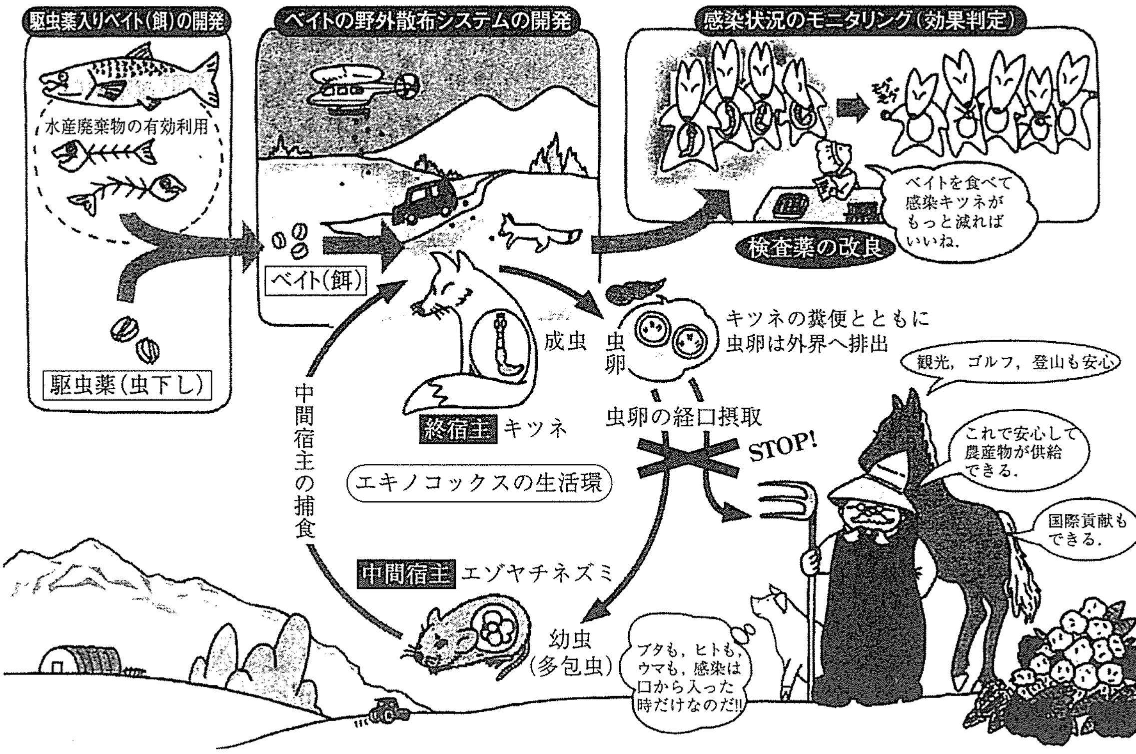

図3 エキノコックス汚染環境の修復

道では，ヒト(虫卵経由)とペット(感染ネズミ経由)への感染リスクが増している．既に，室内飼育犬の感染例や，駆虫後に再感染した例が認められている．これらの感染例は，北海道での飼いイヌへの高い感染圧を示している．すなわち，ヒトの生活圏の環境がエキノコックス虫卵に高度に汚染されており，そこに住む野ネズミが感染し，それを食べるイヌが感染する状況となっている[4]．飼い主，獣医師および行政がこのような状況を十分に認識して，適切な飼育管理と感染予防にあたる必要がある．

イギリスなどは，多包条虫流行国からのペットの持ち込み前の駆虫を義務付けている．我が国でも北海道から本州へ移動するイヌや海外の流行地から輸入されるイヌについても検査・駆虫が必要である．

b. キツネ対策

流行地域におけるキツネの感染率を下げることにより，野ネズミの感染率を下げ，結果として，イヌへの感染リスクを下げることが期待されている．このために，キツネへの駆虫薬入り餌(ベイト)をキツネの巣穴や通り道に散布することにより，野生キツネの駆虫に成功している．

1998年には，オホーツク海に面した田園地帯・小清水町(約200 km^{2}を二分し，実験区と対照区)において駆虫薬(PZQ)を入れた魚肉ソーセージ(ベイト)をキツネの巣穴を中心に散布した．研究者による実験的な試み，1年間延べ1万時間の虫下し作戦が展開された[5]．採取したキツネ糞便から虫卵陽性検体は1/5に，抗原陽性検体は半減し，虫下しの効果が確認された．その後，6年間で，実験区を拡大(2倍)，魚粉などを使ったベイトの機械生産，また，散布方法など様々な改良がなされ，虫卵陽性率を0％に低下させた．リスクの低減，汚染環境修復が可能であることを示した(**図3**)．また，スイス・チューリッヒ市内でも，この方法で効果を上げている[6]．

