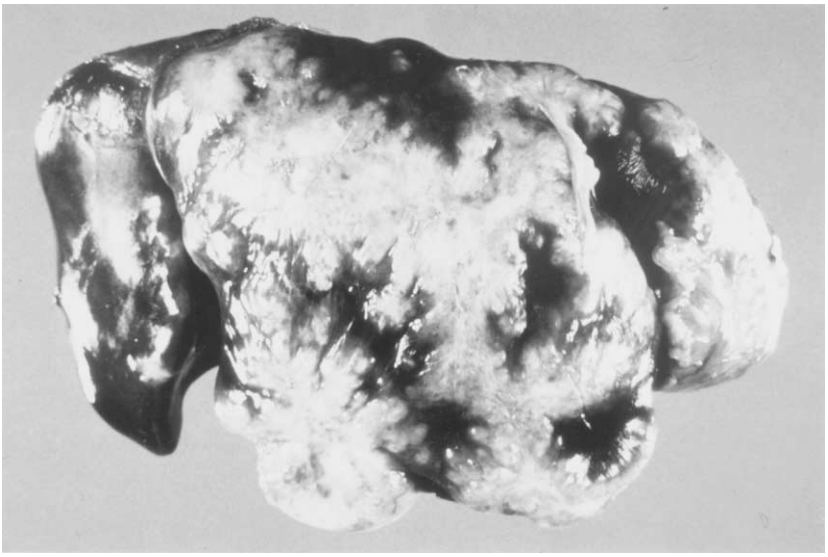

Fig. 2. AE in a captive monkey (*Macaca nigra*): liver almost completely invaded by *E. multilocularis* metacestodes.

E. multilocularis eggs from outside. Another monkey colony in a Swiss zoo was regularly fed with grass from a meadow from a highly endemic area accessible to wild foxes.

9.3. Alveolar echinococcosis in dogs

It is only since the late 1980s that cases of AE (mainly of the liver) have been diagnosed in dogs (Geisel et al., 1990; Deplazes et al., 1997; Losson and Coignoul, 1997; Haller et al., 1998). This is surprising as such infections should have been recognised at necropsy in endemic areas before this time. In Table 3, 10 cases of AE in dogs originating from Switzerland are summarised. Firm, lobulated metacestode tissue containing protoscoleces was found in several cases (Geisel et al., 1990; Haller et al., 1998; Fig. 3, top). However, in at least six of our cases (dogs No. 1, 2, 5, 6, 7, 8) the parasite mass was composed of a large cystic structure containing viscous fluid with necrotic material and thousands of transparent cysts of 2–5 mm in diameter enclosed by a thin Em2-antigen-positive laminated layer (Fig. 3, middle and bottom). In one dog (No. 2), the cyst had collapsed releasing 3 l of fluid into the abdominal cavity, and in two other dogs (Nos. 7 and 8), cysts ruptured during operation. A similar *E. multilocularis* cystic lesion in a dog has been described by Losson and Coignoul (1997). The cases with cystic lesions in dogs are comparable to cases in human (Bresson-Hadni et al., 1994; Ammann and Eckert, 1996; von Sinner and Lewall, 2001) in which the central parts of originally alveolar lesions are degenerating due to liquid necrosis, and only an outer rim of metacestode tissue composed of very small vesicles forms a “cyst wall” and produces small vesicles which are released into the cyst cavity. Concurrent infections of metacestodes in the liver and of adult stages in the intestine of dogs were observed for the first time in two cases (Table 3, Nos. 2 and 4; Deplazes et al.,

