Perspective on control options for *Echinococcus multilocularis* with particular reference to Japan

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SUMMARY

Following a brief introduction of recent advances in molecular and immunological technology for detection of persons and animals infected with *Echinococcus multilocularis* and an overview of the current situation of alveolar echinococcosis (AE) in Japan, perspectives on control options are discussed with reference to different epidemiological situations. AE is considered the most serious parasitic zoonosis in temperate and arctic regions of the northern hemisphere. The number of human cases differs drastically among regions. While high numbers of patients are apparently associated with high *E. multilocularis* prevalence in domestic dogs, e.g. in parts of Alaska and western China, the number of cases is moderate or low in areas where the parasite is mainly transmitted by wild canid species (e.g. in central Europe or temperate North America). However, the severity of the disease, the absence of curative treatment for most cases, the high cost of long-term chemotherapy and the anxiety caused for the population in highly endemic areas call for the development of preventive strategies even in regions where human AE is rare. Furthermore, in view of (1) drastically increasing numbers and infection rates of foxes involved in transmission of *E. multilocularis*, and (2) increasingly close contact between humans and foxes e.g. in Europe and Japan, there is considerable concern that AE incidences may in future increase in these regions. Control options depend on a variety of factors including the species of canid principally responsible for transmission and the socio-economic situation in the region. Where domestic dogs (stray or owned) are the principal hosts for *E. multilocularis*, control options can include those applicable to *E. granulosus*, i.e. reduction of the number of stray dogs, registration and regular preventive chemotherapy of owned dogs, and information campaigns for the population promoting low-risk behaviour for man and dogs. Where *E. multilocularis* is mainly transmitted by wild canids, the situation is far more difficult with preventive strategies still being in trial stage. Integrated control measures could include prevention information campaigns, restricting access of pet animals (dogs and cats) to rodents, chemotherapy of foxes on local or regional scales, and strategies to minimize contacts between people and foxes.

Key words: Alveolar echinococcosis, *Echinococcus multilocularis*, epidemiology, control, Japan.

INTRODUCTION

Following the establishment of *Echinococcus multilocularis* as a distinct species and as the causative agent of alveolar echinococcosis (AE) 50 years ago (Rausch, 1954; Vogel, 1957), the distribution of the parasite was thought to be limited to central Europe, parts of arctic and sub-arctic North America, and Hokkaido in Japan. However, during the past two decades, it was found to cover most of the northern hemisphere of higher latitude (Schantz *et al*. 1995; Eckert *et al*. 2001; Craig & Pawlowski, 2002; Kern *et al*. 2003; Ito *et al*. 2003*b*). Because of the sylvatic life cycle of *E. multilocularis*, involving wild rodents and fox species, AE is not considered an eradicable disease. Human infections are relatively rare accidental spill-overs from this wildlife cycle. It is increasingly recognized that in many regions (e.g. temperate Europe and central China) the transmission of *E. multilocularis* is greatly influenced by anthropogenic modifications of landscape and agricultural practices (Giraudoux *et al*. 2002, this supplement). Due to various developments, the transmission of this parasite has become increasingly synanthropic even where it is maintained by species of wildlife. In view of these developments, and considering increasing fox populations and infection rates, it is essential to establish safer environments for local residents in endemic areas. In this paper, we summarize the present situation of AE in Japan, compare it to the situation in Europe and America, and describe prospects for control of this parasite under different epidemiological situations. Since techniques for detection of *E. multilocularis* infections in humans and animals have greatly advanced in the past decade (reviewed by Eckert *et al*. 2001; Siles-Lucas & Gottstein, 2001; Ito, 2002*a*; Ito & Craig, 2003; McManus, Zhang & Bartley, 2003; Zhang, Li & McManus, 2003), a preceding chapter describes recent advances in diagnostics which are relevant e.g. for the development of control strategies and for the documentation of their effect.

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Table 1. Recent advances in molecular and immunological technology for detection of humans and animals infected with *Echinococcus multilocularis* (modified from Ito & Craig, 2003)

Molecular technology
(a) Detection of copro-DNA of *E. multilocularis* in definitive hosts: Dinkel *et al.* (1998)
(b) Detection of polymorphism of *E. multilocularis* and identification of *Echinococcus* spp.:
Mitochondrial DNA: Mathis *et al.* (1996); Nakao *et al.* (2002)
Microsatellite DNA: Bretagne *et al.* (1996); Nakao *et al.* (2003)
Immunological technology
(c) Detection of copro-antigens of *E. multilocularis* in definitive hosts:
Allan *et al.* (1992); Nonaka *et al.* (1996); Deplazes *et al.* (1999)
(d) Detection of patients infected with *E. multilocularis*:
Gottstein (1985); Frosch *et al.* (1991); Hemmings & McManus (1991);
Gottstein *et al.* (1993); Ito *et al.* (1993, 1999); Lawton *et al.* (1997); Sako *et al.* (2002)*
Imaging analysis
(e) MRI and US for hepatic AE in humans: Pawlowski *et al.* (2001)

* EM4, EMII/3-10 and Em18 are the same protein sequenced by EM10. Em18 is the smallest degenerated product of EM10 by cysteine proteinase and has the least homology to human ERM protein.

RECENT ADVANCES IN MOLECULAR AND IMMUNOLOGICAL TECHNOLOGY FOR DETECTION OF HUMANS AND ANIMALS INFECTED WITH *E. MULTILOCULARIS*

Recent advances in detection of (1) humans infected with metacestodes of *E. multilocularis* and (2) wild and domestic animals infected with adult worms of *E. multilocularis* are summarized into three categories; (a) molecular diagnosis of humans and animals, (b) immunological diagnosis of humans and animals, and (c) image diagnosis for humans. Table 1 is a brief summary of such recent advances in molecular and immunological technology. Combinations of (a), (b) and (c) are recommended for primary screening and for identification of infected persons (Pawlowski *et al.* 2001). As copro-antigen tests for detection of adult worms of *E. multilocularis* in the definitive hosts are reasonably sensitive but not species specific, they may be useful for primary screening (Allan *et al.* 1992; Nonaka *et al.* 1996; Deplazes *et al.* 1999; Raoul *et al.* 2001). However, for diagnosis of individual animals – as opposed to epidemiological applications – it is crucial to introduce more specific diagnostic technology, e.g. copro-DNA tests (Mathis, Deplazes & Eckert, 1996; Dinkel *et al.* 1998). Although these two advanced technologies are essential for field survey, it is also crucial to compare the specificity and sensitivity of these techniques with the real data from necropsy of animals (Romig *et al.* 1999*a*).

Bretagne *et al.* (1996) found three different genotypes of *E. multilocularis* using microsatellite DNA analysis: Central North American, Alaskan and Japanese, and European. However, recent work by Nakao, Sako & Ito (2003) based on analysis of single worms has revealed that two distinct types of *E. multilocularis* and their hybrids exist in the fox population in Hokkaido. Such microsatellite DNA analysis to evaluate intra-specific variation of *E. multilocularis* becomes important for epidemiological work in the future, since such molecular data can document the parasites' dispersal characteristics and spatial dynamics, and is therefore relevant e.g. for the planning and evaluation of control measures.

Detection of infected persons is based on a combination of image analysis and serum antibody detection. It is recommended that image diagnosis for detection of hepatic abnormality is the first choice, followed by (or combined with) serological tests using specific antigens (Pawlowski *et al.* 2001; Ito & Craig, 2003). There are several candidate antigens that are reasonably reliable for differentiation of AE. Most recently, Sako *et al.* (2002) have revealed that one serological marker, Em18, reported from Japan (Ito *et al.* 1993, 1999; Ito, Schantz & Wilson, 1995) is a small component of EM10 (Frosch *et al.* 1991, 1994; Helbig *et al.* 1993) with the least homology to human ezrin, radixin and moesin (ERM) and is expected to contain more B cell epitope activity recognized by AE patients with higher sensitivity than others. All protein antigens for serodiagnosis of AE so far examined independently and produced as recombinant antigens are basically of the same family: EM10 (Frosch *et al.* 1991, 1994; Helbig *et al.* 1993), EM4 (Hemmings & McManus, 1991), Em18 (Ito *et al.* 1993) and EmII/3 (Felleisen & Gottstein, 1994).

There are other antigens of carbohydrate nature (Gottstein, 1985; Sato & Furuya, 1994; Sato, Nagano & Furuya, 1996), and recent work has revealed that a carbohydrate named Em2, a component of the laminated layer of metacestodes of *E. multilocularis* (Gottstein, 1985), is a mucin-type glycoprotein that modulates the host immune response by its T-cell-independent nature (Dai *et al.* 2001; Hulsmeier *et al.* 2002). In contrast to Em18 or EmII/3, Em2 antigen is also recognized in inactive AE cases with calcified lesions (Ito, Schantz & Wilson, 1995; Ito *et al.* 2002*b*; Xiao *et al.* 2003; Gottstein, personal communication). Due to the different nature of both antigens, it is reasonable to recommend the use of

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Fig. 1. Geographic map of the northern part of Japan (see Table 2, reproduced, with permission, from Infectious Agents Surveillance Report 1999).

Em18-ELISA in combination with Em2^{plus}-ELISA (Gottstein *et al*. 1993) or other assays using proteins in the same family (Lawton *et al*. 1997) for monitoring of prognosis. Anti-Em18 antibody becomes undetectable within one year after radical surgery of hepatic AE (Fujimoto *et al*. unpublished; Ishikawa *et al*. unpublished).