おわりに

エキノコックスによる被害は健康被害のみならず，経済問題にまで波及する．我が国の生物リスクとしては，一般に認識されることもなく，その感染源(リスク)へ向けた対策も，ないままで経過している．1930年代から発生したエキノコックス研究の中心は医学であった．その対策も医療が中心であった．初期の対応は仕方がないにしても，現在もその方針が継続されている．環境や野生動物が関与する分野にもかかわらず医学という狭い領域にとどまったために問題解決を遅らせている．行政レベルでは，総合的に'担当する部署がない'ので感染源(リスク)への対応ができていない．また，学会，研究機関レベルでも反省する点は多い．患者発生が増加傾向にあり，野生キツネの半数が感染し，陽性の飼いイヌが報告される北海道の感染レベルを下げることが，本州への流行を阻止し，問題解決に迫る近道である(図4)．開発技術は，国内外を問わず，その他の生物リスクへも応用可能である．この問題に対峙するそれぞれの関係者(ステークホルダー)の貢献が期待されている．

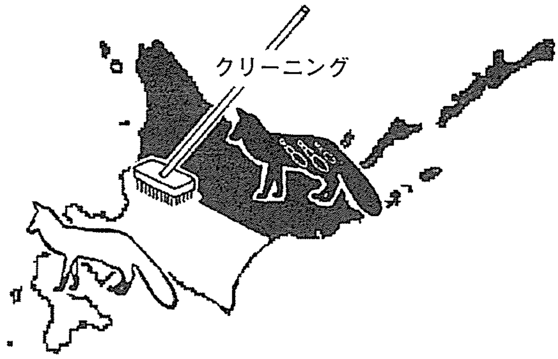

図4 自然界の感染源動物対策：汚染環境修復

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Rev. sci. tech. Off. int. Epiz., 2006, **25** (3), 1055-1066

Current control strategies targeting sources of echinococcosis in Japan

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Submitted for publication: 20 September 2005
Accepted for publication: 21 January 2006

Summary

The authors describe the current control strategies targeting definitive hosts of the most important zoonotic parasite in Japan, *Echinococcus multilocularis*. A dramatic increase in the prevalence of echinococcosis in foxes in Hokkaido (the second largest of Japan's islands), the invasion of wild foxes into urban areas, infection among pet and stray dogs, and the possibility of spreading the disease to the main island of Japan (Honshu) – all these pose significant threats to public health. Previous research findings and current strategies such as control measures against infections in wild foxes, suggest that it will be possible to eliminate echinococcosis in the future. The enforcement of a national reporting system for veterinarians, international collaboration, and the establishment of a Forum on Environment and Animals (FEA) give further reason to believe that success is possible. This is the first report of a multifaceted control strategy against echinococcosis in definitive hosts that includes collaborative efforts with local residents. This model might provide new ideas for Veterinary Services worldwide in their efforts to control other related zoonotic diseases.

Keywords

Baiting trial – Control strategy – Dog – Echinococcosis – Fox – Japan – National reporting system – Private sector cooperation – Zoonosis.

Introduction

Echinococcosis is caused by the tapeworm parasite *Echinococcus multilocularis*. The disease produces primarily hepatic, and to a lesser extent pulmonary and cerebral, disorders in humans. This disease, usually termed alveolar echinococcosis, is considered to be one of the most serious zoonoses in the northern hemisphere. The parasite is maintained in a transmission cycle involving voles (rodent intermediate hosts) and red foxes (definitive hosts); domestic dogs that prey on wild rodents are also presently an emerging concern.

The echinococcosis endemic area in Japan is restricted to the northern island of Hokkaido (total area 83,451 km^2), although sporadic human cases are reported on other islands (7) and infected pigs are documented on the main island of Honshu (14). Lately, the threat that echinococcosis might spread to Honshu has raised fears. Reports claim that 2 out of 69 dogs that were moved from

Hokkaido to Honshu were found positive by *E. multilocularis* coproantigen examination (15), and a dog transported to the main island was found to be excreting *E. multilocularis* eggs. In September 2005, a stray dog in Saitama prefecture (Honshu) tested positive for *E. multilocularis* infection. It has been estimated that nearly ten thousand pet dogs are transported each year to and from Honshu and Hokkaido by plane and ferry; this potentially includes up to 30 *E. multilocularis*-infected animals per year (8). There is no compulsory quarantine for dogs transported within Japan and animals are not examined for the presence of *E. multilocularis*. This problem, however, could be easily dealt with by requiring anyone wanting to move a dog between islands to obtain a certificate from a veterinarian stating that the animal has been treated with praziquantel three to four days before travelling.