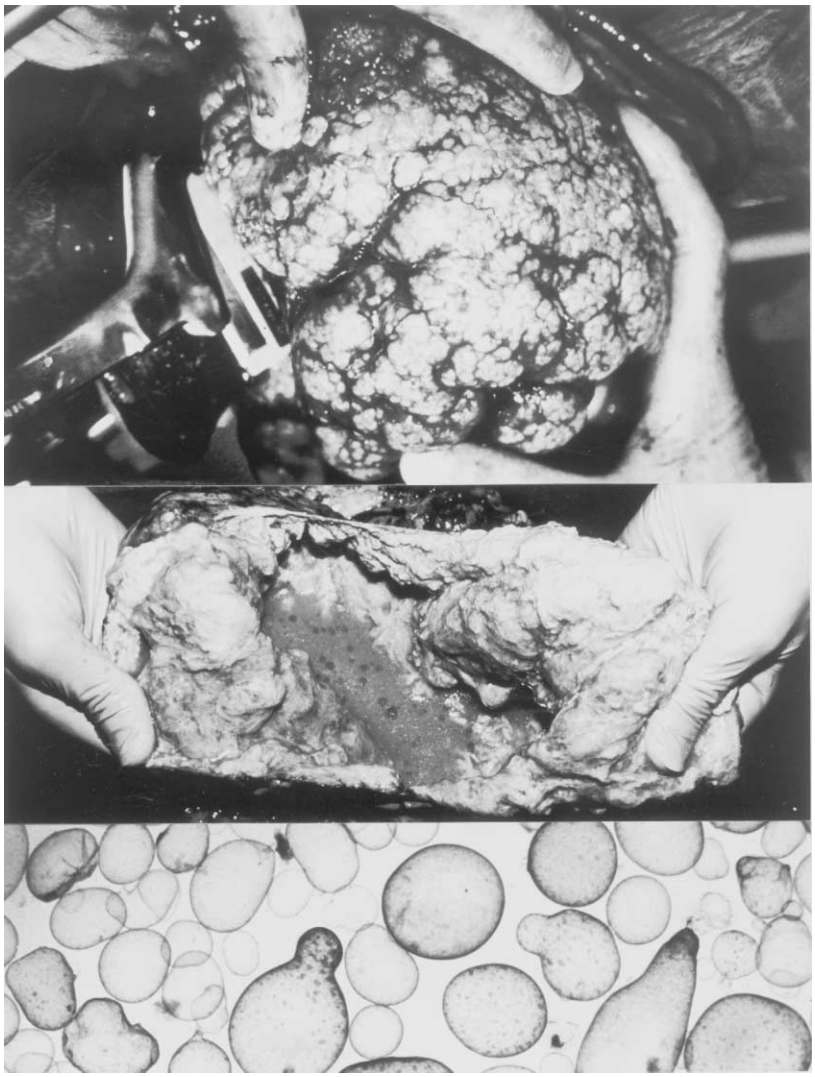

Fig. 3. AE in dogs. Top: intraoperative photograph of a metacestode mass with multiple surface lobulations invading the entire liver lobe (Table 3, case 3). Middle: large metacestode structure (10–15 cm in diameter) with central formation of a cavern containing viscous fluid and thousands of *E. multilocularis* vesicles (Table 3, case 6) (reproduced with permission of C. Bernasconi DVM, Department of Veterinary Surgery, University of Zurich). Bottom: sediment of cavern fluid containing transparent vesicles of 2–5 mm diameter.

1997). In one of these dogs (No. 4), a firm metacestode mass had been partially removed from the liver and abdominal cavity by surgery with two operations, 18 months and again 1 month, before the intestinal infection with *E. multilocularis* was diagnosed. Possible sources of the liver infection of this dog are eggs originating from the environment or from an earlier intestinal infection. Another hypothetical option is an internal autoinfection by

Table 3
Alveolar echinococcosis in dogs, diagnosed in Zurich, Switzerland (*Echinococcus multilocularis*: *E. m.*)

