of mice ($^*P < 0.05$). The induced level of protection was comparable to that of the mice immunized with EM95 (79.0%). The level of protection observed in the EM95 group was consistent with a previous report (78.5–82.9% protection) by Gauci et al. [8]. Thus, we provided the first direct experimental evidence that EMY162 induces a host immune response in *E. multilocularis* infection.

Immunogenicity of EMY162 and EM95

We also analyzed the immunogenicity of EMY162 and EM95 by using sera from AE patients. Western blot analysis was performed with 12 serum samples from AE patients and 6 normal serum samples as negative controls. As shown in Fig. 4A, a total of the 5 of the 12 sera samples (lanes 1, 2, 6, 9, and 10) from AE patients showed a positive reaction to EMY162. No significant reaction was
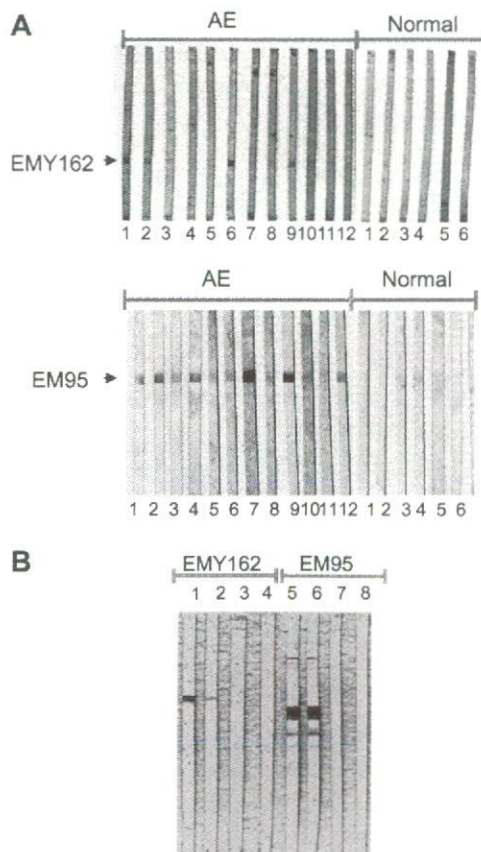

Fig. 4. Serological reactivity of EMY162 and EM95 to the sera from AE patients (A). Serum samples were diluted 400-fold with blocking buffer. Normal sera (numbers 1–6) were included in each assay as a negative control. (B) shows the Western blot analysis of EMY162 and EM95 with each antigen-specific antibody. The assay was performed by using two sera prepared from different mice. Lanes 1–4 show the reactivity of recombinant EMY162 to the anti-EMY162 antibodies (lanes 1 and 2) or anti-EM95 antibodies (lanes 3 and 4). Lanes 5 to 8 show the reactivity of recombinant EM95 to the anti-EM95 antibodies (lanes 5 and 6) or anti-EMY162 antibodies (lanes 7 and 8).

observed between the normal sera and EMY162. Seven of 12 AE patient sera samples (lanes 1, 2, 4, 7, 9, 10, and 12) showed positive reactivity to recombinant EM95. The serum samples in lanes 3, 5, 6, and 8 were judged as weakly positive. Overall, 10 of the 12 sera samples from AE patients showed a positive reaction to recombinant EM95 antigen. No significant reaction was observed between normal sera and EM95. Thus, EMY162 and EM95 possessed notable reactivity to the sera from AE patients. This result indicates that both antigens would be highly immunogenic in AE patients, which is advantageous for developing a vaccine against *E. multilocularis*. In addition, Heath and Lawrence [15] reported that activated oncospheres that are an infective source for humans were deactivated by anti-EG95 antibody-dependent complement-mediated lysis. The plasma membrane of oncosphere can be damaged by antibody and complement [16]. Therefore, when we consider EMY162-based vaccine development against AE, it is crucial to determine if the EMY162-specific antibodies detected in the sera from AE patients were raised by oncosphere invasion, subsequent larval development, or both. The result of our Western blot analyses strongly suggest that EMY162 plays a key role in the host-parasite interaction in human AE: thus, this molecule has a potential as a host-protective vaccine against larval *E. multilocularis* infection.

The amino acid sequence alignment of EMY162 and EM95 revealed several areas of similar sequences that could be common epitopes, such as Leu98 to Tyr104 and Ile124 to Glu131. The peptide sequences cover the linear immunogenic regions of EG95 reported previously [17,18]. To examine whether antigenic differences exist between the EMY162 and EM95 antigens, we analyzed cross-reactivity by Western blot analysis with mouse antisera specific to each recombinant antigen. As shown in Fig. 4B, anti-EMY162 antibodies showed positive reactivity to bands for recombinant EMY162 (lanes 1 and 2), whereas no bands were observed in the lanes 3 and 4 for recombinant EM95. Likewise, anti-EM95 antibodies showed a strong positive reaction to the recombinant EM95 bands (lanes 5 and 6) but did not react with recombinant EMY162 (lanes 7 and 8). Thus, each recombinant antigen-specific antibody distinguished EMY162 and EM95, which indicates that these antigens have a separate antigenicity.

In summary, we performed a vaccine trial of EMY162 (based on the newly isolated clone *emy162*) and demonstrated that EMY162 is a new candidate antigen with potential for use in a vaccine to reduce the risk of human AE. The homology of the amino acid sequences between EMY162 and the EM95 family was less than 30% in the mature form of the protein; thus, the possibility that the vaccine efficacy of EMY162 was induced by cross-reactivity to EM95 could be excluded. Indeed, no cross-reaction between recombinant EMY162 and EM95 was observed in Western blots with mouse antibodies specific to each recombinant antigen. In addition, we analyzed the gene

expression of *emy162* and *em95* in several life-stages and showed that *emy162* was expressed in a stage-nonspecific manner, unlike *em95*. Expression of EMY162 in the oncosphere stage of the parasite has not been examined. However, considering the fact that this molecule is expressed in metacestodes, protoscoleces, and adult worms, it is highly likely that EMY162 plays an essential role for the parasite survival in *E. multilocularis* infection throughout all developmental stages including oncospheres. On the other hand, EM95 is expressed predominantly in oncospheres [12] and thus is supposed to play a key role in the invasion and establishment of the parasite in intermediate hosts. Clear differences in the expression patterns of EMY162 and EM95 suggest functional differences between these molecules, although they are structurally similar to each other. The expression of EMY162 in oncospheres and the functions of this molecule in the host-parasite inter of *E. multilocularis* infection will be investigated in further studies.

Acknowledgments

References

[1] R.C.A. Thompson, A.J. Lymbery, *Echinococcus* and Hydatid Disease, CAB International, Wallingford, United Kingdom, 1995.

[2] K.S. Johnson, G.B.L. Harrison, M.W. Lightowlers, K.L. O'Hoy, W.G. Cougle, R.P. Dempster, S.B. Lawrence, J.G. Vinton, D.D. Heath, M.D. Rickard, Vaccination against ovine cysticercosis using a defined recombinant antigen, Nature 338 (1989) 585–587.

[3] G.B.L. Harrison, D.D. Heath, R.P. Dempster, C. Gauci, S.E. Newton, W.G. Cameron, C.M. Robinson, S.B. Lawrence, M.W. Lightowlers, M.D. Rickard, Identification and cDNA cloning of two novel molecular weight host-protective antigens from *Taenia ovis* oncospheres, Int. J. Parasitol. 26 (1996) 195–204.

[4] M.W. Lightowlers, R. Rolfe, C.G. Gauci, *Taenia saginata*: vaccination against cysticercosis in cattle with recombinant oncosphere antigens, Exp. Parasitol. 84 (1996) 330–338.

[5] M.W. Lightowlers, S.B. Lawrence, C.G. Gauci, J. Young, M.J. Ralston, D. Wass, D.D. Health, Vaccination against hydatidosis using defined recombinant antigen, Parasite Immunol. 18 (1996) 457–462.

[6] M.W. Lightowlers, S.B. Lawrence, C.G. Gauci, J. Young, M.J. Ralston, D. Wass, D.D. Health, Vaccination trials in Australia and Argentina confirm the effectiveness of the EG95 hydatid vaccine in sheep, Int. J. Parasitol. 29 (1999) 531–534.

[7] Y. Katoh, H. Kouguchi, J. Matsumoto, A. Goto, T. Suzuki, Y. Oku, K. Yagi, Characterization of *em*Y162 encoding an immunogenic protein cloned from an adult worm-specific cDNA library of *Echinococcus multilocularis*, Biochim. Biophys. Acta-Gen Subjects, in press.