Previous advances, such as the establishment of a laboratory host model for the complete life cycle of *E. multilocularis*, made it easier and safer to study clinical aspects, diagnosis, and treatment (13). This model led to the development of a monoclonal antibody-based (EmA9) sandwich enzyme-linked immunosorbent assay (ELISA) for coproantigen detection that significantly improved diagnostic capabilities (19). Current control programmes instituted by the World Organisation for Animal Health (OIE) Reference Laboratory for echinococcosis in Japan reveal that an effective countermeasure against the disease is to target the fox definitive host population by anthelmintic baiting. The government of Japan has also responded to the challenges and output of the OIE Reference Laboratory by designating echinococcosis as a Category 4 disease under the Infectious Disease Law, which means that there must be a mandatory national reporting system for the disease. International collaboration enhances the exchange of knowledge and techniques to further redefine control measures against echinococcosis. Cooperation from private veterinarians and local residents through the Forum on Environment and Animals (FEA [see later section]) has also provided a significant contribution to the current control strategies.

Future strategies recommended by the OIE Reference Laboratory for echinococcosis aim to significantly reduce, if not to eliminate, *E. multilocularis* infection in the definitive host. Several measures have been taken and more are in line for evaluation and further investigation. Anthelmintic baiting systems are being studied with the aim of designing strategic modifications which will result in an efficient and cost-effective control programme against infection in fox definitive hosts. The results of research presently being conducted in Honshu are expected to rally behind policies that might require compulsory echinococcosis examination or deworming prior to transportation of dogs and cats from Hokkaido to other locations in Japan.

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* (27). 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 (17). Thereafter, distribution of the parasite gradually expanded and at present, *E. multilocularis* is reported throughout the island of Hokkaido (Fig. 1).

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 400 human cases have been diagnosed in Hokkaido and 5 to 19 (mean = 11) new patients have been reported every year since 1982. Between 1937 and 1997, 373 people underwent surgery to remove multilocular echinococcosis cysts. One study calculated that the rate of occurrence during the endemic period (1988 to 1994) was 48 cases per 100,000 residents every year (20).

The prevalence of echinococcosis in foxes in Hokkaido has dramatically increased over the past two decades. In 1985, less than 10% of foxes were reportedly infected and this rose to 58.4% in 1998. By 2002 the prevalence had fallen to just over 30% (Fig. 2), but it has been steadily increasing again over the past few years. Necropsy surveys conducted by the Laboratory of Parasitology at the Graduate School of Veterinary Medicine of Hokkaido University (situated in the suburbs of Sapporo, the capital city of Hokkaido) showed a high prevalence of infection in foxes, which rose from 54% in 1997 to 56% in 1999 (18, 31).

The invasion of red foxes into urban areas increases the infection risks in densely populated areas. An emerging concern with echinococcosis in foxes is the possibility of an urban cycle of *E. multilocularis* (such a cycle has already been reported in several European cities [11]). Surveys in Sapporo, either by necropsy or coproantigen detection, registered the presence of *Echinococcus*-infected foxes in parks and woodlands within the city (22, 29). Another survey reported that large numbers of fox faeces contaminated with *E. multilocularis* coproantigen were found in urban areas adjacent to recreational parks (10). Furthermore, this same survey reported that suitable intermediate hosts had been trapped in these particular