CURRENT EPIDEMIOLOGICAL SITUATION IN JAPAN

Infection in humans from Hokkaido

The first AE case (a 28 year old woman) in Japan was reported in 1937. The patient was from Rebun Island with an area of 83 km², located 45 km off the northwestern coast of Hokkaido (Figs 1 and 2). The parasite was apparently introduced to the island with 12 pairs of red foxes, imported from the endemic Kuril Islands between 1924 and 1926 for control of voles and production of fox fur (Yamashita, 1978). To date, 131 human AE cases have been recorded from this small island (Table 2, Fig. 1). However, field studies conducted from 1948 only revealed few infected final hosts (dogs and cats), and no metacestodes in rodents. This suggests that transmission of the parasite was already much reduced by that time due to the earlier eradication of foxes (Minagawa, 1999; Doi *et al*. 2000*b*). Today, there are regulations that prohibit dogs or foxes on Rebun Island in order to prevent re-introduction of *E. multilocularis*, and no new human case was reported in the past decade. A second, probably independent introduction of *E. multilocularis* occurred in the eastern most parts of Hokkaido (Nemuro and Kushiro districts) (Figs 1 and 2), where a total of 148 AE cases was diagnosed from 1965 until 1997 with new cases continuing to appear (Table 2, Figs 1 and 2). As the latest development, starting some 15 years ago, human cases are being reported sporadically from the remaining area of Hokkaido (Minagawa, 1997).

In 1972, the local Government in Hokkaido established a reporting system for all AE cases confirmed pathologically. Serological two-step screening systems have been established at Hokkaido Institute of Public Health (HIPH). From 1983, crude antigen-ELISAs were used for primary screening; since 1987, positive sera were then confirmed in an immunoblot assay using the same crude antigens (Sato *et al*. 1983; Furuya *et al*. 1989). This serological screening system in combination with image analysis has served to detect many AE cases in Hokkaido (Suzuki *et al*. 1996); a total of 373 AE patients were confirmed between 1937 and 1997 (Table 2). Until 2002, approximately 10–20 new AE cases were diagnosed per year in Hokkaido (total population: 6 millions). As an example, the screening of 72 801 persons in 1997 resulted in three confirmed cases (Fig. 3) (National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare, 1999).

As will be explained in the following, *E. multilocularis* in foxes has been gradually spreading from the eastern part of Hokkaido over the entire island, with human cases appearing after a certain delay. We therefore believe that cases of AE will continue to appear in Hokkaido due to the ongoing spread of infected foxes. If transmission is truly established in Honshu (central Japan) remains to be seen. Certainly, high prevalence of *E. multilocularis* in foxes is a basic risk factor for accidental infection of humans (Gottstein *et al*. 2001). However, improvements in knowledge, awareness and hygiene may serve to limit new exposures, therefore future incidence rates are impossible to predict. In several studies, farming was recognized as a risk factor, while other presumed risk behaviour (e.g. hunting, eating of raw garden produce etc.) was not clearly correlated to AE incidence (Doi *et al*. 1987; Inaoka *et al*. 1987; Nakao *et al*. 1988; Romig *et al*. 1999*b*; Craig *et al*. 2000; Hildreth *et al*. 2000, Kern *et al*. 2003). Since such data are crucial for the development of strategies for control and prevention, increased surveillance will be necessary with appropriate combination of image analysis and serological tests (Ito, 2001*a*, 2002*a*, *b*; Ito *et al*. 2002*a*, 2003*a*; Sato *et al*. 2003).

Infection in humans from other regions of Japan

76 human AE cases were reported from areas outside Hokkaido between 1926 and 2000 (Takahashi, Yamaguchi & Inaba, 1986; Doi *et al*. 2000*a*). However, the majority of AE cases had a history of previous

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Table 2. Alveolar echinococcosis cases by suspected area of infection* (see Fig. 1, reproduced with permission from Infectious Disease Surveillance Report 1999)

Area	1937–64	1965–74	1975–84	1985–93	1994–97	Total
Rebun Island	111	13	5	2	—	131
Eastern Hokkaido	—	40	40	55	13	148
Northern Hokkaido	—	—	2	15	9	26
Central Hokkaido	—	—	3	8	13	24
Western Hokkaido	—	—	1	1	3	5
Southern Hokkaido	—	—	—	14	5	19
Siberia, Kuril Islands, Sakhalin	5	5	4	—	—	14
Unknown	1	1	1	1	2	6
Total	117	59	56	96	45	373

* Including cases who had been living in different areas at the time of identification.

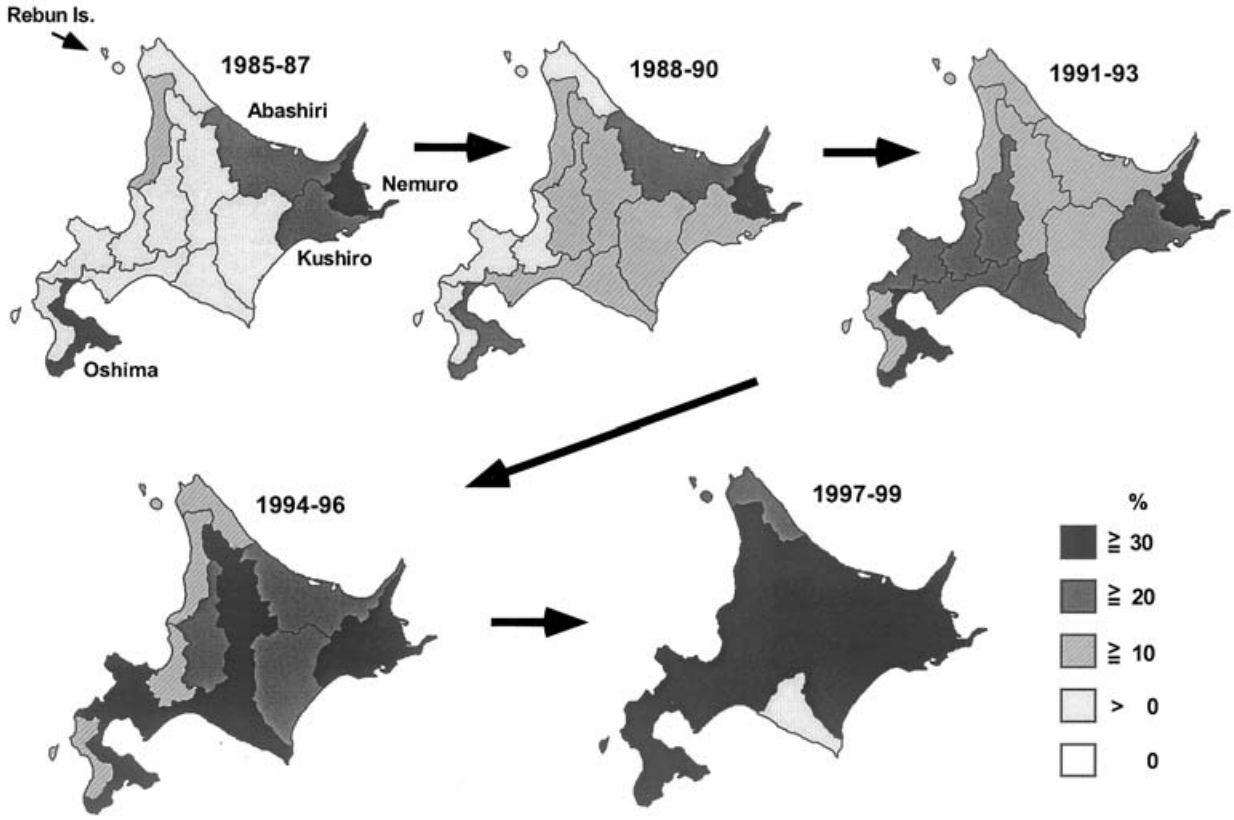

Fig. 2. Changes in prevalence of *E. multilocularis* among foxes of the 14 districts in Hokkaido, Japan from 1985 until 1999 (modified from Takahashi & Mori, 2001).

residence either in Hokkaido, northern China or Russia during the Second World War. Only few cases had apparently never lived in any known endemicity regions. Some of the cases, e.g. one from Okinawa Island, the south end of Japan, and several AE cases from Kyushu Island, were obvious misdiagnoses (Doi *et al*. 2000*b*). Any record from Japan outside Hokkaido without clear pathological confirmation should therefore be treated with caution. Likewise, the fact that most records cluster in Aomori Prefecture of northern Honshu (close to Hokkaido – Fig. 1) is more likely caused by previous residence in Hokkaido, rather than by a southward spread of the parasite. There is however, a report of three infected pigs from a single pig farm in Aomori Prefecture (Kamiya & Kanazawa, 1999), causing concern about a spread of the parasite. However, human cases reported afterwards from this area were incorrectly diagnosed due to serological cross reactions with fasciolosis and cystic echinococcosis (Yoshimura, 2000; Hatakeyama *et al*. 2002; Ito *et al*. 2003*a*). There is, in summary, no unequivocal proof of endemic transmission in Japan outside Hokkaido.