[8] C.G. Gauci, M. Merli, V. Muller, C. Chow, K. Yagi, U. Mackenstedt, M.W. Lightowlers, Molecular cloning of a vaccine antigen against infection with the larval stage of *Echinococcus multilocularis*, Infect. Immun. 70 (2002) 3969–3972.

[9] H. Kouguchi, T. Suzuki, K. Yamano, H. Honma, Y. Sawada, Characterization of various recombinant antigens from *Echinococcus multilocularis* for use in the imunodiagnosis, Protein J. 24 (2005) 57–64.

[10] J.D. Smyth, Z. Davies, In vitro suppression of segmentation in *Echinococcus multilocularis* with morphological transformation of protoscoleces into monozoic adults, Parasitology 71 (1975) 125–135.

[11] M.W. Lightowlers, C.G. Gauci, C. Chow, D.R. Drew, S.M. Gauci, D.D. Heath, D.C. Jackson, D.L. Dadley-Moore, A.J. Read, Molecular and genetic characterization of the host-protective oncosphere antigens of taeniid cestode parasites, Int. J. Parasitol. 33 (2003) 1207–1217.

[12] W. Zhang, J. Li, H. You, Z. Zhang, G. Turson, A. Loukas, D.P. McManus, Short report: *Echinococcus granulosus* from Xinjiang, PR China: cDNAs encoding the EG95 vaccine antigen are expressed in different life cycle stages and are conserved in the oncosphere, Am. J. Trop. Med. Hyg. 68 (2003) 40–43.

[13] A. Merckelbach, M. Wager, R. Lucius, Analysis of cDNAs coding for immunologically dominant antigens from an oncosphere-specific cDNA library of *Echinococcus multilocularis*, Parasitol. Res. 90 (2003) 493–501.

[14] J. Garnier, J.F. Gibrat, B. Robson, GOR method for predicting protein secondary structure from amino acid sequence, Methods Enzymol. 266 (1996) 540–553.

[15] D.D. Heath, S.B. Lawrence, Antigenic polypeptides of *Echinococcus granulosus* oncospheres and definition of protective molecules, Parasite Immunol. 18 (1996) 347–357.

[16] D.D. Heath, B. Holcman, R.J. Shaw, *Echinococcus granulosus*: the mechanism of oncosphere lysis by sheep complement and antibody, Int. J. Parasitol. 24 (1994) 929–935.

[17] D.J. Woollard, C.G. Gauci, D.D. Heath, M.W. Lightwlers, Epitope specificities and antibody responses to the EM95 hydatid vaccine, Parasite Immunol. 20 (1998) 535–540.

[18] D.J. Woollard, C.G. Gauci, M.W. Lightwlers, Synthetic peptides induce antibody against a host-protective antigen of *Echinococcus granulosus*, Vaccine 18 (1999) 785–794.

Research on targeting sources of alveolar echinococcosis in Japan

Masao Kamiya[a,*], Jose Trinipil Lagapa[a,b], Yuzaburo Oku[c]

[a]*OIE Reference Laboratory for Echinococcosis and Laboratory of Environmental Zoology,
Department of Biosphere and Environmental Sciences, Faculty of Environment Systems,
Rakuno Gakuen University, Bunkyodai-midorimachi 582, Ebetsu, Hokkaido 069-8501, Japan*
[b]*Animal Disease Diagnostic Laboratory, Department of Medicine, Surgery and Zootechnics,
College of Veterinary Medicine, Central Mindanao University, Musuan, Bukidnon 8710, Philippines*
[c]*Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine,
Hokkaido University, Kitaku kita 18-jo Nishi 9, Sapporo, Hokkaido 060-0818, Japan*

Abstract

Echinococcus multilocularis is a fatal zoonotic parasite in the Northern Hemisphere. Recently, it has become endemic in many countries in Asia, especially in the northern island of Hokkaido in Japan. The increasing threat of public health due to alveolar echinococcosis has compelled researches for sensitive diagnosis and effective control. This paper reviews on the epidemiology, diagnosis and control of echinococcosis specifically in Japan. International collaborative responses by researchers and government initiatives such as mandatory reporting system for veterinarians who diagnose echinococcosis in dogs are presented. Successful control measures in Japan using anthelmintic fortified baits for foxes are described. Assessment of prevalence rates during control campaigns is analyzed favoring the use of intravital diagnosis rather than the traditional necropsy method from hunting or trapping activities of wild foxes. The novel concept of "endogenous development" by local resident volunteers towards sustainable control of echinococcosis is stressed.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Alveolar echinococcosis; Zoonotic parasite; Fox; Coproantigen; Mandatory reporting system; Anthelmintic; Geographic information system (GIS); Endogenous development

*Corresponding author. Tel.: +81 11 388 4909; fax: +81 11 388 4909.
E-mail address: mkamiya@rakuno.ac.jp (M. Kamiya).

Résumé

Echinococcus multilocularis représente un parasite zoonotique fatal, distribué sur l'hémi-sphère nord. Récemment, le parasite est devenu endémique dans plusieurs pays d'Asie, ainsi aussi sur l'île de Hakkoido au nord du Japon. Due au problème émergeant au niveau de la santé publique, l'échinococcose alvéolaire a été profondément investi avec but de développer de nouveaux modes de contrôle est de diagnostic. Cet article présente une revue de l'épidémiologie, du diagnostic et du contrôle de l'échinococcose au Japon, focalisant sur les travaux en collaboration internationales et sur les initiatives gouvernementales comme le registre obligatoire des cas diagnostiqués par les vétérinaires chez le chien. Sont aussi décrits des mesures de contrôle à la base de traitement anthelminthique par voie d'appât. Le suivi de la prévalence durant les campagnes de contrôle ce réalise préférablement par diagnostic intra vital et non par nécropsie traditionnelle suivant la chasse ou la trappe de renards sauvages par les chasseurs. L'intégration de résidents locaux volontaires dans un nouveau concept de contrôle de l'échinococcose à long terme est renforcée.

Mots clés: Échinococcose alvéolaire; Parasite zoonotique; Renard; Copro-antigène; Registre obligatoire; Anthelminthique; Système d'information géographique; Développement endogène

---

1. Introduction

In mid 1990s, WHO declared that the world was confronted with the risk/crisis of zoonotic infectious diseases spreading on a global scale and no country was safe from that threat. The recent episodes of emerging and re-emerging zoonoses increasingly recognized worldwide, proved that we are now standing face with face of such warnings. Among these emerging infectious diseases, zoonotic infections comprised about 75% with the wildlife constituting as the large reservoir population [1]. These pathogens migrated from wildlife reservoir to domestic animals and/or humans and produced diseases in the latter hosts as a consequence [2]. These situations called for a prompt and effective control measure that was designed and implemented to counter threats to public health. Control strategies were innovative and required the combined efforts of many fields [3] and sectors of the society. Proper analysis and assessment of control strategies have been done to constantly appraise the effects of control measures to develop new technologies for improved and better strategies, thus this review.

Echinococcosis caused by *Echinococcus multilocularis* is a serious parasitic zoonosis in the Northern Hemisphere, with its life cycle mainly maintained in the wildlife. The causative parasite has been reported in several parts of Asia, especially in China, Mongolia and in many of the new independent states of the former Soviet Union. In Japan, the echinococcosis-endemic area is restricted to the northern island of Hokkaido, although sporadic human cases have been reported on other islands [4], and infected pigs have been documented on the main island of Honshu [5].

Human echinococcosis, although relatively rare and generally considered an accidental spill-over from the wildlife, is one of the most difficult invasive helminthic

infections to diagnose, to treat effectively and also to undertake post-treatment evaluation [6]. The disease in humans is characterized by alveolar, hepatic, and cerebral disorders caused by the larval form (metacestode) of the tapeworm *E. multilocularis*. In humans, who may serve as accidental intermediate hosts, the metacestode cells of *E. multilocularis* proliferate like those of tumor cells. When clinical signs are manifested, it becomes very difficult to treat and without therapy the disease is fatal. Complete cure could only be achieved if confirmatory diagnosis is done during the early stage of the disease followed by complete resection of all lesions.