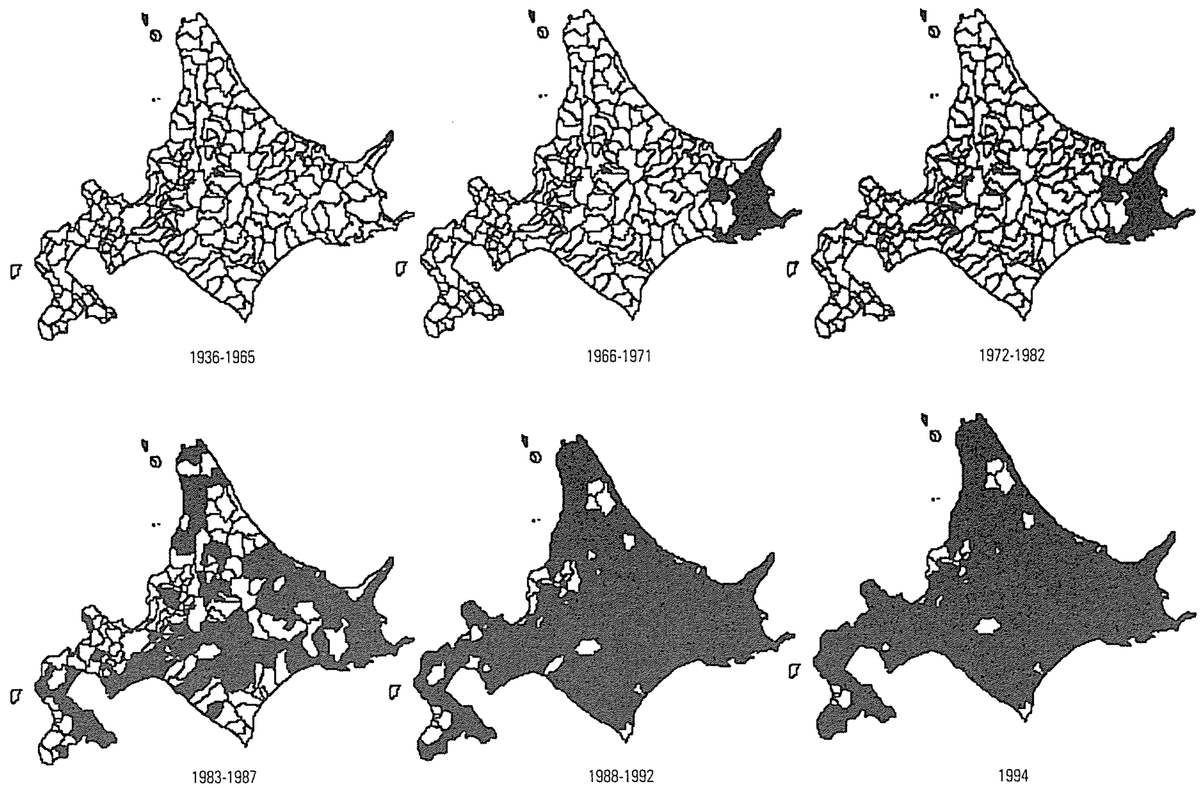


Fig. 1
Expansion of the area in which *Echinococcus multilocularis* was endemic among foxes in Hokkaido (Japan's northernmost island) from 1936 to 1994

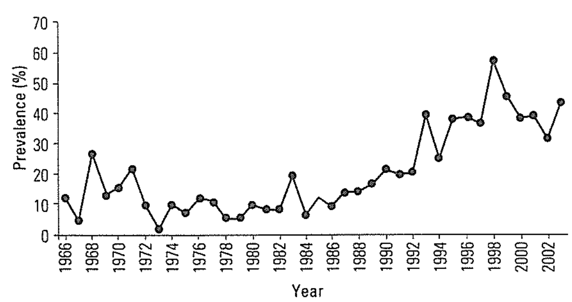


Fig. 2
Changes in the prevalence of *Echinococcus multilocularis* infection in Hokkaido foxes
(necropsy survey data from the Hokkaido government)

areas. Although none of the wild rodents captured were infected, there is a strong indication that an active urban cycle in Sapporo could be in the making. Pet dogs taken for outdoor walks are more likely to prey on infected rodents in suspected areas. This potential infection increase for people in urban areas who frequently visit recreational parks on urban fringes calls for the immediate implementation of control campaigns.

There have been very few studies on the prevalence of echinococcosis in dogs in Hokkaido. A coproantigen survey of *Echinococcus* infection in pet dogs, undertaken from April 2003 to February 2004, found 3 out 1,136 dogs to be positive. Between September 2003 and February 2004, 2 out of 69 dogs were found to be positive for *Echinococcus* coproantigens (M. Kamiya, unpublished findings). Dogs are taken from Hokkaido to other parts of Japan when dog-owners move house or go on holiday. In some endemic areas such as Gansu (the People's Republic of China) and on St. Lawrence Island (Alaska, United States of America), dogs appear to play an important role both in the maintenance and transmission of echinococcosis to humans (5). In one study of low prevalence regions, it was estimated that more than 10% of dogs were infected at least once in their lifetime (6).

In Japan, there has only been one documented case of cats with adult *E. multilocularis* worms (1). Recently, only 2% of cat faeces examined were found positive by coproantigen detection (Y. Morishima, personal communication). In Europe, however, cats were reported to be one of the main sources of infective *Echinococcus* eggs in the environment (31). In Hokkaido, Raccoon dogs (*Nyctereutes procyonoides*) were also reported to be infected with *E. multilocularis* (32). Increased numbers and infection rates of foxes involved in the transmission of *E. multilocularis*, and closer contact between humans and foxes, raise considerable concern that echinococcosis in humans may increase in the future (12). It is clear that updated, continuous, and intensive strategic control measures against echinococcosis are needed in Japan, particularly in Hokkaido.

Current control strategies

Targeting the source of echinococcosis

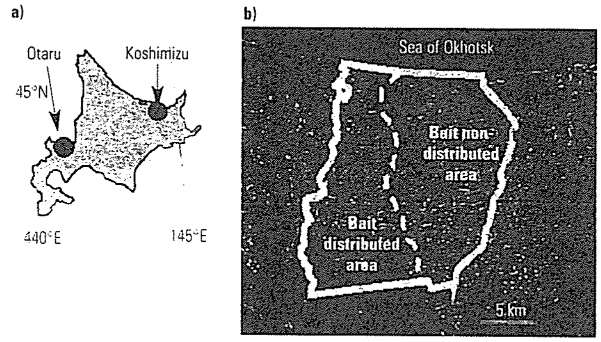
Both cystic and alveolar echinococcoses are important zoonotic parasitic diseases worldwide. In countries where pastoral farming is a major agricultural activity, *E. granulosus*, which is maintained by dogs and herbivore livestock, is more common. In those countries, control strategies include advising farmers not to feed ovine offal to dogs and encouraging them to have their dogs treated regularly with anthelmintic drugs. These steps are necessary because of the numerous cystic hydatid disease patients in these regions. The feasibility of controlling the disease by eradicating it in its intermediate and definitive hosts is relatively high. However, in this case, successful control programmes can only be achieved when implementation is nationwide and all dog-owners give their consent for their animals to be dewormed. In Uruguay, for example, there is a 'dosificador' system, whereby a group of trained personnel moves from farm to farm and deworms dogs with anthelmintic drugs. This system has brought a dramatic decrease in the infection rate of *E. granulosus* among livestock (21).