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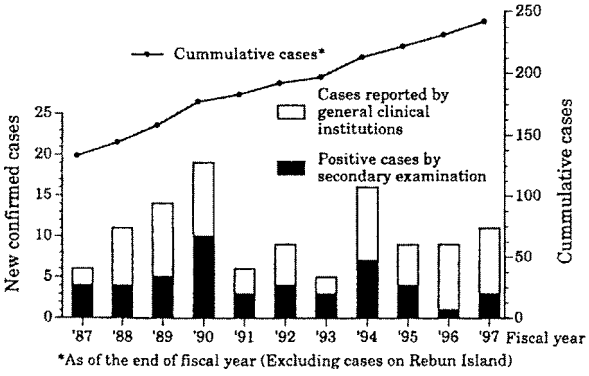

Fig. 3. Incidence of AE in Hokkaido, Japan (reproduced, with permission, from Infectious Agents Surveillance Report 1999).

Epidemiological evidence in animals

The red fox (*Vulpes vulpes*) and the vole genus *Clethrionomys*, especially the gray-sided vole *C. rufocanus*, are the most important hosts for this cestode. From 1966 to 1999, a total of 22 253 foxes were examined in Hokkaido and 3949 (17·7%) were confirmed to be infected (Takahashi & Mori, 2001). Analyses of fox stomach content in Hokkaido have determined that the gray-sided vole is the most important prey species (Abe, 1975; Kondo, Takahashi & Yagi, 1986). The stable predator–prey relationship between the fox and this vole is one of the key factors leading to the high infection rate of foxes. The habitats of the *C. rufocanus* often coincide with the breeding sites of foxes (Takahashi *et al.* 1989; Takahashi & Uraguchi, 1996; Uraguchi & Takahashi, 1998). Furthermore, the association between annual fluctuations in the fox infection rate and vole abundance has been well established. The *C. rufocanus* population greatly fluctuates over time with periodic or aperiodic patterns (Saitoh, Stenseth & Bjornstad, 1998) and the relative abundance of this species directly affects infection rates of *E. multilocularis* in red foxes (Saitoh & Takahashi, 1998).

Dogs are mainly important due to their potential to serve as infection sources for humans. Examination of 9849 dogs in Hokkaido between 1966 and 1999 revealed that 99 (1·0%) were infected with adult worms of *E. multilocularis* (Takahashi & Mori, 2001). The first record of two cats harbouring a single mature worm of *E. multilocularis* each on Rebun Island was reported by Ambo *et al.* (1954). More recently, five (5·5%) of 91 cats examined in Hokkaido were found to be infected; the worms were immature (Yagi, Takahashi & Hattori, 1984; Takahashi & Mori, 2001). Cats appear to be less susceptible to *E. multilocularis* than are foxes and dogs. There are reports of raccoon dogs (*Nyctereutes procyonoides*) infected with *E. multilocularis* in Hokkaido (Yimam *et al.* 2002; Yagi *et al.* personal communication in 1988). Further study is, however, needed to determine the quantitative significance of this species for transmission. Non-human primates are known to be highly susceptible to AE, and several records of infected species from zoos in Hokkaido, including Japanese macaque (*Macaca fuscata*), orangutan (*Pongo pygmaeus*), gorilla (*Gorilla gorilla*) and ring-tailed lemur (*Lemur catta*) (Ohbayashi, 1996; Taniyama *et al.* 1996); presumed infection source is either foodstuff (fruits, leaves etc.) brought in from the environment or contamination of zoos by foxes. Most recently, the case of an infected gorilla in Asahikawa zoo (Hokkaido) was thoroughly reviewed by Kosuge & Bando (2003). As the director of this zoo, Kosuge provided fencing with a concrete base of 1 metre depth for prevention of any fox invasion into the zoo after this accidental death of a gorilla in 1994.

Since 1983, domestic ungulates (pig and horse) infected exclusively with sterile cysts of *E. multilocularis* have been reported in Hokkaido (Sakui *et al.* 1984; Miyauchi *et al.* 1984; Kaji *et al.* 1993). All pigs and horses raised and butchered in Hokkaido are being checked at meat inspection centres, resulting in a total of 18 873 (0·1%) of 18 857 078 pigs and 24 (0·15%) of 15 583 horses found infected from 1983 through 1999 (Takahashi & Mori, 2001). Pigs and horses do not appear to play any role in transmission of the parasite, because the metacestode develops no brood capsules or protoscoleces in these hosts. However, the surveillance of infection in swine is highly informative to monitor the presence of the parasite, especially in areas where AE has not yet been recognized. A number of municipalities have been recognized as endemic areas based on confirmed pig infection cases (Ishige, 1984). Infections of pigs with *E. multilocularis* increased year by year in Hokkaido from 1990 (0·08%) until 1995 (0·25%) but then decreased by 1998 (0·12%). Infection in pigs may depend on factors such as the quality of pig farm facilities and pig handling processes which are highly variable. Therefore, the infection prevalence in pigs may be insufficient for assessment of the epidemiological situation (Takahashi & Mori, 2001; Ito, 2001*b*).

During the past two decades the known geographic range of *E. multilocularis* has expanded drastically (Fig. 2). Following the first record of an infected pig outside the known endemic area in 1983, systematic surveys of the prevalence and geographic distribution of *E. multilocularis* in various animals have been conducted in all parts of Hokkaido by the Hokkaido local government and HIPH. Infection rates in the fox from the Nemuro and Oshima districts in eastern and southern Hokkaido, respectively, exceeded 30% in the 1985–1987 period, while infection rates in most other districts were less than 10%. After 1988, the infection rates in Nemuro and Oshima district remained high, while in other areas they increased year by year (Fig. 2). From these longitudinal surveys we can recognize two developments: in

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the 1980s, a geographical spread of *E. multilocularis* in foxes occurred, while increased prevalence rates are noted in the 1990s (Takahashi, Uraguchi & Yagi, 1999; Takahashi & Mori, 2001).

As in Europe (see below), the invasion of urban areas by foxes appears to be also in progress in Japan and was recently documented for Sapporo, the capital city of Hokkaido. The numbers of foxes killed by traffic accidents increased in Sapporo city since the early 1990s (Uraguchi & Takahashi, 1999). In this city, infections in foxes were estimated by the examination of fox faeces in copro-antigen assay (Tsukada *et al*. 2000) and confirmed by necropsy of foxes killed by traffic accidents (Takahashi *et al*. unpublished).

To avoid exaggerated public anxiety – especially when domestic pets are concerned – it has to be stressed that any diagnosis of *E. multilocularis* in dogs and cats has to be based on specific criteria. These are (1) the morphological detection of adult worms expelled after purging with arecoline (not after dosing with praziquantel) of animals testing positive with coproantigen-assays, or (2) the detection of species specific DNA in fecal samples, or from eggs or adult worms present in the faeces.

COMPARISON WITH OTHER PARTS OF THE WORLD

The low to moderate number of human AE cases in Japan is in agreement with the situation in Europe, and considerably higher than in central North America where human cases are almost non-existent. In all these regions, *E. multilocularis* is mainly transmitted in wildlife cycles. The typical transmission pattern in Europe involves red foxes (*Vulpes vulpes*) as final hosts, and arvicolid rodents (especially the common vole, *Microtus arvalis*, the water vole, *Arvicola terrestris*, and the muskrat, *Ondatra zibethicus*) as intermediate hosts. Most of the parasite biomass is estimated to be present in this wildlife cycle. Dogs and cats are of secondary importance for parasite propagation, but may play an important role in transmission to humans. In the endemic area of central North America (southern Canada to the central USA), red foxes (*V. vulpes*) and coyotes (*Canis latrans*) are the most important final hosts, while intermediate hosts include the meadow vole, *Microtus pennsylvanicus*, and the deer mouse, *Peromyscus maniculatus* (Hildreth, Johnson & Kazacos, 1991). Both in Europe and North America, therefore, the lifecycle of *E. multilocularis* is closely linked to anthropogenic landscapes (pastures and meadows) which are the preferred habitat of the most important intermediate host species. This is in contrast to Japan where – at least in the endemic area of Hokkaido – no rodent species has exploited the abundant open grassland areas: the intermediate hosts (*Clethrionomys* spp.) reach their highest population densities in the undergrowth (e.g. small bamboo species) of forest and bushland.

Despite these differences, the trend toward range expansion and intensification of the transmission appears the same in Japan as in Europe and North America. For some of those regions in Europe, which previously were thought to be non-endemic, recent records of infected foxes may simply be the result of increased investigative efforts, but for several others where high levels on endemicity are found today (e.g. Belgium and northwestern Germany) it is clear that the parasite is either a recent invader, or has drastically increased its prevalence (Romig, 2002). The latter is also true for the European historical 'core area' for AE (southern Germany, eastern France, and parts of Switzerland and Austria), where prevalence in foxes has increased to levels which locally exceed 80%. This is temporally correlated to rising fox population densities, a Europe-wide phenomenon partly caused by successful rabies-control. Also in North America, both range and prevalence rates of *E. multilocularis* appear to increase for reasons which are not well understood. There, the parasite (as in Japan) may be a recent invader from the arctic and subarctic regions, and the colonisation of the new suitable areas may still be in progress (Rausch, 1995; Hildreth *et al*. 2000; Storandt & Kazacos, 1993; Storandt *et al*. 2002).