An *E. multilocularis* epidemic would have the potential to affect the economy of Hokkaido, due to its impact on agricultural and tourist industries [7]. It is thus very important to strengthen dog and fox quarantine processes and to undertake measures aimed at eliminating the source of human infection before the risk of infection increases [8]. The increasing number of infected foxes in cities and villages, in close contacts with domestic pets and humans, could increase the risk of human alveolar echinococcosis [9]. This scenario should prompt for an effective intervention program to control human infection, which up to this date is best introduced to the primary definitive hosts of the parasite, the canids.

2. Aetiology

2.1. Life cycle

The parasite, *E. multilocularis*, is a highly pathogenic cestode of the Family Taeniidae. It is maintained naturally in the wild by predator/prey life cycle. Foxes, dogs, coyotes, raccoon dogs and other canids can serve as definitive hosts. Cats are susceptible to infection but appear to have only a minor role in the maintenance of *E. multilocularis* in endemic areas, and infections in cats may be of minimal public health significance [10].

The definitive hosts of *E. multilocularis* in Hokkaido are known to be the red foxes, *Vulpes vulpes schrenki*, as well as the dogs and cats [11] (Fig. 1). Although immature adult worms were found from naturally infected raccoon dogs, *Nyctereutes procyonoides* [12], red foxes, based on their increasing population in Hokkaido and their high prevalence rate of echinococcosis were considered the major definitive hosts for the cestode in the wildlife [13]. Prevalence rates in cats and dogs were very low, nevertheless their role in transmission of the parasite to humans cannot be overlooked due to proximity and frequency of contacts with humans [14].

Wild rodents such as voles and pikas are known intermediate hosts while pigs, horses, primates and humans can be infected as accidental intermediate hosts. It has long been known that the most important intermediate host of *E. multilocularis* in Hokkaido is the Bedford vole, *Clethrionomys rufocanus bedfordiae* but the parasite has also been detected in *Clethrionomys rutilus* Mikado [11]. Spontaneous infection in intermediate hosts such as *Clethrionomys rex*, *Apodemus argenteus*, *Mus musculus* and *Rattus norvegicus*, as well as in the insectivores, *Sorex unguiculatus* and *Sorex caetiens* has been reported [15–20]. Results of seasonal monitoring of the dynamics of *E. multilocularis* infection in the
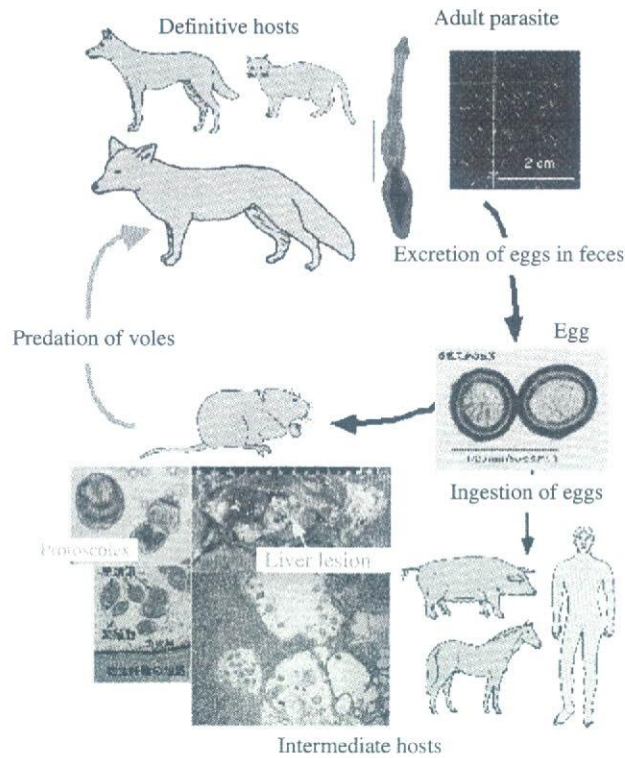
Fig. 1. The life cycle of *Echinococcus multilocularis*.

Bedford voles showed that high prevalence rates occurred in spring and these voles were those that has survived through the previous winter [21].

2.2. Transmission to humans

Despite that only sporadic reports of human cases are reported in Japan, it is predicted that the incidence of alveolar echinococcosis will increase in the near future if no effective preventive measures are put in place [7]. An analysis made on the route of transmission of *E. multilocularis* to humans was made in major dairy cattle and pig farming areas in Hokkaido. Most of the farm waste products and wet garbage were left unattended and ended up as food for foraging foxes. Thus, humans living in these areas were considered in a high-risk group [22,23]. In winter, when food sources of wild foxes are scarce, they depended heavily on farm waste products [24], however, it was pointed out that the food eaten by red foxes in Hokkaido during winter varied depending on areas and availability [25].

Fox trappers and other persons who work with foxes or their fur would appear to be relatively frequently exposed to eggs of *E. multilocularis*, but these occupations have not been associated with higher rates of infection [26–28]. An ELISA test for *E. multilocularis* antibody in these groups of people showed that there was no correlation between the prevalence of the disease and their occupation [22,29]. Depending on the situation, high-risk groups of people varied from one country to another [13].

Humans are infected by ingestion of the parasite eggs, suspectedly through water contaminated with the feces of infected wild red foxes, which have an estimated infection prevalence rate of 54–56% [30]. However, to date, *E. multilocularis* eggs have not been detected in drinking water in Hokkaido. Nevertheless, there is a need to understand the defecation habit of red foxes and the likely places where they tend to shed their feces. Monitoring the activity of foxes using spotlight census method, revealed that they frequently roam around human residential areas [31].

2.3. Immunology

Development of the alveolar hydatid cyst in rodent hosts is accompanied by a pronounced host immune reaction. Although there is a rapid antibody response to *Echinococcus* infection, and antibodies [32] and complement [33] are capable of killing larval protoscolesces (the mature larval stage) in vitro, they alone are not effective in controlling the growth of the hydatid cysts [34,35]. Cell mediated immunity on the other hand, involving granulocytes [37,38], macrophages [39] and lymphocytes [40–42], has a modifying effect on the vitality of larval tissue in vitro, and suppresses the growth of the larval cyst mass. The mechanism for immunosuppression has been variously linked to suppressor cells [43,44], immune complexes [36,38], and secretory products of the larval parasite itself [44].

It was showed that AKR mouse, which is deficient in the fifth component of the complement system, is a very susceptible animal for *E. multilocularis* infection but also demonstrated age resistance to the infection [45]. Furthermore, susceptibility to infection varied for different strains of mice, sex and age of the animals [46]. It was suggested that complement lysis of the protoscolesces plays a very important role in determining the susceptibility of the host and demonstrated that the host complement lysis occurred through the alternative pathway [33].

The importance of thymus or T cell in modulating the outcome of secondary echinococcosis was demonstrated in SCID mice. Increased larval mass and larval development of *E. multilocularis* were shown to be regulated by the T cell [47,48]. The inflammatory reaction surrounding the hydatid lesion such as the infiltration of lymphocytes, histiocytes and eosinophils were also shown to be associated with T cells.

It had been known that the mouse peritoneal macrophage has the ability to kill protoscolesces of *E. multilocularis*. It was demonstrated that the protoscolicidal activity of the macrophage was mediated through nitric oxide (NO) [49]. It was observed also in vitro that the protoscolesces were able to induce immunosuppression through their action on CD8+ cell [50].

3. Epidemiology

3.1. Echinococcosis in Asia

Echinococcosis in China has been reported mainly based on human cases, the most serious of these occurred in an area of Central China and northern Xinjiang

Uygur [51]. Data on infection in animal hosts are very limited, three genera of wild canids, the red fox (*Vulpes vulpes*), sand fox (*V. corsac*) and wolf (*Canis lupus*) have been found infected with adult stages of *E. multilocularis*. In western China, dogs are serving as definitive hosts and have transmitted the infection to humans [52]. Intermediate hosts reported included the highest prevalence in voles (*Microtus brandti*) [53] and pika, (*Ochotona* spp.) [54]. In Tibetan plateau, the increased disease prevalence was likely to be due to the greater population of the very susceptible intermediate host, *Ochotona curzoniae*, as influenced by pasture type of the area that led to more infections in community dogs [55,56].

Foci of *E. multilocularis* have a very patchy distribution in Central Asia [57]. In Kazakhstan there has been an increase in the reported surgical incidence of human echinococcosis [58]. High pathogenicity for people in Kazakhstan was represented with alveolar echinococcosis, distributed by wild predators such as foxes (*Vulpes vulpes*), corsacs (*V. corsac*), spotty cats (*Felis lybica*) and also dogs. Large natural foci are registered in Western Kazakhstan and the significant areas of infections were found on mountains, in coastal zone and the rivers where prevalence rates of echinococcosis in definitive hosts reached up to 11–25%. [59].