In Japan, on the other hand, as well as in the People's Republic of China, Russia, Alaska, and Central Europe, a different parasite is involved. Both its definitive and intermediate hosts are wildlife species and control is considered to be very difficult and costly. Currently, there is no reliable and cost-effective method for sustainable control or eradication of *E. multilocularis* in the sylvatic cycle (10). Deworming of wild foxes had not been attempted in Japan until 1997, but it proved effective and has continued ever since; there are, however, no effective control measures for wild voles. It is not clear whether only deworming foxes would have any impact on the effectiveness of reducing the parasite. 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) throughout the whole island.

Anthelmintic baiting

Germany was the first country to attempt deworming of wild red foxes using anthelmintic (praziquantel) drug-fortified bait distributed to foxes by hand and dropped from the air. After the implementation, a significant reduction in the prevalence of the parasite among wild red foxes was observed (28). This indicates the feasibility of controlling echinococcosis in wildlife. From 1997, the OIE Reference Laboratory started a similar project in Hokkaido to deworm red foxes using anthelmintic-fortified bait. However, conditions in Hokkaido in terms of vegetation, quantity of snowfall, the species of voles involved and their habitat 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 indicates a difference in the foraging behaviour of red foxes. For this reason, bait distribution by aircraft to cover a large area, as done in Germany, is not suitable for Japan and strategic bait distribution plans have to be designed. It will be necessary to develop cheaper baits and modify bait distribution patterns.

In 1998, a pilot area was selected in Hokkaido (Koshimizu, 200 km^2) (Fig. 3a) where a survey was first undertaken to locate fox dens (30). 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 (Fig. 3b). Commercial fish sausages containing fish meat, lard, gelatin and some spices were used as bait (1.5 cm long) and were embedded with 25 mg of 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 (Fig. 4a). 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



Bait non-distributed area: 109.4 km^2; 20 fox families
Bait distributed area: 90 km^2; 18 fox families

Fig. 3
Trial areas for controlling *Echinococcus multilocularis* in foxes with anthelmintic bait
a) map of Hokkaido
b) baited and non-baited areas in Koshimizu