The adaptation of foxes to urban environments – known in Britain since the 1940s – occurred rather recently in continental Europe (as in Japan), possibly because the development was prevented by the low population density of foxes during the rabies period. Today 'urban foxes' are known from many towns and cities of south-central Europe, e.g. in southern Germany and Switzerland. Casual observations and basic research have shown that population densities can be much higher than in rural habitats due to abundant availability of food. Infection rates with *E. multilocularis* can be high (44% of 388 foxes in Zurich; Hofer *et al*. 2000), but appear to be limited by the presence of habitats suitable for voles. However, due to the high population density the absolute number of infected foxes may be higher than in agricultural landscapes, and the close proximity of foxes and man poses a considerable risk to the human population. Transmission to humans may not only occur directly from infected foxes, but also from pet dogs and cats which become infected by catching rodents in city parks and gardens: 9% of 889 *A. terrestris* were found infected in the urban to peri-urban areas of Zurich (Stieger *et al*. 2002).

In summary, the superficially very similar epidemiological situation differs between Japan and other endemic regions of the word, most conspicuously due to different ecological requirements for the wildlife cycle. Therefore, control methods developed

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e.g. in Europe may not necessarily exhibit the same efficacy under Japanese conditions, and any control measures introduced require careful analysis.

CONTROL OPTIONS

Options to control the transmission of *E. multilocularis* vary according to the species of final host which is thought to be the predominant source of human infection, and according to control objectives. Concerning the final host species, two fundamental patterns exist: situations where domestic dogs are substantially involved in the lifecycle of *E. multilocularis* (synanthropic cycle), and situations where the involvement of domestic dogs is only marginal or does not exist at all (sylvatic cycle).

Synanthropic life cycles

Where dogs are quantitatively important links in the cycle, frequent close contact with infected dogs is likely to result in relatively high infection pressure on humans. Where owned or stray dogs show high infection rates, human infection is relatively common. This is the case in rather few regions; examples include parts of Alaska and western China. There, the parasite's lifecycle is maintained by owned or semi-feral dogs kept in villages, which frequently feed on rodents inside or near human habitations. In Alaska (St. Lawrence Island), commensal populations of the arctic vole, *Microtus oeconomus*, are of principal importance, while in China a range of species of rodents (*Microtus* spp.) and pica (*Ochotona* spp.) are proven or suspected as intermediate hosts. Although sylvatic transmission usually also occurs in such areas and is linked with the dog-rodent cycle, the relative frequency of human contact with the wild canid species is negligible, and the contribution of wild canids to human morbidity is thought to be small. A special situation has recently arisen in Europe and Japan where red foxes (*V. vulpes*) increasingly adapt to urban environments, creating a wildlife-based form of synanthropic transmission (discussed above).

Sylvatic life cycles

In most regions, wild canids (foxes, coyotes etc.) carry by far the largest part of the parasite biomass. In these situations, domestic dogs and cats may be marginally involved in the lifecycles, but prevalence rates are usually very low. With infected dogs being rare, and with human contact with infected wildlife negligible, the number of human AE cases is comparatively small. This is, still, the case in Europe, Japan and temperate North America.

Control objectives

The overall aim of *E. multilocularis* control is the reduction or elimination of infection pressure to the human population. This may be achieved in two fundamentally different ways: the interruption of specific transmission routes to humans, or the suppression of the entire lifecycle of the parasite (with the ultimate aim of eradication). Which approach may be most applicable or desirable in the locally prevailing situation depends not only on the actual infection risk and the type of life cycle, but also on socio-cultural factors such as risk perception, human-wildlife interaction and economic conditions. Among communities where synanthropic transmissions with high levels of human AE exposure exist, the elimination of dogs as the main infection source (e.g. by dog dosing or elimination of strays) may be perceived as sufficient, and the remaining small risk emanating from wildlife may be considered unimportant. Such a risk, however, is not acceptable in Europe or Japan where by public pressure enormous efforts are spent to contain even objectively minor mortality risks, e.g. BSE or rabies.

Control options in the synanthropic situation

Principal final hosts in synanthropic cycles are domestic dogs. Many options for control can therefore be borrowed from control programmes against cystic echinococcosis (CE) which is predominantly transmitted by dogs. However, since intermediate hosts for *E. granulosus* are usually domestic ungulates, an important part of CE control focuses on preventing access of dogs to offal of slaughtered livestock; vaccination of intermediate hosts may be a future option (Lightowlers *et al.* 1999; Gauci *et al.* 2002; Siles-Lucas *et al.* 2003). With *E. multilocularis* there are no practical intervention measures targeted at the intermediate host (rodent) side, making the dog the exclusive control target. Control measures which have proven effective against CE include information campaigns about the parasite, its transmission routes and applicable preventive behaviour. Other effective measures are the elimination of stray dogs, reduction in the number of owned dogs, and registration and regular dosing with cestodicidal drugs of the remaining population. In all control efforts, continuous surveillance of the control effect is essential, e.g. by coproantigen surveillance of dog faeces. However, the applicability of such measures depends on the regions' cultural and social backgrounds. While destruction of stray dogs may be viewed as welcome pest control in one culture, it may be totally unacceptable in others, e.g. Buddhist communities in parts of Asia. Dog registration, regular dosing and surveillance require considerable funds and a certain level of organizational infrastructure which are not available everywhere. In conclusion, there is no universally applicable approach to control synanthropic *E. multilocularis* transmission. The strategy has to be specifically designed for the local situation by selecting the appropriate

combination from the menu of possible measures listed above. An intrinsic drawback for all control measures which concentrate on dogs is neglect of the transmission persisting in sylvatic cycles in the surrounding areas, and which is likely to occasionally interlink with the dog-rodent cycle. Therefore, local eradication appears unfeasible due to constant reintroduction of the parasite, in contrast to the situation with *E. granulosus* where in most endemic areas wildlife species do not maintain transmission independently of the domestic cycle. Therefore, intervention strategies focusing on dogs exclusively have to be viewed as long-term commitments with no prospect of regional eradication.

The efficacy of dog dosing for *E. multilocularis* control was demonstrated in a 10-year study by Rausch, Wilson & Schantz (1990) on St. Lawrence Island (Alaska) where owned dogs kept within a village were dosed at monthly intervals with praziquantel (5 mg/kg). The effect was measured by the prevalence rate of the intermediate hosts, commensal voles (*M. oeconomous*) which shared the habitat with the dogs. *E. multilocularis* prevalence in the voles was 29% during three pre-control years and decreased after two years of dosing to a relatively stable level of 5% for a further five years. The remaining level of infection – and the rebound after discontinuation of the treatment schedule (Schantz *et al.* 1995) – was attributed to the interaction with the sylvatic transmission outside the village, maintained by arctic foxes (*Alopex lagopus*). For the efficacy of the method, the authors stress the importance of strict adherence to the dosing schedule and destruction of non-dosed stray dogs.

Control options in the sylvatic situation

Several species of wild canids are known to be natural hosts for *E. multilocularis*. In temperate regions throughout the parasite's range, the red fox (*V. vulpes*) appears to be principally responsible for maintaining the life cycle (together with the coyote, *C. latrans*, in North America). As in the synanthropic cycles, theoretical control measures include population reduction of the hosts, and chemotherapy ('deworming'). While contemporary traditional hunting appears ineffective for the reduction of fox populations, more radical measures like poisoning or gassing of dens are environmentally destructive and are considered unethical in most countries. Furthermore, widespread attempts at fox eradication for rabies control in central Europe in the mid-20th century demonstrated that such methods are neither practical to achieve the elimination of foxes nor of the parasite.

A first attempt at chemotherapy of wild foxes was made in southern Germany by Schelling *et al.* (1997). The background hypothesis was that increasing the mortality rate of adult worms in foxes by repeated chemotherapy with the cestodicidal drug praziquantel (Andrews & Thomas, 1983) would eventually decrease the prevalence in the rodent population and, consequently, lead to diminishing infection pressure to foxes – a process which may eventually lead to local extinction of the parasite. Bait pellets containing 50 mg praziquantel each were distributed in densities of 15–20 baits/km^2 within an endemic area of 566 km^2. Baits were distributed by hand six times during a 14-month period, and achieved a decrease of *E. multilocularis* prevalence in sampled foxes from 32% (C.I.95: 16–52%) to 4% (C.I.95: 2–7%). While demonstrating the baiting efficacy in principle, the period and area size of this study were insufficient for further conclusions on the practicality of a large-scale application. Therefore, an additional five-year study was done in a much larger area (3500 km^2) of the same region. In the second trial, small aircraft were used for distribution of praziquantel baits (20/km^2) according to the protocol of concurrent rabies immunization campaigns. After 1·5 years of repeated baiting at six-week intervals, a 75% reduction of prevalence in shot foxes was achieved from 64% (C.I.95: 59–69%) to 18% (C.I.95: 13–24%) (Romig *et al.* 1999*c*). During a further 1·5 years of baiting at three-month intervals, the prevalence remained low with 15% (C.I.95: 10–21%), while within two years of gradual discontinuation of baiting, the prevalence rebound to almost pre-control levels (Romig *et al.* unpublished).

The results of this study, conducted in a highly endemic region of southwestern Germany, are in agreement with a similar large-scale trial in northeastern Germany. There, the study area of 5000 km^2 contained two circumscribed endemic foci and a low endemic periphery. Bait distribution was done closely similar to the study above, for one year at six-week intervals, followed by another year at three-month intervals. Baiting efficacy was analyzed separately for the endemic and low endemic areas, with prevalences in foxes in the year before control of 15·6–26·8% and 4·1–7·1%, respectively. Drastic reduction, but no eradication was achieved under both conditions, with prevalences in the last year of control ranging from 1·9–6·2% in the endemic area, and 0·0–1·0% in the low endemic area (Tackmann *et al.* 2001).