Case No.	Year of diagnosis	Breed	Age (years) /sex	Clinical symptoms/findings (C) and diagnostic method for parasite identification (D)[a]	History/follow-up/ pathological findings
1	1988	Sheepdog	5/f	C: ascites, mass occupying the entire abdominal cavity. D: post mortem, histology	Euthanised during exploratory laparotomy. Metacestode mass of 5 × 8 cm containing necrotic liquid and small *E. m.* cysts
2	1995	Crossbreed	3/f	C: intermittent diarrhoea, abdominal enlargement, ascites. D: diagnosis of an intestinal *E. m.* infection. EmG11-Ag-ELISA from fine needle biopsy material	Euthanised: metacestode tissue invading most of the liver parenchyma, 3 l ascites fluid containing about 1 l sediment of small *E. m.* cysts
3	1995	Dachshund	10/m	C: gradually progressing abdominal enlargement, exercise intolerance. D: histology of resected liver and lobulated firm mass (13–15 cm in diameter) occupying the abdominal space	Complete lobectomy of the affected liver lobes. Two years chemotherapy with albendazole (daily 10 mg/kg b.w., p.o.) without any sign of recurrence (Haller et al., 1998)
4	1996	Crossbreed	6/f	C: gradually progressing abdominal enlargement. D: diagnosis of an intestinal *E. m.* infection. EmG11-Ag-ELISA and EmG11-FITC from fine needle biopsy material	Incomplete resection of firm metacestode masses (∼15 cm in diameter) in 1994 and again in 1996, treated with albendazole (daily 10 mg/kg b.w., p.o.) without any sign of recurrence for 3.5 years
5	1998	Hunting dog	4/m	C: ascites, hepatomegaly, fluid filled liver cavernas. D: post mortem, histology	Euthanised, metacestode mass of 10 cm in diameter in the liver
6	1998	Crossbreed	8/f	C: abdominal enlargement, mass occupying the entire abdominal space. D: as in case No. 4	Incomplete resection of metacestode cyst of 10–15 cm in diameter, treated with albendazole (daily 10 mg/kg b.w., p.o.) without any sign of recurrence for 1.5 years (euthanised for other reasons)
7	2000	Miniature Schnautzer	9/m	C: progressive abdominal enlargement, ascites, dispnoe. D: PCR and EmG11-Ag-ELISA from fine needle biopsy material	Resection of two large metacestode masses containing necrotic liquid. Adhesions with several abdominal organs. Complicated operation with cyst rupture. Fatal outcome 1 day after operation
8	2000	Boxer	2/f	C: nausea, vomiting, tense abdominal wall. D: as in case No. 7	Complete lobectomy including a metacestode mass (∼12 cm in diametre with liquid filled cavity). Since 5 months treatment with albendazole (daily 10 mg/kg b.w., p.o.) without any sign of recurrence but persisting nausea
9	2000	Belgian Sheepdog	6/f	C: vomiting, loss of weight, mass occupying the abdominal space. D: as in case No. 7	Complete resection of two large masses (∼12 and 8 cm in diameter) of metacestode, treated with albendazole (daily 10 mg/kg b.w., p.o.) without sign of recurrence for 1 year
10	2000	Crossbreed	3/f	C: abdominal enlargement, mass in the abdominal space. D: post mortem, histology	Euthanised with a putative tumor diagnosis

[a] EmG11-Ag-ELISA, EmG11-FICT: tests detecting the species-specific antigen EmG11 (Em2) (Deplazes and Gottstein, 1991), PCR for specific detection of *E. m.* DNA (Dinkel et al., 1998).

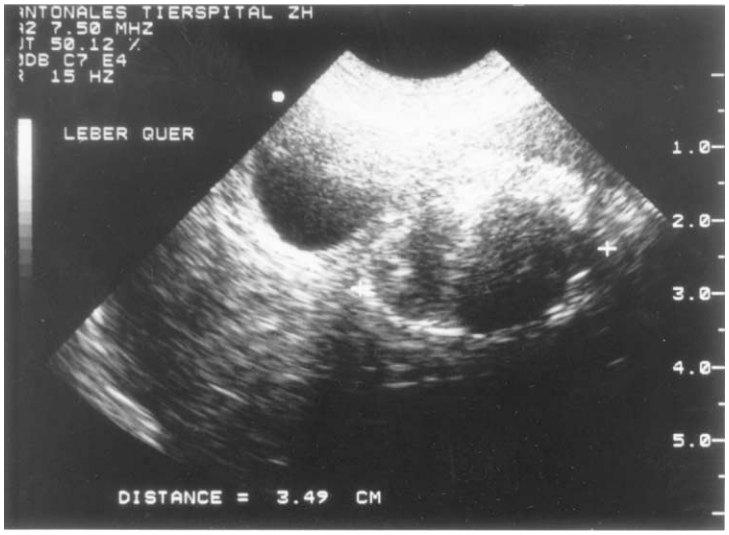

Fig. 4. US image of a dog liver showing residual metacestode structure after operation (Table 3, case 4) (reproduced with permission of M. Flückiger PD DVM, Section of Diagnostic Imaging, Veterinary Faculty, University of Zurich).

proliferation of metacestode tissue to the bile ducts and release of protoscoleces, which might have reached the intestine via the Ductus choledochus. The parameters influencing the susceptibility of dogs to *E. multilocularis* metacestode infection, the incubation period of canine AE and other factors are still unknown.