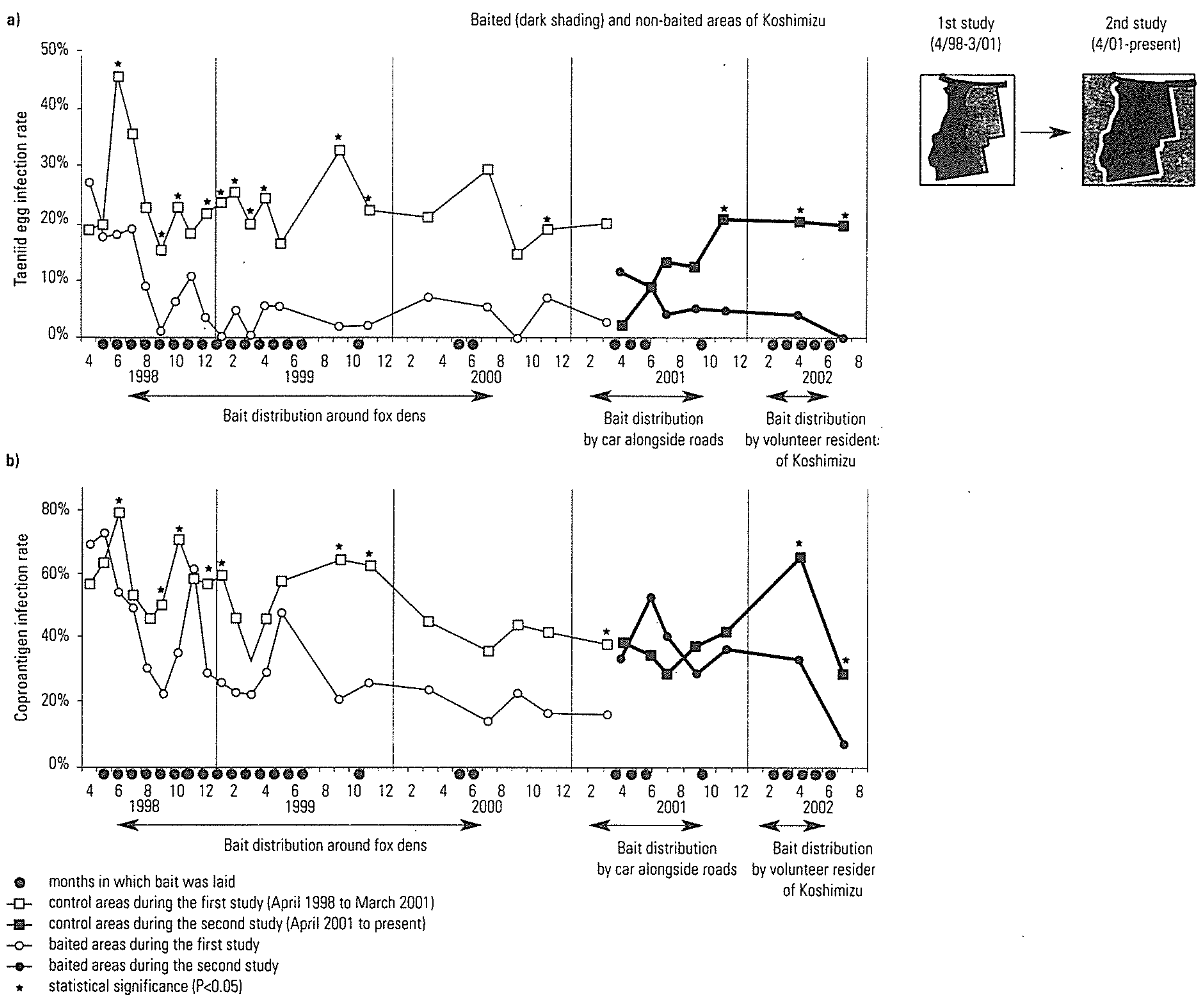


Fig. 4

Changes in the taeniid egg positive rates (Fig. 4a) and coproantigen positive rates (Fig. 4b) among foxes in Koshimizu during the first and second studies of bait distribution undertaken by the OIE Reference Laboratory for echinococcosis

(Fig. 4b). The trial found that voles born after bait distribution had a significantly lower prevalence of infection than their older counterparts (30).

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 in 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 (Fig. 4). The significant reduction of taeniid egg infection rates, however, was not observed until six months after the start of bait distribution, and a lowering of coproantigen positive rates came about almost a year later (Oku *et al.*, unpublished findings). Nevertheless, distribution of bait by car 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 fast, did not require large numbers of personnel, and is highly effective for controlling disease over large areas.

It was demonstrated in these trials that distribution of praziquantel-fortified bait to foxes could reduce egg contamination by *E. multilocularis* and the potential risk for human echinococcosis. Thus, it is 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 multilocular echinococcosis in Hokkaido.

Deplazes *et al.* (6) have suggested that new approaches for efficient deworming are needed, such as suitable, target-specific bait or slow-release praziquantel applications. The current implementation of the new baiting strategy is under study in the OIE Reference Laboratory for echinococcosis to see if it could be extended to cover a larger area like Otaru City. Machine-made bait embedded with 50 mg of praziquantel is produced from fishery waste and laced with tetracycline to monitor fox consumption by examination of tetracycline line formation in canine teeth. Bait is distributed along roads in the city (20 baits/km) (Fig. 5) and the prevalence in foxes is evaluated by necropsy of animals captured by local hunters, and by ELISA coproantigen test. Preliminary results show an effective reduction in the prevalence of *E. multilocularis* in foxes. In addition, a majority of the foxes with a tetracycline line in their canine teeth were found negative for *Echinococcus* infection.

The results of the baiting strategies discussed above suggest that when used in combination with the coproantigen detection system they could be highly effective in reducing or even eliminating the source of infection in the fox population of Hokkaido. This would be possible if a larger-scale operation with a proper combination of machine-made bait, an efficient distribution method, and an accurate evaluation system could be implemented.

Improving baiting strategies is an important step in reducing the risks posed by zoonoses that are transmitted by wild and domestic canine populations. Germany's strategy for controlling alveolar echinococcosis was largely modelled from previous rabies vaccination campaigns. In the case of Japan, strategies against echinococcosis are vital should other zoonotic diseases (e.g. rabies) emerge in the country. Presently, Japan is free from rabies, nevertheless, the illegal trafficking of dogs, and foxes wandering across drift ice from Russia, might become potential threats.

The recent upsurge of proteomic studies has helped explain biochemical host-parasite interactions that might contribute to disease control (2). The construction of a cDNA library for *E. multilocularis* is ongoing at the OIE Reference Laboratory, with collaboration from the University of Tokyo and the Parasitology Laboratory in Sapporo. The upcoming result is expected to propel research and trials to identify proteins for immunodetection of infection in definitive hosts.