Studies on small-scale application of praziquantel to foxes were reported from Japan. In a 90 km^2 area in Hokkaido, praziquantel tablets embedded in a fish-meal matrix were placed near fox dens. Baiting was repeated every month for a 13-month period. Efficacy was estimated by taeniid egg detection in fox faeces, which decreased from 27% to 6%, and by coproantigen positivity rate, decreasing from 60% to 30% (Tsukada *et al.* 2002). An additional trial using praziquantel-baits has been done in a 135 km^2 area of Nemuro city, eastern Hokkaido, a high endemic area of AE, and a decrease of *E. multilocularis* in

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foxes was observed by necropsy (Takahashi *et al*. 2002).

In the European studies of chemotherapeutic intervention against the sylvatic cycle, infection rates in foxes decreased drastically, but eradication was not achieved under the conditions tested. While such a decrease is likely to relieve the infection pressure for human AE, a linear relation between fox infection and AE incidence is unproven because of the rarity of human AE data in regions with sylvatic transmission and the direct relevance of *E. multilocularis* prevalence in wildlife to human risk is unknown. In any case, in order to improve control efficacy there is a need to address the potential factors responsible for persistence of the transmission. One such factor appears important: in the studies summarized above, baiting frequency and distribution density remained constant throughout the year, while fox populations gradually decrease towards the reproductive period in spring, and increase drastically thereafter due to the appearance of juveniles. Apart from the numerical change, in both cited studies the role of juvenile foxes in the summer months was recognized to be of importance for transmission during the non-intervention period and for the persistence of the lifecycle during control. In endemic and high endemic areas, both prevalence and infection intensity (worm burden) was higher in juveniles during all project phases. In the study from northeastern Germany, after two years of baiting no infected adult fox was found, but juveniles maintained prevalence of 11·6% in the endemic and 1·8% in the low endemic area (Tackmann *et al*. 2001). Changing the baiting schedules in order to specifically target the juvenile fox populations, therefore, appears to be one of several options to improve efficacy. However, to estimate fully the impact of such parameter changes would require a series of large-scale studies which are expensive and time consuming. The development of mathematical models of the *E. multilocularis* life cycle is therefore a priority in order to understand limiting factors for transmission, to optimize large-scale baiting and to adapt the method to geographical and seasonal conditions. Recently, a spatially explicit simulation model was developed taking into account the observed clustering of the parasite on the intermediate host level (Hansen *et al*. 2001), which is expected to be a valuable tool for optimization of control strategies. However, theoretical considerations on a lifecycle of such complexity are necessarily an incomplete image of reality, and have to be further compared to field data in a continuous process.

Synanthropic foxes

As described above, the 'urban fox phenomenon' has recently become conspicuous both in Europe and in Japan. Such foxes are less likely to be targeted by baits dispersed by aircraft outside settled areas, although they may be exposed to *E. multilocularis* infection via rodent populations in or near towns. Because of the close contact with humans and the resulting risk of AE transmission, the targeting of such synanthropic fox populations of cities, towns and villages in control strategies is a matter of priority. *E. multilocularis* control in urban foxes may be achieved by large-scale baiting as described above or a strategy targeted at areas of intense fox-man contact. Studies on fox biology and the synanthropic *E. multilocularis* transmission are presently in progress in cities (e.g. Zurich, Sapporo) and in rural settlements (e.g. on the Swabian Jura in southwestern Germany). Although these studies are still in a preliminary phase, the options of fox reduction or elimination in urban environments appear unfeasible for a variety of reasons (including human attitudes towards wildlife and the non-applicability of trapping or shooting). Chemotherapy with praziquantel baits may be a more acceptable method.

As mentioned earlier, the role of dogs and cats in exposure of humans in regions of sylvatic transmission is not completely understood. While their contribution to the overall maintenance of the parasite is likely to be small, they may act as important agents of human exposure. This could be especially true in situations of urban fox-based *E. multilocularis* transmission, since large numbers of pet dogs and cats in suburban environments are now exposed to an infection which was previously restricted to rural areas. A quantitative estimate of the contribution of pet dogs and cats to the *E. multilocularis* lifecycle in such situations has not been done. Such data, however, are necessary to decide whether regular chemotherapy of these species would be a substantial contribution to control of the parasite, or would rather be a measure to reduce human exposure to infective eggs.

Cost–benefit considerations

While the benefits of control measures are obvious under conditions of synanthropic, dog-based transmission with relatively high incidence of human AE, the situation is different in regions of sylvatic transmission where AE in humans is comparatively rare. Public demand for the implementation of control programmes may partly be driven by exaggerated risk perception in 'developed' countries where lethal infectious diseases are almost non-existent. However, previous population surveys for AE in highly endemic regions of Germany and France calculated prevalence figures of 40/100 000 and 152/100 000, respectively (Romig *et al*. 1999*b*; Bresson-Hadni *et al*. 1994). When considering the cost of drug treatment (often life-long chemotherapy) which can range from US$ 5500 to 17 800 annually, and an estimated US$ 300 000 a mean the total medical cost

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per case (Reuter *et al*. 1998; Romig *et al*. 1999*b*), it is apparent that, at least in highly endemic areas the economic costs are not trivial (even when additional economic losses due to disability and mortality are not included). However, in the absence of data on the correlation between prevalence of *E. multilocularis* infection in wildlife and the incidence of AE in humans (which are unlikely to be available soon), it remains a political decision whether control measures should be implemented, and which of the approaches summarized above are most appropriate for the local conditions.

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REFERENCES

ABE, H. (1975). Winter food of the red fox, *Vulpes vulpes schrencki* Kishida (Carnivora: Canidae), in Hokkaido, with special reference to vole populations. *Applied Entomology and Zoology* **10**, 40–51.

ALLAN, J. C., CRAIG, P. S., GARCIA-NOVAL, J., MENCOS, F., LIU, D., WANG, Y., WEN, H., ZHOU, P., STRINGER, R., ROGAN, M. & ZEYHLE, E. (1992). Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. *Parasitology* **104**, 347–356.

AMBO, H., ICHIKAWA, K., IIDA, H. & ABE, N. (1954). On echinococcosis alveolaris, endemic parasitosis in Rebun Island. *Special Report of Hokkaido Institute of Public Health* **4**, 1–19 (in Japanese).

ANDREWS, P. & THOMAS, H. (1983). Praziquantel. *Medical Research Review* **3**, 147–200.

BRESSON-HADNI, S., LAPLANTE, J. J., LENYS, D., ROHMER, P., GOTTSTEIN, B., JACQUIER, P., MERCET, P., MEYER, J. P., MIGUET, J. P. & VUITTON, D. A. (1994). Seroepidemiologic screening of *Echinococcus multilocularis* infection in a European area endemic for alveolar echinococcosis. *American Journal of Tropical Medicine and Hygiene* **51**, 837–846.

BRETAGNE, S., ASSOULINE, B., VIDAUD, D., HOUIN, R. & VIDAUD, M. (1996). *Echinococcus multilocularis*: microsatellite polymorphism in U1 snRNA genes. *Experimental Parasitology* **82**, 324–328.

CRAIG, P. S., GIRAUDOUX, P., SHI, D., BARTHOLOMOT, B., GARNISH, G., DELATTRE, P., QUÉRE, J. P., HARRAGA, S., BAO, G., WNAG, W., LU, F., ITO, A. & VUITTON, D. A. (2000). An epidemiological and ecological study of human alveolar echinococcosis transmission in south Gansu, China. *Acta Tropica* **77**, 167–177.

CRAIG, P. S. & PAWLOWSKI, Z. S. (2002). *Cestode Zoonoses: Echinococcosis and Cysticercosis An Emergent and Global Problem*. 395 pp. NATO Science Series, IOS Press, Amsterdam.

DAI, W. J., HEMPHILL, A., WALDVOGEL, A., INGOLD, K., DEPLAZES, P., MOSSMANN, H. & GOTTSTEIN, B. (2001). Major carbohydrate antigen of *Echinococcus multilocularis* induces an immunoglobulin G response independent of alphabeta+ CD4+ T cells. *Infection and Immunity* **69**, 6074–6083.

DEPLAZES, P., ALTHER, P., TANNER, I., THOMPSON, R. C. & ECKERT, J. (1999). *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog and cat populations. *Journal of Parasitology* **85**, 115–121.

DINKEL, A., NICKISCH-ROSENEGK, M., BILGER, B., MERLI, M., LUCIUS, R. & ROMIG, T. (1998). Detection of *Echinococcus multilocularis* in the definitive host: coprodiagnosis by PCR as an alternative to necropsy. *Journal of Clinical Microbiology* **36**, 1871–1876.

DOI, M., KANDA, E., NIHEI, N. & UCHIDA, A. (2000*a*). Occurrence of alveolar hydatid disease (multilocular echinococcosis) outside of Hokkaido and a proposal for its prevention. *Japanese Journal of Public Health* **47**, 111–126 (in Japanese with English summary).

DOI, M., NAKAO, M., INAOKA, T., OHNISHI, K., KUTSUMI, H., ARAKAWA, K., AMOH, K., ISHIMARU, O., SEO, H. & FUKUYAMA, Y. (1987). Epidemiology of multilocularis echinococcosis in Hokkaido (1) a sero-epidemiological study of hunters. *Japanese Journal of Public Health* **34**, 357–365 (in Japanese with English summary).