3.2. Echinococcosis in Japan

The history of echinococcosis in Japan started when Rebun Island, a small northern island off Hokkaido, became an endemic region. Foxes were introduced from the Kurile Islands between 1924 and 1926 for fur production and to control the vole population primarily *Clethrionomys rufocanus* [60]. A high prevalence of *Echinococcus* infection was reported not only among the red fox population (*Vulpes vulpes*) but also among stray dogs. The parasite was completely eliminated by intensively hunting foxes and culling all dogs on the island. In 1966, however, echinococcosis was diagnosed for the first time in the eastern part of Hokkaido. Re-introduction of the parasite into Japan is believed to have been from infected foxes wandering on drift ice from Russian islands or from infected dogs transported with repatriates after World War II [61]. Thereafter, distribution of the parasite gradually expanded and at present, *E. multilocularis* is reported throughout the island of Hokkaido (Fig. 2).

Alveolar echinococcosis in humans is endemic in Japan; however, the causal agent, *E. multilocularis*, has been restricted to the northernmost insular prefecture of Hokkaido, where the Tsugaru Strait acts as a natural physical barrier against migration to the mainland. The early human cases of alveolar echinococcosis were reported from Sendai on northern Honshu in 1926 and on Rebun Island in 1937. Since then, around 500 human cases have been diagnosed in Hokkaido and 5–19 (mean = 11) new patients have been reported every year since 1982. Between 1937 and 1997, 373 people underwent surgery to remove alveolar echinococcosis cysts. One study calculated that the rate of occurrence during the endemic period was 48 cases per 100,000 residents every year [62].

The finding of 19 autochthonously acquired cases of alveolar echinococcosis in prefectures other than Hokkaido [63] implied that the parasite exists in other areas.

Studies of the increased spread of the parasite in most countries have traditionally focused on the epidemiology of echinococcosis in foxes [64]; however, these cases may also have been spread by domestic dogs from disease-endemic areas.

In September 2005, a stray dog in Saitama prefecture in mainland Honshu was found to be positive for *E. multilocularis* infection by PCR (mitochondria 12S RNA gene) [65]. The sequence was identical to the Hokkaido isolate (GenBank accession no. AB244598). This raised an alarm because the area in which the infection was found is adjacent to the Tokyo metropolis, the most populous zone in Japan. Reports also claimed that 2 of 69 dogs moved from Hokkaido to Honshu were positive for *E. multilocularis* by coproantigen examination [8]. Moreover, a non-Hokkaido resident dog became infected with *E. multilocularis* despite being permitted to roam freely for just a few hours during its 5-day stay in Hokkaido [66]. This finding suggested a high infection pressure of *E. multilocularis* to domestic dogs within the area. Furthermore, in April 2004 to August 2005, from a total of 1460 domestic dogs examined, 4 (0.27%) were confirmed positive to echinococcosis by PCR, all from Hokkaido [67].

While the threat of echinococcosis spreading into Honshu had raised fears, an emergent concern is the possible role of domestic dogs in dispersing the disease from disease-endemic areas during relocation of residences by owners or when accompanying owners during domestic travel. Nearly 10,000 pet dogs were estimated to have been transported in 1 year to and from Honshu and Hokkaido by planes and
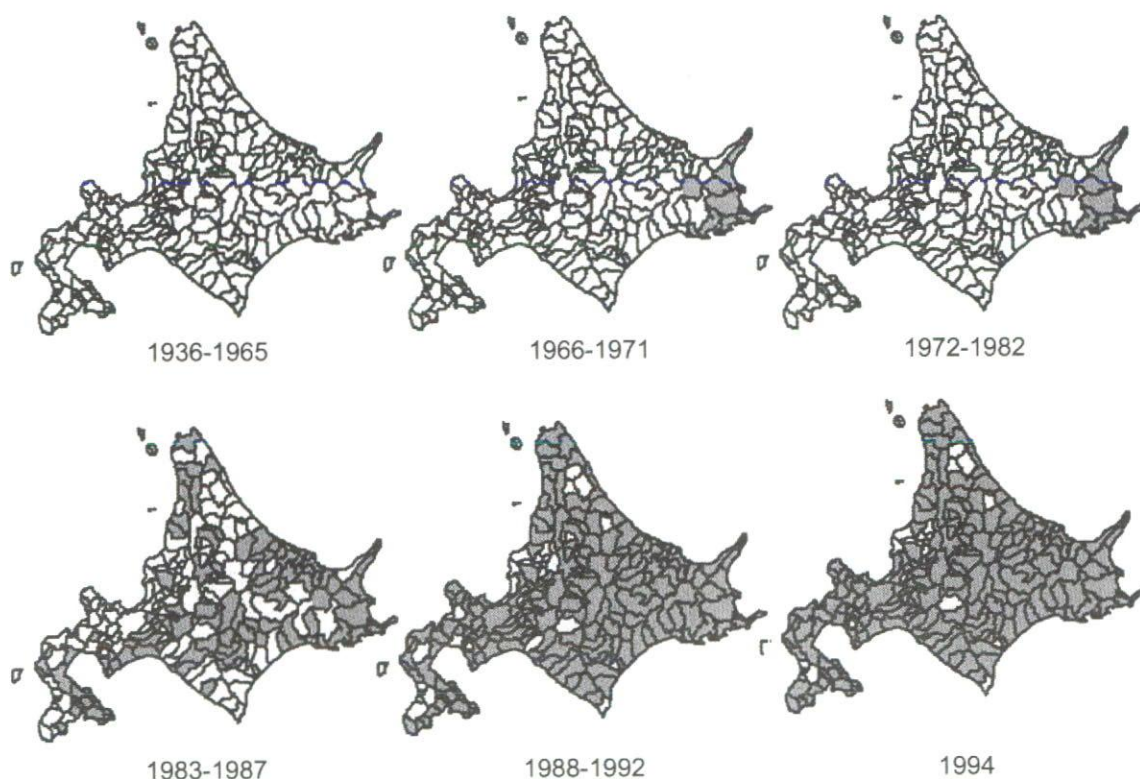

Fig. 2. Expansion of the endemic area of *E. multilocularis* among foxes in Hokkaido Island from 1936 to 1994.

ferries; this presumably included up to 30 *E. multilocularis*—infected pet dogs per year [68]. Even so, no compulsory quarantine or *Echinococcus* examination is enforced for dogs transported within Japan. A compulsory requirement of a certificate from a veterinarian stating that the animal has been treated with praziquantel within 48 hours before traveling would be a helpful preventive measure.

Confirmed cases of infection in dogs further showed the potential threat of domestic dogs transmitting *E. multilocularis* to humans in this region, as well as the potential for dispersal to other islands of Japan if proper preventive measures are not implemented. These scenarios pressed a nationwide appeal for the government to take steps for the prevention and control of echinococcosis in Japan. The realization of a mandatory reporting system on echinococcus infection in dogs was put to limelight during the symposia as discussed below.

4. Responses by Asian researchers

Researchers involved with echinococcosis problem made several unified moves to control the disease. Worthy to mention are the symposia on Echinococcosis in Central Asia: problems and solution [69] and the Japan–Taiwan Symposium on Infectious Diseases of Animals and Quarantine, on 2004 [70]. The symposia focused mainly on echinococcosis and other zoonotic diseases, human health, food safety and quarantine.

A significant highlight of the latter symposium was the presentation of a control policy against echinococcosis on the mandatory reporting of infection in dogs, which became part of a revised law that became effective after October 1, 2004. This implementation is the first of its kind in the world.

These symposia brought together researchers, government officials, and members of the public to facilitate exchange of information, with the understanding that technology that is applicable to both regions can also be extrapolated to the whole world. With the current pace of globalization, the concepts demonstrated in these symposia can serve as worldwide models.

5. Diagnosis

5.1. E. multilocularis full length cDNA library

The recent upsurge of proteomic studies has helped explain biochemical host–parasite interactions that might contribute to disease control [71]. Recombinant antigens may not only enhance diagnostic accuracy but may provide candidate proteins for vaccination, particularly for dogs and cats.