9.4. Diagnosis of alveolar echinococcosis in animals

The post mortem diagnosis of AE in aberrant hosts is based on pathognomonic macroscopic and histological findings, and in doubtful cases on results of immunological and molecular tests (Deplazes and Gottstein, 1991; Dinkel et al., 1998). More or less characteristic lesions can be diagnosed intra vitam by US examination of the liver (Fig. 4) and other abdominal organs in certain animal species, such as monkeys and dogs. Furthermore, serum antibodies against *E. multilocularis* antigens can be detected in several aberrant host species. In our laboratory, we use specific antigens of *E. multilocularis* (Em2, EmG11, II/3 10) in the ELISA for detecting specific antibodies in pigs, dogs or monkeys (Haller et al., 1998, unpublished data). However, these tests have not yet been sufficiently evaluated for the diagnoses of AE in animals, especially concerning sensitivity.

In routine practice, abdominal masses of unclear origin in dogs (or other animals) are normally punctured by fine needle biopsy for tumour identification or exclusion. In two of our cases routinely performed cytological investigation of biopsy material did not lead to the diagnosis of a metacestode infection. However, a specific metacestode antigen (Em2 or EmG11) could be captured from the biopsy material by a sandwich-ELISA or by visualisation of fragments of the laminated layer using the EmG11 monoclonal antibody (Deplazes

and Gottstein, 1991). Furthermore, PCR was shown to be very useful as a confirmatory test (Dinkel et al., 1998).

9.5. Chemotherapy of AE in animals

Based on data from experimental studies with laboratory rodents, benzimidazoles have been routinely used in recent years for chemotherapy of AE in humans (see above). In three of our dogs (Nos. 3, 8, 9, Table 3), albendazole was applied at daily doses of 10 mg/kg b.w. for 5–24 months after anticipated complete surgical removal of the metacestode tissue from the liver, and no recurrence was observed. However, in two dogs (Nos. 4 and 6), the metacestode could not be totally resected as visualised by us in one of these animals (Fig. 4). In both dogs, parasite development was controlled by an identical albendazole treatment over 3.5 and 1.5 years of observation, respectively (Table 3).

10. Concluding remarks

As outlined in this review, human AE and *E. multilocularis* infection in animals are of specific veterinary interest not only from the epidemiological point of view, but also because of the infectivity and pathogenicity of the metacestode stage to wild and domestic aberrant host animals. Such cases have emerged in Europe, and this may be a further indication that epidemiological factors have changed in recent years possibly leading to a higher infection risk also for humans. Scientists have expressed their concern about this potential risk, but it remains the task of health authorities and governments to initiate adequate countermeasures with the aim of reducing the infection risk for humans.

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References

Ammann, R.W., Eckert, J., 1996. Cestodes: *Echinococcus*. In: Weinstock, J.V. (Ed.), Parasitic Diseases of the Liver and Intestines. Gastroenterology Clinics of North America. Saunders, Philadelphia, PA, pp. 655–689.

Ammann, R., Fleiner Hoffmann, A., Eckert, J., Schwiez, Echinokokkose-Studiengruppe, 1999. Schweizerische Studie für Chemotherapie der alveolären Echinokokkose-Rückblick auf ein 20 jähriges klinisches Forschungsprojekt. Schweiz. Med. Wschr. 129, 323–332.

Arru, E., Garippa, G., Manger, B.R., 1990. Efficacy of epsiprantel against *Echinococcus granulosus* infections in dogs. Res. Vet. Sci. 49, 378–379.

Artois, M., Langlais, M., Suppo, C., 1997. Simulation of rabies control within an increasing fox population. Ecol. Mod. 97, 23–34.

Bontadina, F., Contesse, P., Gloor, S., 2001. Beeinflusst die persönliche Betroffenheit die Einstellung gegenüber Stadtfüchsen? — Aspekte zur Akzeptanz eines Wildtieres im Siedlungsgebiet. For. Snow Landscape Res., in press.

Breitenmoser, U., Müller, U., Kappeler, A., Zanoni, R.G., 2000. Die Endphase der Tollwut in der Schweiz. Schweiz. Arch. Tierheilk. 147, 447–453.