DOI, M., NAKAO, M., NIHEI, N. & KUTSUMI, H. (2000*b*). Epidemiology of alveolar hydatid disease (AHD) and estimation of infected period of AHD in Rebun Island, Hokkaido. *Japanese Journal of Public Health* **47**, 145–152 (in Japanese with English summary).

ECKERT, J., GEMMELL, M. A., MESLIN, F.-X. & PAWLOWSKI, Z. S. (2001). *WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern*. Office International des Epizooties, Paris, 265 pp.

FELLEISEN, R. & GOTTSTEIN, B. (1994). *Echinococcus multilocularis*: molecular and immunochemical characterization of diagnostic antigen II/3–10. *Parasitology* **107**, 335–342.

FROSCH, P. M., FROSCH, M., PFISTER, T., SCHAAD, V. & BITTER-SUERMANN, D. (1991). Cloning and characterization of an immunodominant major surface antigen of *Echinococcus multilocularis*. *Molecular and Biochemical Parasitology* **48**, 121–130.

FROSCH, P. M., MUHLSCHEGEL, F., SYGULLA, L., HARTMANN, M. & FROSCH, M. (1994). Identification of a cDNA clone from the larval stage of *Echinococcus granulosus* with homologies to the *E. multilocularis* antigen EM10-expressing cDNA clone. *Parasitology Research* **80**, 703–705.

FURUYA, K., SASAKI, S., HONMA, H., KUMAGAI, M., SATO, N., TAKAHASHI, M. & UCHINO, J. (1989). Serologic investigations of human alveolar hydatid disease by western blotting and indirect histo-immunoperoxidase techniques. *Japanese Journal of Parasitology* **38**, 184–193.

GAUCI, C., MERLI, M., MULLER, V., CHOW, C., YAGI, K., MACKENSTEDT, U. & LIGHTOWLERS, M. W. (2002). Molecular cloning of a vaccine antigen against infection with the larval stage of *Echinococcus multilocularis*. *Infection and Immunity* **70**, 3969–3972.

GIRAUDOUX, P., DELATTRE, P., TAKAHASHI, K., RAOUL, F., QUÉRE, J. P., CRAIG, P. & VUITTON, D. (2002). Transmission ecology of *Echinococcus multilocularis* in wildlife: what can be learnt from comparative studies and multi-scale approaches? In *Cestode Zoonoses: An Emergent and Global Problem* (ed. Craig, P. S. & Pawlowski, Z.), pp. 251–262. NATO Science Series, IOS Press, Amsterdam.

GOTTSTEIN, B. (1985). Purification and characterization of a specific antigen from *Echinococcus multilocularis*. *Parasite Immunology* **7**, 201–212.

GOTTSTEIN, B., JACQUIER, P., BRESSON HADNI, S. & ECKERT, J. (1993). Improved primary immunodiagnosis of alveolar echinococcosis in humans by an enzyme-linked immunosorbent assay using Em2^{plus} antigen. *Journal of Clinical Microbiology* **31**, 373–376.

GOTTSTEIN, B., SAUCY, F., DEPLAZES, P., REICHEN, J., DEMIERRE, G., BUSATO, A., ZUERCHER, C. & PUGIN, P. (2001). Is high prevalence of *Echinococcus multilocularis* in wild and domestic animals associated with disease incidence in humans? *Emerging Infectious Diseases* **7**, 408–412.

HANSEN, F., TACKMANN, K., JELTSCH, F., STAUBACH, C. & THULKE, H. H. (2001). If space changes all – the small-scale epidemiology of the fox tapeworm. *Proceedings, SVEPM conference*, pp. 73–85. Nordwijkerhout.

HATAKEYAMA, Y., SATO, N., OYAMA, Y., INOUE, N., TAKENOSITA, S., TAKEUCHI, S. & ITO, A. (2002). A surgical case report of hepatic cystic echinococcosis. *Shujutsu* **56**, 819–823 (in Japanese).

HELBIG, M., FROSCH, P., KERN, P. & FRSOCH, M. (1993). Serological differentiation between cystic and alveolar echinococcosis by use of recombinant larval antigens. *Journal of Clinical Microbiology* **31**, 3211–3215.

HEMMINGS, L. & McMANUS, D. P. (1991). The diagnostic value and molecular characterisation of an *Echinococcus multilocularis* antigen gene clone. *Molecular and Biochemical Parasitology* **44**, 56–62.

HILDRETH, M. B., JOHNSON, M. D. & KAZACOS, K. R. (1991). *Echinococcus multilocularis*: A zoonosis of increasing concern in the United States. *Compendium on Continuing Education for the Practising Veterinarian* **13**, 727–740.

HILDRETH, M. B., SRIRAM, S., GOTTSTEIN, B., WILSON, M. & SCHANTZ, P. M. (2000). Failure to identify alveolar echinococcosis in trappers from South Dakota in spite of high prevalence of *Echinococcus multilocularis* in wild canids. *Journal of Parasitology* **86**, 75–77.

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 Zürich, Switzerland. *Parasitology* **120**, 135–142.

HULSMEIER, A. J., GEHRIG, P. M., GEYER, R., SACK, P., GOTTSTEIN, B., DEPLAZES, P. & KOHLER, P. (2002). A major *Echinococcus multilocularis* antigen is a mucin-type glycoprotein. *Journal of Biological Chemistry* **277**, 5742–5748.

INAOKA, T., NAKAO, M., OHNISHI, K., DOI, M. & KUTSUMI, H. (1987). Epidemiological survey of multilocularis echinococcosis intended for tanners and taxidermists in Hokkaido, Japan. *Journal of Northern Occupational Health* **36**, 9–12 (in Japanese with English summary).

ISHIGE, M. (1984). Incidence of swine multilocular echinococcosis in Hokkaido. *Report of the Hokkaido Institute of Public Health* **34**, 70–71 (in Japanese).

ITO, A. (2001*a*). Introduction of ongoing research projects on echinococcosis at Asahikawa Medical College and some comments on the surveillance, prevention and control of alveolar echinococcosis in Japan. *Hokkaido Journal of Medical Science* **76**, 3–8 (in Japanese with English summary).

ITO, A. (2001*b*). Problems on echinococcosis. *Asahikawa Medical Forum* **2**, 13–19 (in Japanese with English summary).

ITO, A. (2002*a*). Serologic and molecular diagnosis of zoonotic larval cestode infections. *Parasitology International* **51**, 221–235.

ITO, A. (2002*b*). Up-to-date situation and problems of cysticercosis and echinococcosis in the world as emerging and re-emerging parasitic diseases. *Japanese Journal of Clinical Environment* **10**, 59–66 (in Japanese with English summary).

ITO, A. & CRAIG, P. S. (2003). Immunodiagnostic and molecular approaches for the detection of taeniid cestode infections. *Trends in Parasitology* **19**, 377–381.

ITO, A., MA, L., SCHANTZ, P. M., GOTTSTEIN, B., LIU, Y. H., CHAI, J. J., ABDEL-HAFEZ, S. K., ALTINTAS, N., JOSHI, D. D., LIGHTOWLERS, M. W. & PAWLOWSKI, Z. S. (1999). Differential serodiagnosis for cystic and alveolar echinococcosis using fractions of *Echinococcus granulosus* cyst fluid (antigen B) and *E. multilocularis* protoscolex (Em18). *American Journal of Tropical Medicine and Hygiene* **60**, 188–192.

ITO, A., NAKAO, M., KUTSUMI, H., LIGHTOWLERS, M. W., ITOH, M. & SATO, S. (1993). Serodiagnosis of alveolar hydatid disease by western blotting. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **87**, 170–172.

ITO, A., SAKO, Y., ISHIKAWA, Y., NAKAO, M., NAKAYA, K. & YAMASAKI, H. (2002*a*). Differential serodiagnosis for alveolar echinococcosis by Em18-immunoblot and Em18-ELISA in Japan and China. In *Cestode Zoonoses: Echinococcosis and Cysticercosis – An Emergent and Global Problem* (ed. Craig, P. S. & Pawlowski, Z.), pp. 147–155. NATO Science Series, IOS Press, Amsterdam.

ITO, A., SAKO, Y., YAMASAKI, H., MAMUTI, W., NAKAYA, K., NAKAO, M. & ISHIKAWA, Y. (2003*a*). Development of Em18-immunoblot and Em18-ELISA for specific diagnosis of alveolar echinococcosis. *Acta Tropica* **85**, 173–182.

ITO, A., SCHANTZ, P. M. & WILSON, J. F. (1995). Em18, a new serodiagnostic marker for differentiation of active and inactive cases of alveolar hydatid disease. *American Journal of Tropical Medicine and Hygiene* **52**, 41–44.

ITO, A., URBANI, C., QIU, J. M., VUITTON, D. A., QIU, D. C., HEATH, D. D., CRAIG, P. S., FENG, Z. & SCHANTZ, P. M. (2003*b*). Control of echinococcosis and cysticercosis: a public health challenge to international cooperation in China. *Acta Tropica* **86**, 3–17.