Full-Echinococcus, a database for full-length cDNAs from the parasite, *E. multilocularis*, has been produced (Fig. 3). The full-length cDNA library was produced using the Vector-trapper method from hydatid cysts developed in cotton rats that were infected with *E. multilocularis*. A total of 10,966 5′ end-one-pass
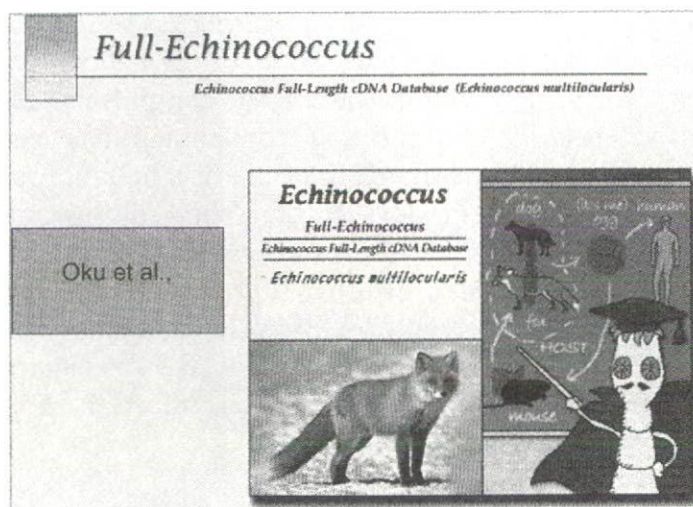
Fig. 3. Homepage of the Echinococcus full length cDNA database website (http://fullmal.hgc.jp/em/index.html).

sequences were compared with the non-redundant database of DDBJ/Genbank/EMBL using BLAST and TBLASTX programs. Two-thirds of the sequences were considered to be derived from *Echinococcus*, while the remaining one-third represented host genes. Many of the former clones represent full-length cDNAs that are expressed in the larva stage. These clones are available for further analysis and experiments.

5.2. Geographic information system (GIS)-based surveillance

We are presently processing information on the location of fox habitats using a geographic information system (GIS) so that bait can be distributed more accurately. Recently, GIS has emerged as an innovative and important component of many projects in public health and epidemiology [72]. Analysis of spatial data in relation to epidemiology is an important tool in the control and eradication of echinococcosis. It was reported that were a significant correlation between landscape data and *E. multilocularis* transmission and epidemiology existed. In Europe, the densities of fox and grassland rodent populations and the interactions between highly influenced *E. multilocularis* transmission rates were shown by spatial analysis [73].

The development of methods for disease mapping using GIS has progressed considerably in recent years [74]. We designed a GIS based mapping of echinococcus environmental contamination primarily to aid the current deworming campaigns conducted in selected areas of Hokkaido. A digital map downloaded to a hand-held Global Positioning System (GPS) displaying the exact location of fox fecal contamination would allow anyone to reach the site with ease and accuracy. This is most significant for local resident volunteers who are collaborating during deworming campaigns. Furthermore, this system could help in establishing monitoring sites to evaluate effectiveness of control measures and to detect re-emergence of the disease over time.

5.3. Coproantigen detection

There are several methods employed for the diagnosis of *E. multilocularis* in definitive hosts. Traditionally, flotation method is the simplest and enables the detection of the presence of taeniid eggs. Fecal egg examination, however, is difficult to confirm because the morphology of taeniid eggs is indistinguishable from those of *E. multilocularis* [75], and intermittent egg excretion occurred even after the worms mature. Parasitological examination of the small intestine by necropsy is considered the most reliable method to diagnose *E. multilocularis* in definitive hosts. While sedimentation and counting of worms is considered a "gold standard" [76], the issue of biohazard is still a critical consideration. Besides, during necropsy, very low infections of *Echinococcus* maybe missed [77]. Predilection site of the parasite should also be considered, results of survey and experimental infection of *E. multilocularis* in red foxes revealed that parasites tend to be present at the posterior portion of small intestine [78].

Other method that had been used for the diagnosis of cestode infection in the definitive host is the detection of the excretory/secretory products by the adult tapeworm in the gut by using the immunological method. Theoretically, it was suggested that excretory/secretory antigens are not directly associated with parasite reproduction, should be present even when reproductive material (like eggs) is absent from the feces and should disappear shortly after a successful treatment of the infection [76]. Subsequent coproantigen-based immunodiagnostic studies have been done for *E. multilocularis* [79–83].

Previous advances, such as the establishment of a laboratory host model for the complete life cycle of *E. multilocularis* [84], made it easier and safer to study clinical aspects, diagnosis, and treatment. This model led to the development of a monoclonal antibody-based sandwich enzyme-linked immunosorbent assay (ELISA) for coproantigen detection that significantly improved diagnostic capabilities [80]. The test basically used a monoclonal antibody (EmA9) specific for *E. multilocularis* adult worm (85) that detects the parasite coproantigen in the feces of definitive hosts. Coproantigen ELISA was reported to detect *E. multilocularis* antigens in fecal material already during the prepatent period and coproantigen excretion was closely correlated with the presence of intestinal immature and mature parasitic stages and their numbers [86]. In addition, the coproantigen detection test could also be used to diagnose the definitive host when the parasite eggs shed had been reduced to so few that it had become very difficult to detect under the microscope [85–88]. The stability of the coproantigen to chemical and physical treatment was evaluated and the test was found applicable in the field [80]. The validity of the test for diagnosis of echinococcosis in red foxes was confirmed by comparing the results of autopsy, egg examination and coproantigen ELISA using rectal fecal samples [30,89]. This method had proven useful for primary screening and was documented to have 94.9% sensitivity and 100% specificity for echinococcosis in wild red foxes in Hokkaido [30].

Another advantage of coproantigen ELISA is implied by the fact that it enables to examine a large number of the samples in comparison to the time and labor

consuming necropsy [90]. It is also very useful for real time monitoring on the prevalence of the echinococcosis during surveillance and control operations.

The introduction of copro-DNA tests has improved the sensitivity for the diagnosis of echinococcosis in definitive hosts [91–95]. Nonetheless, this technique is cost and time consuming to be used as a routine test and may be troublesome to apply at some laboratories for several reasons such as lack of equipment. It was suggested that coproantigen ELISA should be used as a primary screening test, and the PCR as secondary test [86].

In response to the critical demand of a rapid and accurate diagnosis, we have designed in house tests that could enhance the pace in screening suspected *E. multilocularis* infected definitive hosts. One was the development of a rapid ELISA kit for detection of *E. multilocularis* coproantigen. This kit was designed to perform diagnosis in laboratories that lacks more sophisticated instrumentation such as ELISA washer and other necessary instruments for the preparation of reagents. The kit is also capable for doing mass or individual diagnosis rapidly compared to the standard procedure. Another was a rapid visual assay for the qualitative detection of *E. multilocularis* coproantigen (Fig. 4). This immunochromatography method provided an ease and rapid individual diagnosis of suspected echinococcosis infected definitive hosts. With the mandatory reporting system implemented in Japan
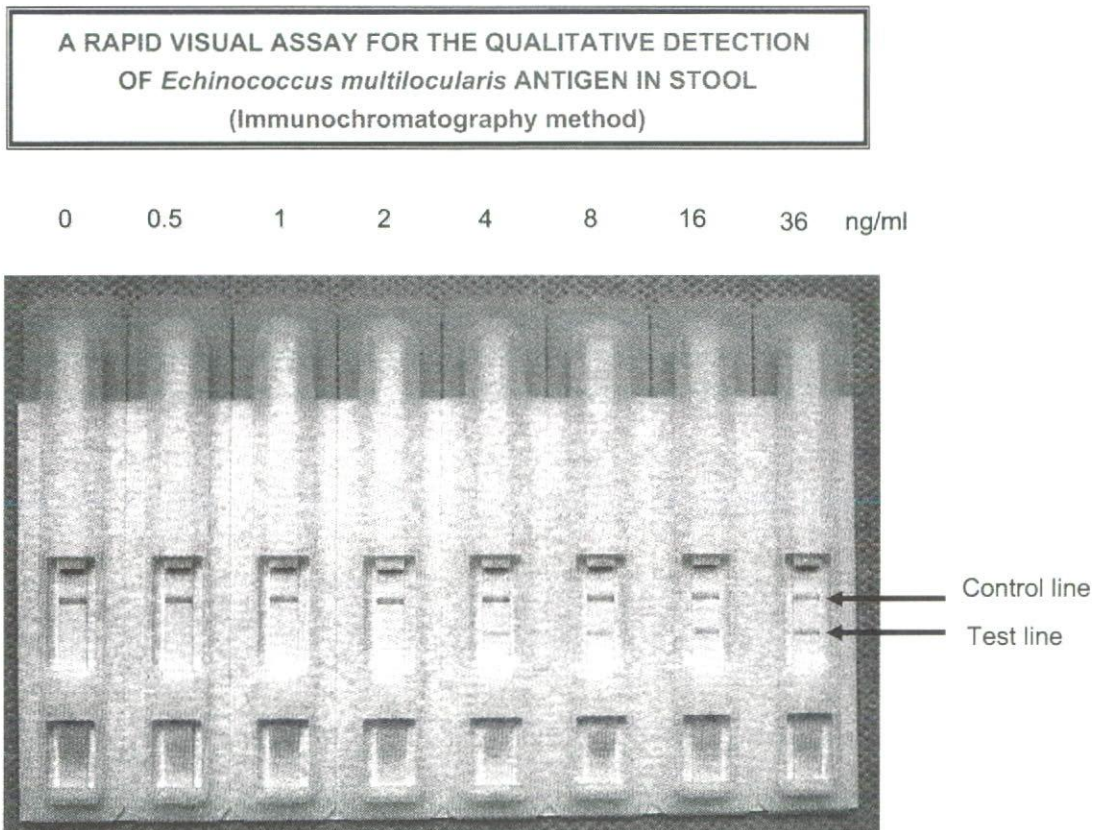