Bresson-Hadni, S., Laplante, J.-J., Lenys, D., Rohmer, P., Gottstein, B., Jacquier, P., Mercet, P., Meyer, J.-P., Miguet, J.-P., Vuitton, D., 1994. Seroepidemiologic screening of *Echinococcus multilocularis* infection in a European area endemic for alveolar echinococcosis. Am. J. Trop. Med. Hyg. 51, 837–846.

Bretagne, S., Guillou, J.P., Morand, M., Houin, R., 1993. Detection of *Echinococcus multilocularis* DNA in fox faeces using DNA amplification. Parasitology 106, 193–199.

Craig, P.S., Rogan, M.T., Allan, J.C., 1996. Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. Adv. Parasitol. 38, 169–250.

Craig, P.S., Giraudoux, P., Shi, D., Bartholomot, B., Barnish, G., Delattre, P., Quere, J.P., Harraga, S., Bao, G., Wang, Y., Lu, F., Ito, A., Vuitton, D.A., 2000. An epidemiological and ecological study of human alveolar echinococcosis transmission in south Gansu, China. Acta Trop. 77, 167–177.

Deplazes, P., Eckert, J., 1996. Diagnosis of the *Echinococcus multilocularis* infection in final hosts. Appl. Parasitol. 37, 245–252.

Deplazes, P., Gottstein, B., 1991. A monoclonal antibody against *Echinococcus multilocularis* Em2 antigen. Parasitology 103, 41–49.

Deplazes, P., Mathis, A., 1999. Methods for direct diagnosis of *Echinococcus multilocularis* in definitive hosts. Arch. Int. Hidatid. 33, 6–10.

Deplazes, P., Thompson, R.C.A., Constantine, C.C., Penhale, W.J., 1994. Primary infections of dogs with *Echinococcus granulosus*: systemic and local (Peyer's patches) immune responses. Vet. Immunol. Immunopathol. 40, 171–184.

Deplazes, P., Arnold, P., Kaser-Hotz, B., Gardelle, O., Guscetti, F., Haller, M., Eckert, J., 1997. Concurrent infections of the liver and the intestine with *Echinococcus multilocularis* in dogs. Arch. Int. Hidatid. 32, 201–202.

Deplazes, P., Alther, P., Tanner, I., Thompson, R.C.A., Eckert, J., 1999. *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog and cat populations. J. Parasitol. 85, 115–121.

Dinkel, A., Von Nickisch-Rosenegk, M., Bilger, B., Merli, M., Lucius, R., Romig, T., 1998. *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR is an alternative to necropsy. J. Clin. Microbiol. 36, 1871–1876.

Eckert, J., 1996. *Echinococcus multilocularis* and alveolar echinococcosis in Europe (except parts of eastern Europe). In: Uchino, J., Sato, N. (Eds.), Alveolar Echinococcosis. Strategy for Eradication of Alveolar Echinococcosis of the Liver. Fuji Shoin, Sapporo, Japan, pp. 27–43.

Eckert, J., 1998. Alveolar echinococcosis (*Echinococcus multilocularis*) and other forms of echinococcosis (*Echinococcus oligarthrus* and *Echinococcus vogeli*). In: Palmer, S.R., Soulsby, E.J.L., Simpson, D.I.H. (Eds.), Zoonoses. Oxford University Press, Oxford, UK, pp. 689–716.

Eckert, J., Deplazes, P., 1999. Alveolar echinococcosis in humans: the current situation in central Europe and the need for countermeasures. Parasitol. Today 15, 315–319.

Eckert, J., Conraths, F.J., Tackmann, K., 2000. Echinococcosis — an emerging or re-emerging zoonosis. Int. J. Parasitol. 30, 1283–1294.

Eckert, J., Thompson, R.C.A., Bucklar, H., Bilger, B., Deplazes, P., 2001. Prüfung der Wirkung von Epsiprantel (Cestex®) gegen *Echinococcus multilocularis* bei Hunden und Katzen. Berl. München. Tierärztl. Wochenschr. 114, 1–6.

EurEchinoReg, 1999. European Network for Concerted Surveillance of Alveolar Echinococcosis: final report. Community Program for Prevention of AIDS and Some Other Communicable Diseases. European Commission. Unité de Recherche SERF, Université de Franche-Comté, F-25030 Besançon. http://www.eurechinoreg.org.