ITO, A., XIAO, N., LIANCE, M., SATO, M. O., SAKO, Y., MAMUTAI, W., ISHIKAWA, Y., NAKAO, M., YAMASAKI, H., NAKAYA, K., BARDONNET, K., BRESSOPN-HADNI, S. & VUITTON, D. A. (2002*b*). Evaluation of an enzyme-linked immunosorbent assay (ELISA) with affinity-purified Em18 and an ELISA with recombinant Em18 for

differential diagnosis of alveolar echinococcosis: results of a blind test. *Journal of Clinical Microbiology* **40**, 4161–4165.

KAJI, Y., TANIYAMA, H., MATSUKAWA, K., OKADA, H., TSUNODA, S., TAGAMI, M. & AKITA, H. (1993). First incidence of multilocular echinococcosis in a race horse in Japan. *Japanese Journal of Veterinary Medical Science* **55**, 869–870.

KAMIYA, H. & KANAZAWA, T. (1999). The first detection of *Echinococcus* infection among pigs on the main island of Japan, August 1998 – Aomori. *Infectious Agents Surveillance Report* **20**, 248–249 (in Japanese).

KERN, P., BARDONNET, K., RENNER, E., AUER, H., PAWLOWSKI, Z., AMMANN, R. W., VUITTON, D. A., KERN, P. & EUROPEAN ECHINOCOCCOSIS REGISTRY (2003). European echinococcosis registry: human alveolar echinococcosis, Europe, 1982–2000. *Emerging Infectious Diseases* **9**, 343–349.

KONDO, N., TAKAHASHI, K. & YAGI, K. (1986). Winter food of the red fox, *Vulpes vulpes schrencki* Kishida in the endemic area of multilocular echinococcosis. *The Bulletin of Preparative Office of Nemuro Municipal Museum* **1**, 23–31 (in Japanese with English summary).

KOSUGE, M. & BANDO, G. (2003). A case report of alveolar echinococcosis of a Gorilla at Asahikawa Zoo and strategy for prevention of accidental infection of zoo animals and visitors from wild foxes. *Japan Veterinary Medical Association* **56**, 46–51 (in Japanese).

LAWTON, P., HEMPHILL, A., DEPLAZES, P., GOTTSTEIN, B. & SARCIRON, M. E. (1997). *Echinococcus multilocularis* metacestodes: immunological and immunocytochemical analysis of the relationships between alkaline Phosphatase and the Em2 antigen. *Experimental Parasitology* **87**, 142–149.

LIGHTOWLERS, M. W., JENSEN, O., FERNANDEZ, E., IRIATE, J. A., WOOLARD, D. J., GAUCI, C. G., JENKINS, D. J. & HEATH, D. D. (1999). Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep. *International Journal for Parasitology* **29**, 531–534.

MATHIS, A., DEPLAZES, P. & ECKERT, J. (1996). An improved test system for PCR-based specific detection of *Echinococcus multilocularis* eggs. *Journal of Helminthology* **70**, 219–222.

McMANUS, D. P., ZHANG, W. & BARTLEY, P. B. (2003). Echinococcosis. *Lancet* (in press).

MINAGAWA, N. (1997). Survey of echinococcosis in Hokkaido and measure against it. *Hokkaido Journal of Medical Science* **72**, 569–581 (in Japanese with English summary).

MINAGAWA, N. (1999). The reconsideration of natural history of echinococcosis on Rebun Island. *Hokkaido Journal of Medical Science* **74**, 113–134 (in Japanese with English summary).

MIYAUCHI, T., SAKUI, M., ISHIGE, M., FUKUMOTO, S., UEDA, A. & OHBAYASHI, M. (1984). A case of multilocular echinococcosis in a horse. *Japanese Journal of Veterinary Research* **32**, 171–173.

NAKAO, M., INAOKA, T., DOI, M., KUTSUMI, H., ARAKAWA, K. & OHNISHI, K. (1988). Epidemiology of multilocular echinococcosis in Hokkaido (2) Seroepidemiological survey of residents in hog raising areas in Asahikawa city. *Japanese Journal of Public Health* **35**, 184–192 (in Japanese with English summary).

NAKAO, M., SAKO, Y. & ITO, A. (2003). Isolation of polymorphic microsatellite loci from the tapeworm *Echinococcus multilocularis*. *Infection, Genetics and Evolution* **3**, 159–163.

NAKAO, M., YOKOYAMA, N., SAKO, Y., FUKUNAGA, M. & ITO, A. (2002). The complete mitochondrial DNA sequence of the cestode *Echinococcus multilocularis* (Cyclophyllidea: Taeniidae). *Mitochondrion* **1**, 497–509.

NATIONAL INSTITUTE OF INFECTIOUS DISEASES AND TUBERCULOSIS AND INFECTIOUS DISEASES CONTROL DIVISION, MINISTRY OF HEALTH, LABOUR AND WELFARE (1999). Multilocular echinococcosis in Hokkaido, Japan. *Infectious Agents Surveillance Report* **20**, 1′–2′.

NONAKA, N., IIDA, M., YAGI, K., ITO, T., OOI, H. K., OKU, Y. & KAMIYA, M. (1996). Time course of coproantigen excretion in *Echinococcus multilocularis* infection in foxes and alternative host, golden hamsters. *International Journal for Parasitology* **26**, 1271–1278.

OHBAYASHI, M. (1996). Host animals of *Echinococcus multilocularis* in Hokkaido. In *Alveolar Echinococcosis: Strategy for Eradication of Alveolar Echinococcosis of the Liver* (ed. Uchino, J. & Sato, N.), pp. 59–64. Sapporo, Fujishoin.

PAWLOWSKI, Z. S., ECKERT, J., VUITTON, D. A., AMMANN, R. W., KEMP, P., CRAIG, P. S., DAR, K. F., DE ROSA, F., FILICE, C., GOTTSTEIN, B., GRIMM, F., MACPHERSON, C. N. L., SATO, N., TODOROV, T., UCHINO, J., VON SINNER, W. & WEN, H. (2001). Chapter 2 Echinococcosis in humans: clinical aspects, diagnosis and treatment. In *WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern* (ed. Eckert, J., Gemmell, M. A., Meslin, F.-X. & Pawlowski, Z. S.), pp. 20–66. Paris, Office International des Epizooties.

RAOUL, F., DEPLAZES, P., NONAKA, N., PIARROUX, R., VUITTON, D. A. & GIRAUDOUX, P. (2001). Assessment of the epidemiological status of *Echinococcus multilocularis* in foxes in France using ELISA coprotests on fox faeces collected in the field. *International Journal for Parasitology* **31**, 1579–1588.

RAUSCH, R. L. (1954). Studies on the helminth fauna of Alaska. XX. The histogenesis of the alveolar larva of *Echinococcus* species. *Journal of Infectious Diseases* **94**, 178–186.

RAUSCH, R. L. (1995). Life cycle patterns and geographic distribution of *Echinococcus* species. In *Echinococcus and Hydatid Disease* (ed. Thompson, R. C. A. & Lymbery, A. J.), pp. 88–134. Wallingford, CAB International.

RAUSCH, R. L., WILSON, J. F. & SCHANTZ, P. M. (1990). A programme to reduce the risk of infection by *Echinococcus multilocularis*: the use of praziquantel to control the cestode in a village in the hyperendemic region of Alaska. *Annals of Tropical Medicine and Parasitology* **84**, 239–250.

REUTER, S., KRATZER, W., KURZ, S., WELLINGHAUSEN, N. & KERN, P. (1998). Chemotherapie der alveolären Echinokokkose mit Benzimidazolen. *Medizinische Klinik* **93**, 463–467 (in German).

ROMIG, T. (2002). Spread of *Echinococcus multilocularis* in Europe? In *Cestode Zoonoses: Echinococcosis and Cysticercosis – An Emergent and Global Problem*

(ed. Craig, P. & Pawlowski, Z.), pp. 65–80. NATO Science Series, Amsterdam, IOS Press.

ROMIG, T., BILGER, B., DINKEL, A., MERLI, M. & MACKENSTEDT, U. (1999*a*). *Echinococcus multilocularis* in animal hosts: new data from western Europe. *Helminthologia* **36**, 185–191.

ROMIG, T., BILGER, B., MERLI, M., DINKEL, A., LUCIUS, R. & MACKENSTEDT, U. (1999*c*). Bekämpfung von *Echinococcus multilocularis* in einem Hochendemiegebiet Süddeutschlands. In *Neuere Methoden und Ergebnisse zur Epidemiologie von Parasitosen*, pp. 172–183. Giessen, Deutsche Veterinärmedizinische Gesellschaft (in German).

ROMIG, T., KRATZER, W., KIMMIG, P., FROSCH, M., GAUS, W., FLEGEL, W. A., GOTTSTEIN, B., LUCIUS, R., BECKH, K. & KERN, P. (1999*b*). An epidemiologic survey of human alveolar echinococcosis in southwestern Germany. *American Journal of Tropical Medicine and Hygiene* **61**, 566–573.

SAITOH, T., STENSETH, N. C. & BJORNSTAD, O. N. (1998). The population dynamics of the vole *Clethrionomys rufocanus* in Hokkaido, Japan. *Research in Population Ecology* **40**, 61–76.

SAITOH, T. & TAKAHASHI, K. (1998). The role of vole populations in prevalence of the parasite (*Echinococcus multilocularis*) in foxes. *Research in Population Ecology* **40**, 97–105.