Fig. 4. Newly developed immunochromatography method as an in-house test for *E. multilocularis* in definitive hosts.

for veterinarians to report each case of *E. multilocularis* infected dogs to the health authorities, a rapid method is necessary to arrive with a tentative diagnosis. Confirmation can be done using standard assays.

6. Control strategies

6.1. Fox hunting

Since 1970, an intensive control program for red foxes has been conducted in Nemuro City, an eastern part of Hokkaido island. Foxes had been killed for control purposes by hunters and trappers during winter occurring from November to February. Pups in the population were not subjected to control measures, while juvenile foxes were hunted when they reached about 7 months of age. Prior to 1970, fox hunting was not intensive, but there was an increased harvest from 1971 than that in 1979. Reportedly, the number of hunting kills was about 16 times larger than in 1969 [96].

In spite of the long implementation of that program, echinococcosis is still detected in foxes although at a low prevalence rate. Even though intensive hunting pressure resulted to a decrease in the survival rate of adult foxes it was also contributed to the increased proportion of young foxes in the age distribution [96]. Likewise, it was noted that the proportion of juveniles to adults were higher in intensive fox control areas than in areas with light control [97]. A decrease in proportion of adult to subadult foxes in areas under heavy trapping pressure was also observed in other regions [98].

Conversely, a nation-wide hunting ban for foxes in the United Kingdom did not have a measurable impact on fox numbers in randomly selected areas [99]. Thus, it indicated that fox culling by hunting or trapping do not play a role in limiting the number of foxes and more so in controlling *Echinococcus* prevalence in an area. From this context, another method should be applied to control echinococcosis in foxes aside from the traditional fox culling approach.

6.2. Anthelmintic baiting

Currently, there is no reliable and cost-effective method for sustainable control or eradication of *E. multilocularis* in the sylvatic cycle [100]. From 1997, we started a study in Hokkaido to deworm red foxes using anthelmintic-fortified bait. A pilot area was selected in Hokkaido (Koshimizu, 200 km²) where a survey was first made to locate fox dens. Thereafter, examination for the presence of taeniid eggs and *Echinococcus* coproantigen in fox faeces collected around the vicinity of fox holes was conducted. The following year, anthelmintic-fortified bait was distributed on a monthly basis around fox holes in about half of the total study area. Commercial fish sausages containing fish meat, lard, gelatin and some spices were used as bait (1.5 cm long) and were embedded with half tablet, 25 mg praziquantel (Droncit®, Bayer Co.). It was observed that there was a decrease in the taeniid egg infection rate in

foxes and this suppressive effect was also seen in the following years despite a decrease in the number of times the bait was distributed. Results revealed that coproantigen-positive rates in fox faeces from the baited area were significantly lowered after a month of bait distribution, as compared to non-baited areas. The trial found that voles born after bait distribution had a significant lower prevalence of infection than their older counterparts [101].

In a follow-up study from April 2001, praziquantel-fortified bait was distributed throughout the whole area of Koshimizu by car alongside roads, at intersections and at wind-shield forests. Faeces from fox families outside this area were used as controls. Taeniid egg infection rates in foxes were significantly decreased together with coproantigen infection rates. The significant reduction of taeniid egg infection rates, however, was not observed until 6 months after the start of bait distribution, and a lowering of coproantigen positive rates came about almost a year later. This indicated that continuous baiting campaign was necessary. Recently, after continuous annual bait distribution in the area by local residents, the coproantigen positive rate in 2006 was brought down to zero (Oku et al., unpublished findings). Distribution of bait by local residents alongside roads, at intersections between roads, and in wind-shield forests proved to be effective in suppressing the infection rate of *E. multilocularis* in wild red foxes. This method was economical, the workforce was provided by the local resident volunteers and was seen as highly effective for controlling echinococcosis in other areas of Hokkaido [102].

A similar control strategy was conducted in Kutchan, another echinococcosis endemic area of Hokkaido. Prior to the bait distribution, a baseline survey was made in July, September and November of 2005. Fox feces were collected from the 100 km study area. Rates of taeniid egg and *E. multilocularis* coproantigen positive feces were 7% (19/268) and 21% (55/268), respectively. From May to November of 2006 approximately 1500 baits were distributed throughout the study area by volunteers from the residents of Kutchan, through their Non-Profit Organization (NPO), WAO. Remarkably, the rates of taeniid egg and coproantigen positive feces dropped to 0% and 2% in less than a year of baiting campaign (Okazaki et al. (WAO) personal comm.). This is so significant for Kutchan being an internationally known favorite area for winter sports.

It was demonstrated in these studies that distribution of praziquantel-fortified bait to foxes could reduce egg contamination by *E. multilocularis* and the potential risk for human echinococcosis. Thus, it was an effective way to stamp out the transmission sources of echinococcosis. These findings showed that this method could possibly be applied to overall control of the transmission source of alveolar echinococcosis in Hokkaido [102] (Fig. 5).

In all these campaigns, the initiative of local residents through their NPO was highly instrumental in carrying out the bait distribution. The unprecedented move of volunteers was imperative towards a successful eradication of echinoccocosis in wild foxes. Zoonotic diseases are important concerns for the public health personnel and the individual residents posed to the threat of getting the infection. The involvement of local volunteers in the intervention strategy was the key to an endogenous and sustainable system of public health protection and safety. This novel concept of
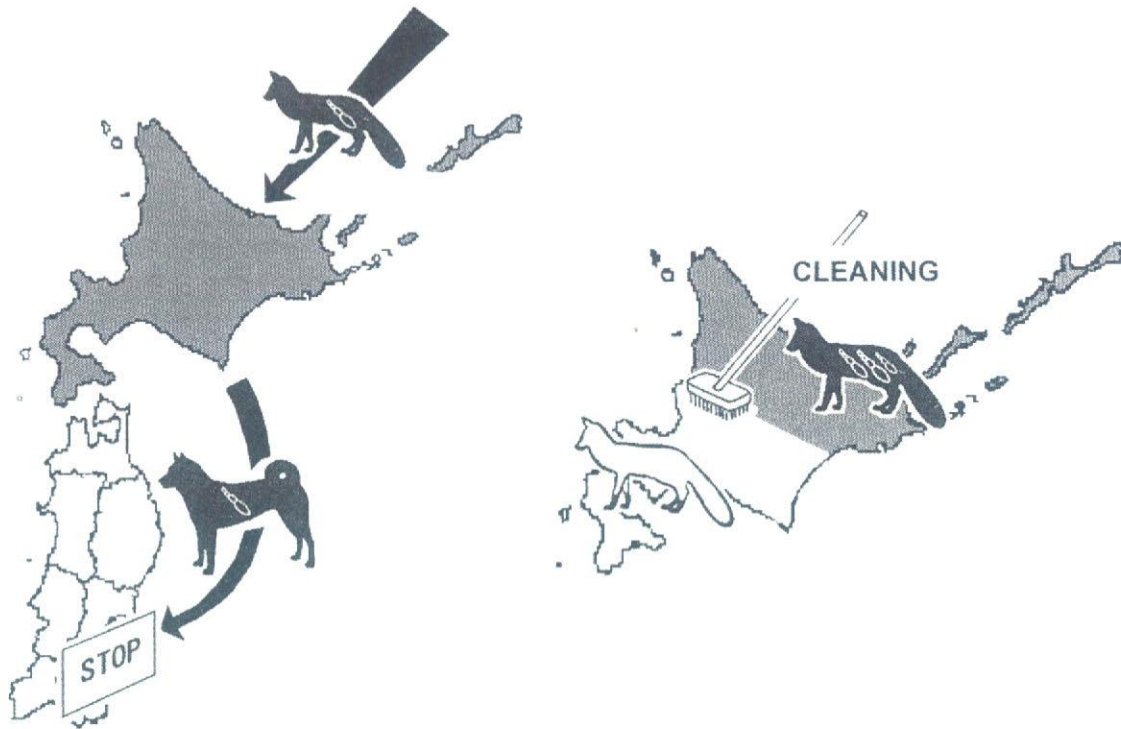
Fig. 5. A two-fold target in the control of Echinococcosis in definitive hosts of Japan: immediate prevention of spread towards Honshu and "cleaning" of Hokkaido island through improved strategic baiting campaigns.