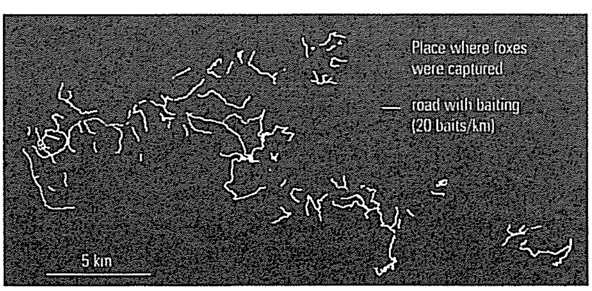


Fig. 5
Area covered by the current echinococcosis control programme in Koshimizu

Recombinant antigens may not only enhance diagnostic accuracy but may provide candidate proteins for vaccination, particularly for dogs and cats.

The OIE Reference Laboratory is presently processing information on the location of fox habitats using a geographical 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 (25). Analysis of spatial data in relation to epidemiology is an important tool in the control and eradication of echinococcosis. It is reported that there is a significant correlation between landscape data and *E. multilocularis* transmission and epidemiology (4). In Europe, spatial analysis has shown that *E. multilocularis* transmission rates increase in areas with dense populations of foxes and grassland rodents (23). Furthermore, GIS is a technology that provides Veterinary Services with the information necessary to properly handle control strategies against zoonotic diseases.

Compulsory reporting system for echinococcosis-infected dogs

The results of a research project in the OIE Reference Laboratory for echinococcosis entitled 'Prevention on the spread of areas that are endemic for zoonotic parasitic diseases' suggested that there was a strong possibility that dogs infected with *Echinococcus* through faecal contamination could transmit the infection to their owners. The overall results of this study led the Ministry of Health, Labour and Science to direct the Hokkaido Prefectural Government to take the necessary measures for the prevention of infection in pet dogs. As part of the campaign, the public was also made aware of the potential for infected dog faeces to be a source of infection to humans.

During amendment of the Infectious Disease Law in Japan, certain specific zoonotic diseases were included in the 4th category of diseases, i.e. diseases which must be reported. Echinococcosis in dogs was incorporated into this category along with bacterial dysentery in primates and West Nile fever in birds. In its 20th session, the Infectious Disease Evaluation Committee of the Ministry of Health, Labour, and Science, passed a resolution that makes it mandatory for veterinarians who diagnose echinococcosis in dogs to report the case to the health authorities. Thus, a national reporting system for dogs infected with *E. multilocularis* has been implemented by veterinarians since October 2004 to monitor and control the occurrence of infection in dogs throughout the country.

The Ministry of Health, Labour, and Science, with the assistance of the OIE Reference Laboratory, published guidelines on standard procedures to follow and diagnostic measures to be taken when reports are submitted by veterinarians. The guidelines have been distributed to local health offices as well as to practicing veterinarians throughout Japan. Thus, it is equally important to utilise research output for lobbying policy-makers into implementing laws that strengthen Veterinary Services.

Forum on Environment and Animals

The full implementation of measures to control or eliminate echinococcosis is dependent on cooperation from all sectors of society. In 1999, the OIE Reference Laboratory organised a scheme called FEA (Fig. 6). This scheme links important organisations including government and non-governmental organisations, academic institutions, international agencies (e.g. the OIE), government and private veterinarians and local residents. Although involvement of private sectors in Veterinary Services has been reported in some parts of Africa, Europe, and South America, the focus is on livestock animal health programmes (3, 9, 24). Present experience in Japan has unified various sectors with the aim of reducing the prevalence of *Echinococcus* infection in definitive hosts and keeping agriculture safe for public health protection.

The FEA is presently serving as a hub for private veterinarians involved in small animal practice around the country, specifically for the diagnosis of echinococcosis. In cooperation with the Hokkaido Small Animal Veterinary Association, veterinarians who suspect *Echinococcus* infection in dogs, cats, or other susceptible definitive hosts send faecal samples for laboratory examination. Small animal clinics in the Tohoku district of northern Honshu have recently agreed to submit samples for echinococcosis examination in the light of fears that the disease might spread to the mainland. Three criteria for diagnosis are stipulated in the national reporting system, and a positive result in any of these should be reported to health authorities. The three criteria are as follows:

a) locating the parasite body which can be morphologically identified

b) detecting parasite DNA from eggs or a part of the parasite body

c) detecting parasite coproantigen, which should give a negative result due to deworming.

This avenue provided by the FEA enhances accurate diagnosis and proper monitoring of echinococcosis in