Gasser, R.B., Jenkins, D.J., Paolillo, E., Parada, L., Cabrera, P., Craig, P.S., 1993. Serum antibodies in canine echinococcosis. Int. J. Parasitol. 23, 579–586.

Geisel, O., Barutzki, D., Minkus, G., Hermanns, W., Löscher, T., 1990. Hunde als Finnenträger (Intermediärwirt) von *Echinococcus multilocularis*. Kleintierprax 35, 235–280.

Giraudoux, P., Vuitton, D.A., Bresson-Hadni, S., Craig, P., Bartholomot, B., Barnish, G., Laplante, J.-J., Zhong, S.D., Lenys, D., 1996. Mass screening and epidemiology of alevolar echinococcosis in France, western Europe, and Gansu, central China: from epidemiology towards transmission ecology. In: Uchino, J., Sato, N. (Eds.), Alveolar Echinococcosis. Strategy for Eradication of Alveolar Echinococcosis of the Liver. Fuji Shoin, Sapporo, Japan, pp. 197–211.

Gloor, S., Bontadina, F., Hegglin, D., Deplazes, P., Breitenmoser, U., 2001. The rise of urban fox populations in Switzerland. Mamm. Biol., 66, in press.

Gottstein, B., Hemphill, A., 1997. Immunopathology of echinococcosis. In: Freedman, D.O. (Ed.), Immunopathogenetic Aspects of Disease Induced by Helminth Parasites. Chem. Immunol., Vol. 66, Karger, Basel, pp. 177–208.

Gottstein, B., Wunderlin, E., Tanner, I., 1994. *Echinococcus multilocularis*: parasite-specific humoral and cellular immune response in mouse strains susceptible (AKR, C57BI/6J) or resistant (C57BI/10) to secondary alveolar echinococcosis. Clin. Exp. Immunol. 96, 245–255.

Gottstein, B., Saucy, F., Deplazes, P., Reichen, J., Demierre, G., Zürcher, Ch., Busato, A., Pugin, P., 2001. Is a high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with increased disease incidence in humans? Emerg. Inf. Dis., 7, in press.

Haller, M., Deplazes, P., Guscetti, F., Sardinas, J.C., Reichler, I., Eckert, J., 1998. Surgical and chemotherapeutic treatment of alveolar echinococcosis in a dog. J. Am. Anim. Hosp. Ass. 34, 309–314.

Harris, S., 1981. An estimation of number of foxes (*Vulpes vulpes*) in the city of Bristol, and some possible factors affecting their distribution. J. Appl. Ecol. 18, 455–465.

Harris, S., Rayner, J.M.V., 1986. Urban fox (*Vulpes vulpes*) population estimates and habitat requirements in several British cities. J. Anim. Ecol. 55, 575–591.

Heath, D.D., 1995. Immunology of *Echinococcus* infections. In: Thompson, R.C.A., Lymbery, A.J. (Eds.), *Echinococcus* and Hydatid Disease. CAB International, Wallingford, UK, pp. 183–232.

Hofer, S., Gloor, S., Müller, U., Mathis, A., Hegglin, D., Deplazes, P., 2000. High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. Parasitology 120, 135–142.

Jenkins, D.J., Romig, T., 2000. Efficacy of Droncit® spot-on (praziquantel) 4% (w/v) against immature and mature *Echinococcus multilocularis* in cats. Int. J. Parasitol. 30, 959–962.

Kapel, C.M.O., Saeed, I., 2000. *Echinococcus multilocularis* — en ny zoonotisk parasiti Danmark. Dansk Veterinaertidsskrift 83, 14–16.

Kohno, H., Sakai, H., Okomoto, M., Ito, M., Oku, Y., Kamiya, M., 1995. Development and characterization of murine monoclonal antibodies to *Echinococcus multilocularis* adult worms and its use for the coproantigen detection. Jpn. J. Parasitol. 44, 404–412.

Kolarova, L., 1999. *Echinococcus multilocularis*: new epidemiological insights in central and eastern Europe. Helminthologia 36, 193–200.

Kondo, H., Wada, Y., Bando, G., Kosuge, M., Oku, Y., 1996. Alveolar hydatidosis in a gorilla and a ring-tailed lemur in Japan. J. Vet. Med. Sci. 58, 441–449.