using local resources such as manpower by NPO's leads to what is known as "endogenous development".

It was not clear what system of deworming the wild foxes could make an impact on the effectiveness of reducing the parasite prevalence rates. Deworming of wild foxes had not been attempted in Germany until 1990, besides, there had been no effective control approach for wild voles yet. Conditions in Hokkaido in terms of vegetation, quantity of snowfall, the species of voles involved and their habitat, however, were different from those in Germany. A difference in the life cycle of *E. multilocularis* in Europe and Japan is mainly due to the intermediate hosts involved, and indicated a difference in the foraging behavior of red foxes. The first successful reduction of *E. multilocularis* prevalence in foxes (from 32% to 4%) was reported in southern Germany [103]. Baiting using pellets containing praziquantel was distributed within the endemic area six times during a 14-month period. It was observed in this baiting campaign that a stronger effect was observed in 156 km core area than in the 6–10 km border area. The immigration of young, infected foxes was suggested to have caused the border effect [104].

A larger scale of campaign followed which utilized small aircraft for distribution of praziquantel baits based on rabies immunization protocols [105]. Reduction of prevalence rates was maintained low at 15% for 1.5 years at 3 months interval. These trials reduced the prevalence considerably of *E. multilocularis* in foxes, however, they failed to eliminate the parasite in the study region. Following the control trial in

northern Germany, prevalence rates recovered unexpectedly and rapidly, reaching pre-control levels five quarters (15 months) after the end of control [106].

It must be noted that in both these trials, assessment of reduction on prevalence rates was done by necropsy of foxes hunted in the study area. Such hunting pressure as emphasized earlier might have contributed to the increase of population in young, susceptible foxes. While some studies indicated no significant age-dependent differences in *E. multilocularis* infection, other studies found juvenile foxes to be significantly more frequently infected than adults [107]. Under high-endemic conditions, young foxes were significantly more frequently infected with *E. multilocularis* than under low-endemic conditions [105]. It was reported also that subadult foxes carried significantly higher worm burdens than adult foxes which is an indication for the acquisition of a partial immunity after repeated infections [108]. In contrast, our previously described baiting campaigns in Japan do not disturb the ecological niche of foxes because the assessment of efficacy was done by coproantigen detection from fox feces collected in the environment. Immigration rate especially for young foxes was suggested to be influenced by hunting pressure [104]. This was also observed in another trial of the Hokkaido government at Nemuro peninsula of Hokkaido where praziquanatel baits were distributed in a 135 km area and assessment of prevalence rate was by necropsy of hunted foxes [109]. Until recently, a decrease in prevalence rate was noted but the parasite was still detected in foxes. We predicted that fox culling or hunting for evaluation of control efficacy is detrimental to the success of the control campaign itself. Thus, the use of intravital diagnostics such as coproantigen ELISA is the must component in efficacy assessment of control interventions.

In Hokkaido, only partial culling of red foxes and stray dogs has been carried out. These partial measures have resulted in the spread of the disease from eastern Hokkaido (where it had been endemic since the 1960s) towards the whole island based on recent surveys.

6.3. Monitoring of domestic definitive host

The Forum on Environment and Animals (FEA) was established to meet the demand for accurate and rapid diagnosis of echinococcosis in definitive hosts, especially domestic dogs. FEA (Fig. 6) has been serving as a hub for veterinary practitioners around the country for confirmation of *E. multilocularis* infection in definitive hosts, especially dogs but also cats. Feces submitted other than from foxes, are from dogs and cats that are suspected to be infected or have wandered in parks and woodlands and likely preyed on wild rodents. Examinations are performed weekly, and results are immediately forwarded to the submitting veterinarians. Before examination, fecal samples are sterilized by heating for 12 h at 70 °C. Fecal egg examination is conducted by using centrifugal flotation [110] with sucrose solution with a specific gravity of 1.27. Sandwich ELISA using a monoclonal antibody EmA9 [42] is used for *E. multilocularis* coproantigen detection. Egg- and ELISA-positive fecal samples from dogs are subjected to PCR amplification Echinococcus Risk Control Center (ERCC) is still underway in its planning and implementation. This section will serve as the arm of FEA to advance research and
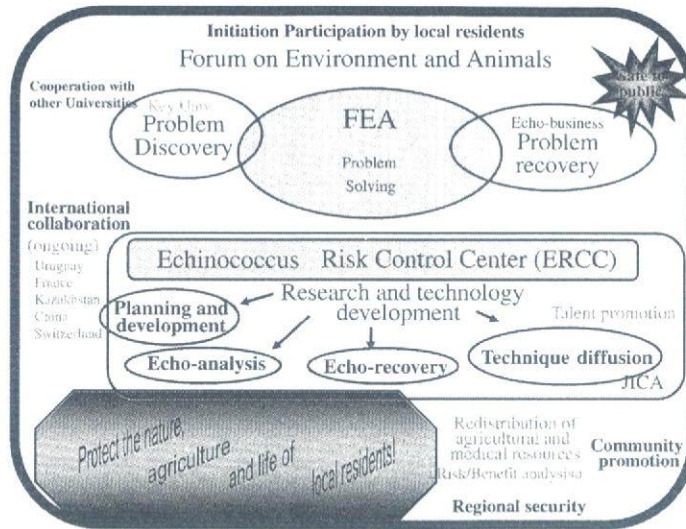
Fig. 6. Composition of Forum on Environment and Animals (FEA).

technology development in the prevention and control of alveolar echinococcosis not only in Japan but also in the international setting.

7. Conclusions

The emergent threat of echinococcosis infection on humans not only by the wildlife hosts but the domestic hosts, as well, is a compelling reason to advance researches on the intervention programs to control human infections. While traditional control measures (fox culling/hunting) are still implemented, modern technologies such as proteonomics, GIS, and tactical bait distribution should be harnessed to address this global problem. The initiative by local residents as a form of endogenous development and the able assistance of FEA are significant factors towards an effective and sustainable control measure of echinococcosis.

A delay in the implementation of a control measure can possibly result in massive economic loss as demonstrated by the recent confusion generated in the implementation of control measures against Bovine Spongiform Encephalopathy (BSE) in Japan. It is imperative that echinococcosis control policy be first evaluated for its maximum cost effectiveness through risk analysis and communication. This should be done without antagonism towards the inhabitants at risk while simultaneously protecting them against infection.

References

[1] Chomel BB, Belotto A, Meslin F-X. Wildlife, exotic pets, and emerging zoonoses. Emerg Infect Dis 2007;13:6–11.

[2] Artois M, Caron A, Leighton FA, Bunn C, Vallat B. Wildlife and emerging diseases. Rev Sci Tech Off Epi 2006;25:897–912.

[3] Kahn LH. Confronting zoonosis, linking human and veterinary medicine. Emerg Infect Dis 2006;12:556–61.

[4] Doi R, Nakao M, Nihei N, Kutsumi H. Epidemiology of alveolar hydatid disease (AHD) and estimation of infected period of AHD on Rebun Island, Hokkaido. Nippon Koshu Eisei Zasshi 2000;47:145–52.