SAKO, Y., NAKAO, M., NAKAYA, K., YAMASAKI, H., GOTTSTEIN, B., LIGHTOWLERS, M. W., SCHANTZ, P. M. & ITO, A. (2002). Alveolar echinococcosis: characterization of diagnostic antigen Em18 and serological evaluation of recombinant Em18. *Journal of Clinical Microbiology* **40**, 2760–2765.

SAKUI, M., ISHIGE, M., FUKUMOTO, S., UEDA, A. & OHBAYASHI, M. (1984). Spontaneous *Echinococcus multilocularis* infection in swine in north-eastern Hokkaido, Japan. *Japanese Journal of Parasitology* **33**, 291–296.

SATO, C. & FURUYA, K. (1994). Isolation and characterization of a diagnostic polysaccharide antigen from larval *Echinococcus multilocularis*. *Japanese Journal of Medical Science and Biology* **47**, 65–71.

SATO, C., NAGANO, H. & FURUYA, K. (1996). A polysaccharide antigen diagnostic in human alveolar hydatid disease. In *Alveolar Echinococcosis: Strategy for Eradication of Alveolar Echinococcosis of the Liver* (ed. Uchino, J. & Sato, N.), pp. 129–134. Sapporo, Fujishoin.

SATO, H., MITAMURA, H., ARAI, J. & KUMAGAI, M. (1983). Serological diagnosis of human hydatid disease by enzyme-linked immunosorbent assay (part 1) enzyme-linked immunosorbent assay by multilocular echinococcus antigen. *Report of the Hokkaido Institute of Public Health* **33**, 8–15 (in Japanese with English summary).

SATO, N., OGASAWARA, K., KAMIYAMA, T., MATSUSHITA, M. & TODO, S. (2003). Echinococcosis. *Nippon Rinsho* **61** (Suppl. 2), 636–643 (in Japanese).

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 *Echinococcus and Hydatid Disease* (ed. Thompson, R. C. A. & Lymbery, A. J.), pp. 233–331. Wallingford, CAB International.

SCHELLING, U., FRANK, W., WILL, R., ROMIG, T. & LUCIUS, R. (1997). Chemotherapy with praziquantel has the potential to reduce the prevalence of *Echinococcus multilocularis* in wild foxes (*Vulpes vulpes*). *Annals of Tropical Medicine and Parasitology* **91**, 179–186.

SILES-LUCAS, M. & GOTTSTEIN, B. (2001). Molecular tools for the diagnosis of cystic and alveolar echinococcosis. *Tropical Medicine and International Health* **6**, 463–475.

SILES-LUCAS, M., MERLI, M., MACKENSTEDT, U. & GOTTSTEIN, B. (2003). The *Echinococcus multilocularis* 14-3-3 protein protects mice against primary but not secondary alveolar echinococcosis. *Vaccine* **21**, 431–439.

STIEGER, C., HEGGLIN, D., SCHWARZENBACH, G., MATHIS, A. & DEPLAZES, P. (2002). Spatial and temporal aspects of urban transmission of *Echinococcus multilocularis*. *Parasitology* **124**, 631–640.

STORANDT, S. T. & KAZACOS, K. R. (1993). *Echinococcus multilocularis* identified in Indiana, Ohio, and east-central Illinois. *Journal of Parasitology* **79**, 301–305.

STORANDT, S. T., VIRCHOW, D. R., DRYDEN, M. W., HUGNSTOROM, S. E. & KAZACOS, K. R. (2002). Distribution and prevalence of *Echinococcus multilocularis* in wild predators in Nebraska, Kansas, and Wyoming. *Journal of Parasitology* **88**, 420–422.

SUZUKI, K., UCHINO, J., SATO, N. & TAKAHASHI, H. (1996). Development and efficacy of mass screening of alveolar echinococcosis in Hokkaido. In *Alveolar Echinococcosis: Strategy for Eradication of Alveolar Echinococcosis of the Liver* (ed. Uchino, J. & Sato, N.), pp. 213–217. Sapporo, Fujishoin.

TACKMANN, K., LÖSCHNER, U., MIX, H., STAUBACH, C., THULKE, H.-H., ZILLER, M. & CONRATHS, F. J. (2001). A field study to control *Echinococcus multilocularis*-infections of the red fox (*Vulpes vulpes*) in an endemic focus. *Epidemiology and Infection* **127**, 577–587.

TAKAHASHI, K. & MORI, C. (2001). Host animals and prevalence of *Echinococcus multilocularis* in Hokkaido. *Public Health in Hokkaido* **27**, 73–80 (in Japanese).

TAKAHASHI, K. & URAGUCHI, K. (1996). Ecological factors influencing prevalence of larval *E. multilocularis* in vole. In *Alveolar Echinococcosis: Strategy for Eradication of Alveolar Echinococcosis of the Liver* (ed. Uchino, J. & Sato, N.), pp. 75–77. Sapporo, Fujishoin.

TAKAHASHI, K., URAGUCHI, K., ROMIG, T., HATAKEYAMA, H. & TAMURA, M. (2002). Preliminary report on *Echinococcus multilocularis* control by fox baiting with praziquantel. *Report of the Hokkaido Institute of Public Health* **52**, 61–63 (in Japanese).

TAKAHASHI, K., URAGUCHI, K. & YAGI, K. (1999). Prevalence of *Echinococcus multilocularis* in animals in Hokkaido. Alveolar Echinococcosis in Hokkaido – a Fiftieth-anniversary Publication – Hokkaido Institute of Public Health, pp. 24–38 (in Japanese).

TAKAHASHI, K., YAGI, K., URAGUCHI, K. & KONDO, N. (1989). Infection of larval *Echinococcus multilocularis* in red-backed vole *Clethrionomys rufocanus bedfordiae* captured around fox dens. *Report of the Hokkaido Institute of Public Health* **39**, 5–9 (in Japanese with English summary).

TAKAHASHI, A., YAMAGUCHI, T. & INABA, T. (1986). A review of multilocularis echinococcosis cases reported from Honshu, Japan, during a period from 1926 to 1984. *Japanese Journal of Parasitology* **35**, 95–107 (in Japanese with English summary).

TANIYAMA, H., MORIMITSU, Y., FUKUMOTO, S., ASAKAWA, M. & OHBAYASHI, M. (1996). A natural case of larval

echinococcosis caused by *Echinococcus multilocularis* in a zoo orangutan (*Pongo pygmaeus*). In *Alveolar Echinococcosis : Strategy for Eradication of Alveolar Echinococcosis of the Liver* (ed. Uchino, J. & Sato, N.), pp. 65–67. Sapporo, Fujishoin.

TSUKADA, H., HAMAZAKI, K., GANZORIG, S., IWAKI, T., KONNO, K., LAGAPA, J. T., MATSUO, K., ONO, A., SHIMIZU, M., SAKAI, H., MORISHIMA, Y., NONAKA, N., OKU, Y. & KAMIYA, M. (2002). Potential remedy against *Echinococcus multilocularis* in wild red foxes using baits with anthelmintic distributed around fox breeding dens in Hokkaido, Japan. *Parasitology* **125**, 1–11.

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.

URAGUCHI, K. & TAKAHASHI, K. (1998). Den site selection and utilization by the red fox in Hokkaido, Japan. *Mammal Study* **23**, 31–40.

URAGUCHI, K. & TAKAHASHI, K. (1999). Ecology of the red fox in Hokkaido. Alveolar Echinococcosis in Hokkaido – A Fiftieth-Anniversary Publication – Hokkaido Institute of Public Health, pp. 39–48 (in Japanese).

VOGEL, H. (1957). Studies on the *Echinococcus multilocularis* of South Germany. I. The tapeworm stage of strains of human and animal origin. *Zeitschrift für Tropenmedizin und Parasitologie* **8**, 404–456 (in German).

XIAO, N., MAMUTI, W., YAMASAKI, H., SAKO, Y., NAKAO, M., NAKAYA, K., GOTTSTEIN, B., SCHANTZ, P. M., LIGHTOWLERS, M. W., CRAIG, P. S. & ITO, A. (2003). Evaluation of recombinant Em18 and affinity-purified Em18 for serological differentiation of alveolar echinococcosis from cystic echinococcosis and other parasitic infections. *Journal of Clinical Microbiology* **41**, 3351–3353.

YAGI, K., TAKAHASHI, K. & HATTORI, K. (1984). A case of immature *Echinococcus multilocularis* in a domestic cat in Nemuro, eastern Hokkaido, Japan. *Report of the Hokkaido Institute of Public Health* **34**, 68–69 (in Japanese).

YAMASHITA, J. (1978). *Echinococcus*. Sapporo, Hokkaido University Press, 246 pp. (in Japanese).

YIMAM, A. E., NONAKA, N., OKU, Y. & KAMIYA, M. (2002). Prevalence and intensity of *Echinococcus multilocularis* in red foxes (*Vulpes vulpes schrencki*) and raccoon dogs (*Nyctereutes procyonoides albus*) in Otaru city, Hokkaido, Japan. *Japanese Journal of Veterinary Research* **49**, 287–296.

YOSHIMURA, K. (2000). A confirmation of fascioliasis for a case reported as echinococcosis in October 1999 – Akita. *Infectious Agents Surveillance Report* **21**, 170–171 (in Japanese).

ZHANG, W., LI, J. & MCMANUS, D. P. (2003). Concepts in immunology and diagnosis of hydatid disease. *Clinical Microbiology Reviews* **16**, 18–36.