Ligthowlers, M.W., Gottstein, B., 1995. Echinococcosis/hydatidosis: antigens, immunological and molecular diagnosis. In: Thompson, R.C.A., Lymbery, A.J. (Eds.), *Echinococcus* and Hydatid Disease. CAB International, Wallingford, UK, pp. 355–410.

Losson, B.J., Coignoul, F., 1997. Larval *Echinococcus multilocularis* infection in a dog. Vet. Rec. 141, 49–50.

Lucius, R., Bilger, B., 1995. *Echinococcus multilocularis* in Germany: increased awareness or spreading of a parasite? Parasitol. Today 11, 430–434.

Macdonald, D.W., Newdick, M., 1982. The distribution and ecology of foxes, *Vulpes vulpes* in urban areas. In: Bornkamm, R., Lee, J.A., Seeward, M.R. (Eds.), Urban Ecology. Blackwell Scientific Publications, Oxford, UK, pp. 123–135.

Mathis, A., Deplazes, P., Eckert, J., 1996. Improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. J. Helminthol. 70, 219–222.

Nonaka, N., Iida, M., Yagi, K., Ito, T., Ooi, H.K., Oku, Y., Kamiya, M., 1996. Time course of coproantigen excretion in *Echinococcus multilocularis* infections in foxes and an alternative definitive host, golden hamsters. Int. J. Parasitol. 26, 1271–1278.

Nonaka, N., Tsukada, H., Abe, N., Oku, Y., Kamiya, M., 1998. Monitoring of *Echinococcus multilocularis* in red foxes in Shiretoko, Japan, by coproantigen detection. Parasitology 117, 193–200.

Ohbayashi, M., 1996. Host animals of *Echinococcus multilocularis* in Hokkaido. In: Uchino, J., Sato, N. (Eds.), Alveolar Echinococcosis. Strategy for Eradication of Alveolar Echinococcosis of the Liver. Fuji Shoin, Sapporo, Japan, pp. 59–64.

Pétavy, A.F., Tenora, F., Deblock, S., Sergent, V., 2000. *Echinococcus multilocularis* in domestic cats in France: a potential risk factor for alveolar hydatid disease contamination in humans. Vet. Parasitol. 87, 151–156.

Pfister, T., Schad, V., Schelling, U., Lucius, R., Frank, W., 1993. Incomplete development of larval *Echinococcus multilocularis* (Cestoda, Taeniidae) in spontaneously infected wild boars. Parasitol. Res. 79, 37–39.

Raoul, F., Deplazes, P., Nonaka, N., Vuitton, D., Giraudoux, P., 2000. Can *Echinococcus multilocularis* coproantigen detection in fox faecal samples collected in the field be used to identify high and low endemicity areas? Acta Parasitol. 45, 238.

Rietschel, W., Kimmig, P., 1994. Alveoläre Echinokokkose bei einem Javaneraffen. Tierärztl. Parxis. 22, 85–88.

Romig, T., Bilger, B., Dinkel, A., Merli, M., Mackenstedt, U., 1999a. *Echinococcus multilocularis* in animal hosts: new data from western Europe. Helminthologia 36, 185–191.

Romig, T., Kratzer, W., Kimmig, P., Frosch, M., Gaus, W., Flegel, W.A., Gottstein, B., Lucius, R., Beckh, K., Kern, P., Römerstein Study Group, 1999b. An epidemiologic survey of human alveolar echinococcosis in southwestern Germany. Am. J. Trop. Med. Hyg. 61, 566–573.

Sakai, H., Nonaka, N., Yagi, K., Oku, Y., Kamiya, M., 1998. Coproantigen detection in a routine fox survey of *Echinococcus multilocularis* infection in Hokkaido, Japan. Parasitol. Inter. 47, 47–51.

Sakui, M., Ishige, M., Fukumoto, S., Ueda, A., Ohbayashi, M., 1984. Spontaneous *Echinococcus multilocularis* infection in swine in north-eastern Hokkaido, Japan. Jpn. J. Parasitol. 33, 291–296.

Schantz, P.M., Chai, J., Craig, P.S., Eckert, J., Jenkins, D.J., Macpherson, C.N.L., Thakur, A., 1995. Epidemiology and control of hydatid disease. In: Thompson, R.C.A., Lymbery, A.J. (Eds.), *Echinococcus* and Hydatid Disease. CAB International, Wallingford, UK, pp. 233–331.