[5] Kamiya H, Kanazawa T. The first detection of *Echinococcus* infection among pigs on the main island of Japan, August 1998—Aomori. Infect Agents Surveillance Rep 1999;20:248–9.

[6] Craig PS. Epidemiology of echinococcosis in western China. In: Torgerson P, Shaikenov B, editors. Echinococcosis in Central Asia: Problems and Solution. INTAS Network Project, Zurich Almaty, 2004. p. 43–58.

[7] Konno K, Oku Y, Tamashiro H. Prevention of alveolar echinococcosis—ecosystem and risk management perspectives in Japan. Acta Trop 2003;89:33–40.

[8] Kamiya M, Nonaka N, Ganzorig S, Oku Y. Effective countermeasures against alveolar echinococcosis in the red fox population of Hokkaido, Japan. In: Torgerson P, Shaikenov B, editors. Echinococcosis in Central Asia: problems and solution. Zurich: Almaty; 2004. p. 273–82.

[9] Deplazes P, Gloor S, Stieger C, Hegglin D. Urban transmission of *Echinococcus multilocularis*. In: Craig P, Pawlowsky Z, editors. Cestode zoonoses: echinococcosis and cysticercosis. Amsterdam: IOS Press; 2002. p. 287–97.

[10] Thompson RC, Kapel CM, Hobbs RP, Deplazes P. Comparative development of *Echinococcus multilocularis* in its definitive hosts. Parasitology 2006;132(Part 5):709–16.

[11] Yamashita J. Echinococcus and echinococcosis. In: Morishita K, Komiya Y, Matsubayashi H, editors. Progress of medical parasitology in Japan, vol. 5. Tokyo: Meguro Parasitological Museum; 1973. p. 67–123.

[12] Yagi K, Takahashi K, Hattori K, Kondo N, Oku Y, Ito M. A natural infection of *Echinococcus multilocularis* in a raccoon dog, *Nyctereutes procyonoides albus* in Hokkaido, Japan. Jpn J Parasitol 1988;37:79 [in Japanese].

[13] Oku Y, Kamiya M. Biology of echinococcus. In: Otsuru M, Kamegai S, Hayashi S, editors. Progress of medical parasitology in Japan, vol. 8. Tokyo: Meguro Parasitological Museum; 2003. p. 293–318.

[14] Ohbayashi M. Echinococcosis—especially multilocular echinococcosis in Hokkaido. Progr Vet Sci 1986;59–72 [in Japanese].

[15] Kamiya H, Ohbayashi M, Sugawara K, Hattori K. An epidemiological survey of multilocular echinococcosis in small mammals of eastern Hokkaido, Japan. Jpn J Parasitol 1977;26:148–56 [in Japanese with English summary].

[16] Inaoka T, Ohnishi K, Kutsumi H. Detection of echinococcal infection in rodents and shrews caught in Asahikawa and Kushiro districts. Hokkaido J Med Sci 1984;59:728–33 [in Japanese with English summary].

[17] Okamoto M, Fujita O, Arikawa J, Kurosawa T, Oku Y, Kamiya M. Natural *Echinococcus multilocularis* infection in a Norway rat, *Rattus norvegicus*, in southern Hokkaido, Japan. Int J Parasitol 1992;22:681–4.

[18] Iwaki T, Hatakeyama S, Nonaka N, Miyaji S, Yokohata Y, Okamoto M, et al. Survey on larval *Echinococcus multilocularis* and other hepatic helminthes in rodents and insectivores in Hokkaido, Japan from 1985 to 1992. Jpn J Parasitol 1993;42:502–6.

[19] Takahashi K, Nakata K. Note on the first occurrence of larval *Echinococcus multilocularis* in *Clethrionomys rex* in Hokkaido, Japan. J Helminthol 1995;69:265–6.

[20] Ohbayashi M. Host animals of *Echinococcus multilocularis* in Hokkaido. In: Uchino J, Sato N, editors. Alveolar echinococcosis strategy for eradication of alveolar echinococcosis in the liver. Sapporo, Japan: Fuji-shoin; 1996. p. 59–64.

[21] Takahashi K, Uraguchi K. Ecological factors influencing prevalence of *E. multilocularis* in vole population. In: Uchino J, Sato N, editors. Alveolar echinococcosis strategy for eradication of alveolar echinococcosis in the liver. Sapporo, Japan: Fuji-shoin; 1996. p. 75–7.

[22] Doi R, Seo H, Fukuyama Y, Nakao M, Inaoka T, Kutsumi S, et al. Epidemiology of multilocular echinococcosis in Hokkaido (1) a sero-epidemiological study of hunters. Jpn J Pub Health 1987;34:357–65 [in Japanese].

[23] Nakao M, Inaoka T, Kutsumi H, Doi R, Arakawa K, Ohnishi K. Epidemiology of multilocular echinococcosis in Hokkaido (2) seroepidemiological survey of residents in hog raising areas in Asahikawa City. Jpn J Pub Health 1988;35:184–92 [in Japanese].

[24] Abe H. Winter food of the red fox, *Vulpes vulpes schrencki* Kishida (Carnivora: Canidae), in Hokkaido, with special reference to vole population. Appl Ent Zool 1975;10:40–51.

[25] Kondo N, Takahashi K, Yagi K. Winter feeding property of foxes (*Vulpes vulpes schrencki* Kishida) in an endemic area of multilocular echinococcosis. Rep Nemuro Municipal Museum 1986;1:23–31 [in Japanese].

[26] Gamble WG, Segal M, Schantz PM, Rausch RL. Alveolar hydatid disease in Minnesota. First human case acquired in the contiguous United States. JAMA 1979;241:904–7.

[27] Hildreth MB, Sriram S, Gottstein B, Wilson M, Schantz PM. Failure to identify alveolar echinococcosis in trappers from South Dakota in spite of high prevalence of *Echinococcus multilocularis* in wild canids. J Parasitol 2000;86:75–7.

[28] Schantz PM. Progress in diagnosis, treatment and elimination of echinococcosis and cysticercosis. Parasitol Int 2006;55:S7–S13.

[29] Inaoka T, Nakao M, Ohnishi K, Doi R, Kutsumi H. Epidemiological survey of multilocular echinococcosis intended for tanners and taxidermist in Hokkaido, Japan. J North Occup Health 1987;36:9–12 [in Japanese].

[30] Morishima Y, Tsukada H, Nonaka N, Oku Y, Kamiya M. Evaluation of coproantigen diagnosis for natural *Echinococcus multilocularis* infection in red foxes. Jpn J Vet Res 1999;46:185–9.

[31] Uraguchi K, Takahashi K. The distance from the house to the red fox observed with spotlight census technique. Rep Hokkaido Inst Pub Hlth 1989;39:1–4 [in Japanese with English summary].

[32] Kassis AI, Tanner CE. Novel approach to the treatment of hydatid disease. Nature 1976;262:588.

[33] Kamiya H, Kamiya M, Ohbayashi M. Studies on the host resistance to infection with *Echinococcus multilocularis* 2. Lytic effect of complement and its mechanism. Jpn J Parasitol 1980;29:169–79.

[34] Pauluzzi S. Serologic response of mice and rats to secondary experimental hydatid disease. Am J Trop Med Hyg 1969;18:7–12.

[35] Liu D, Lightowlers MW, Rickard MD. Examination of murine antibody response to secondary hydatidosis using ELISA and immunoelectrophoresis. Parasite Immunol 1992;14:239–48.

[36] Treves S, Ali-Khan Z. Characterization of the inflammatory cells in progressing tumor-like alveolar hydatid cysts I. Kinetics and composition of inflammatory infiltrates. Tropenmed Parasitol 1984;34:183–8.

[37] Devouge M, Ali-Khan Z. Intraperitoneal murine alveolar hydatidosis: relationship between the size of the larval cyst mass, immigrant inflammatory cells, splenomegaly and thymus involution. Tropenmed Parasitol 1983;34:15–20.

[38] Alkarmi T, Behbehani K. *Echinococcus multilocularis*: inhibition of murine neutrophil and macrophage chemotaxis. Exp Parasitol 1989;69:16–22.

[39] Baron RW, Tanner CE. *Echinococcus multilocularis* in themouse: the in vitro protoscolicidal activity of peritoneal macrophages. Int J Parasitol 1977;7:489–95.

[40] Ali-Khan Z. *Echinococcus multilocularis*: cell-mediated immune response in early and chronic alveolar murine hydatidosis. Exp Parasitol 1978;46:157–65.