Schelling, U., Schäfer, E., Pfister, T., Frank, W., 1991. Zur Epidemiologie des *Echinococcus multilocularis* im nordöstlichen Baden-Württemberg. Tieräzrtl. Umschau 46, 673–676.

Sydler, T., Mathis, A., Deplazes, P., 1998. *Echinococcus multilocularis* lesions in the livers of pigs kept outdoors in Switzerland. Eur. J. Vet. Pathol. 4, 43–46.

Tackmann, K., Löschner, U., Mix, H., Reimer, K., Straubach, C., Thulke, H.-H., Conraths, F., 1997. Efficacy of praziquantel baits on *Echinococcus multilocularis* infections of red foxes in a moderate endemic focus. Arch. Int. Hidatidol. 32, 264.

Tackmann, K., Löschner, U., Mix, H., Staubach, C., Thulke, H.-H., Conraths, F.J., 1998. Spatial distribution patterns of *Echinococcus multilocularis* (Leuckart 1863) (Cestoda: Cyclophyllidea: Taeniidae) among red foxes in an endemic focus in Brandenburg (Germany). Epidemiol. Infect. 120, 101–109.

Taniyama, H., Morimitsu, Y., Fukumoto, S.-I., Asakawa, M., Ohbayashi, M., 1996. A natural case of larval echinococcosis caused by *Echinococcus multilocularis* in a zoo orangutan (*Pongo pygmaeus*). In: Uchino, J., Sato, N. (Eds.), Alveolar Echinococcosis. Strategy for Eradication of Alveolar Echinococcosis of the Liver. Fuji Shoin, Sapporo, Japan, pp. 65–67.

Thompson, R.C.A., 1995. Biology and systematics of *Echinococcus*. In: Thompson, R.C.A., Lymbery, A. J. (Eds.), Biology and Hydatid Disease. CAB International, Wallingford, UK, pp. 1–50.

Thompson, R.C.A., Eckert, J., 1983. Observations on *Echinococcus multilocularis* in the definitive host. Z. Parasitenk. 69, 335–345.

Thompson, R.C.A., Reynoldson, J.A., Manger, B.R., 1991. In vitro and in vivo efficacy of epsiprantel against *Echinococcus granulosus*. Res. Vet. Sci. 51, 332–334.

Tsukada, H., Morishima, Y., Nonaka, N., Oku, Y., Kamiya, M., 2000. Preliminary study of the role of red foxes in *Echinococcus multilocularis* transmission in the urban area of Sapporo, Japan. Parasitology 120, 423–428.

Van der Giessen, J.W.B., Rombout, Y.B., Franchimont, J.H., Limper, L.P., Homan, W.L., 1999. Detection of *Echinococcus multilocularis* in foxes in The Netherlands. Vet. Parasitol. 82, 49–57.

Viel, J.-F., Giraudoux, P., Abrial, V., Bresson-Hadni, S., 1999. Water vole (*Arvicola terrestris* Scherman) density as risk factor for human alveolar echinococcosis. Am. J. Trop. Med. Hyg. 61, 559–565.

Vogel, H., 1957. Über den *Echinococcus multilocularis* Süddeutschlands. I. Das Bandwurmstadium von Stämmen menschlicher und tierischer Herkunft. Z. Tropenmed. Parasitol. 8, 404–454.

von Sinner, W., Lewall, D., 2001. Hydatid Disease (Echinococcosis). In: Palmer, P.E.S., Reeder, M.M. (Eds.), The Imaging of Tropical Diseases, 2nd Edition. Springer, Berlin, pp. 205–353.

WHO, 1996. Guidelines for treatment of cystic and alveolar echinococcosis. WHO Informal Working Group on Echinococcosis. Bull. World Hlth. Org. 74, 231–242.

WHO/OIE, 2001. WHO/OIE Manual on Echinococcosis in Humans and Animals. In: Eckert, J., Gemmell, M.A., Meslin, F.-X., Pawlowski, Z. (Eds.), Office International des Épizooties, Paris, in press.

Yagi, K., Ito, T., Ishige, M., 1996. A survival strategy of *Echinococcus multilocularis* presumed by experimental studies. In: Uchino, J., Sato, N. (Eds.), Alveolar Echinococcosis. Strategy for Eradication of Alveolar Echinococcosis of the Liver. Fuji Shoin, Sapporo, Japan, pp. 97–99.