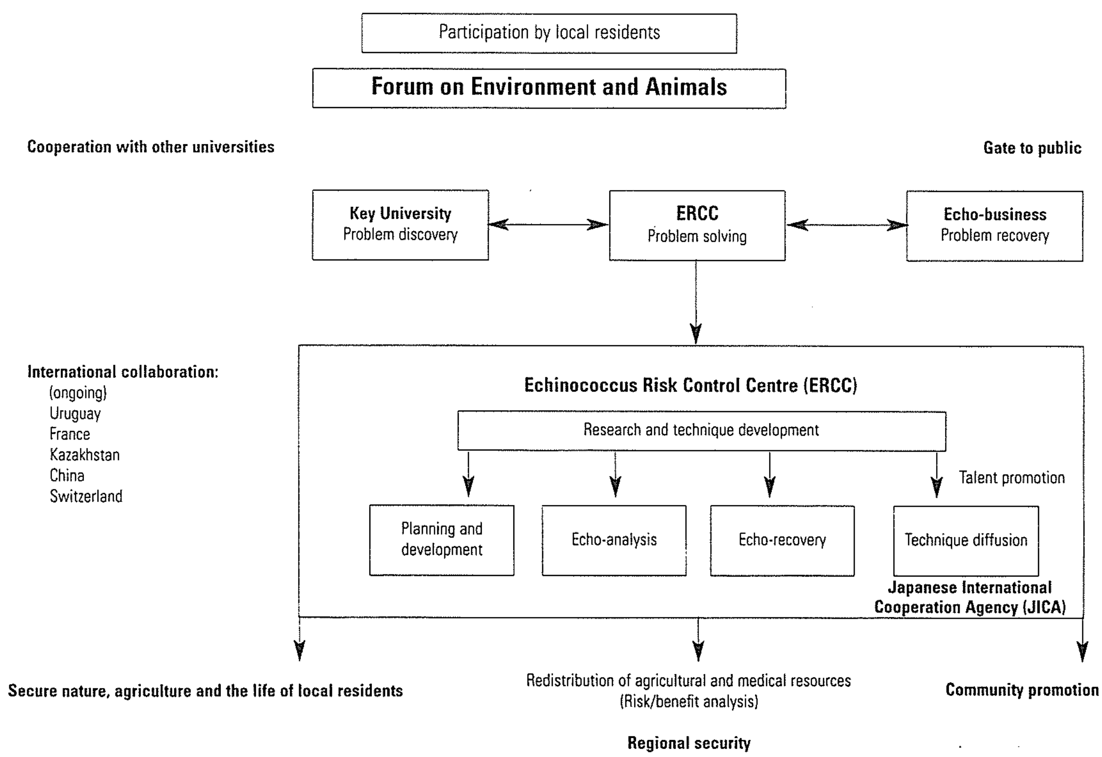


Fig. 6
Composition and aims of the Forum on Environment and Animals

Japan. It is envisioned that an *Echinococcus* Risk Control Centre will be established for the purpose of facilitating research and development related to control programmes protecting nature, agriculture, and public health.

Symposia focusing on the danger and threat of echinococcosis have brought about positive responses from local residents. The growing number of volunteers from among local residents, who constitute the population at risk, is a significant step towards successfully controlling echinococcosis. They serve as a potential workforce for bait distribution, fox monitoring, and reporting. Thus, it is claimed that successful surveillance systems should rely on networks of different people whose activities would include collecting, transmitting, analysing, and disseminating disease information (24).

Moreover, successful bait distribution in Koshimizu challenged local resident volunteers to continue the campaign with support from the OIE Reference Laboratory and the FEA. Recently, local residents of Kutchan conducted a preliminary survey on the prevalence of echinococcosis in foxes with technical assistance from the FEA. A proposed deworming campaign is now being formulated by Kutchan local residents and the FEA. It is expected that after successful campaigns in these areas, more volunteers from *Echinococcus*-endemic localities will follow. The current practice of involving local residents is believed to be a driving force for large-scale control in the near future.

International collaboration

Lessons learned from other countries have been useful in defining strategic approaches to controlling *E. multilocularis* in Japan. Aside from local control strategies, foreign models have broadened perspectives on control strategies. Collaboration with a national control programme in Uruguay's fight against *E. granulosus* provided insights into the delivery of large-scale programmes. Collaborating projects and exchange of information on control strategies against echinococcosis/hydatidosis in countries like Kazakhstan, the People's Republic of China, France, and Switzerland strengthen capabilities and increase the likelihood that echinococcosis will be completely eliminated from Japan within a few years.

A programme of technical cooperation between Japan and Kazakhstan has recently been undertaken to establish control measures against echinococcosis. A dramatic increase in the prevalence of human cystic echinococcosis in Kazakhstan is due to a sharp transformation in its social and economic conditions (26). After the break-up of the Soviet Union, privatisation of former state farms concentrated animals around human settlements, causing a growing problem of zoonoses and public health.

International symposia related to echinococcosis and other zoonotic diseases are being continuously organised. The Japan Interchange Association, in conjunction with the OIE Reference Laboratory and Hokkaido University, organised the Japan–Taiwan Symposium on Infectious Diseases in Animals and Quarantine on 20 and 21 October 2004. The issues discussed were the following:

a) infectious diseases of animals that have caused economic loss, and diseases that threaten the health of companion animals

b) infectious diseases that are transmitted from animals to humans

c) emerging infectious diseases that have been reported recently in world news, such as bovine spongiform encephalopathy and avian influenza

d) animal quarantine measures to prevent the spread of the aforementioned diseases (16).

These kinds of symposia have allowed researchers, government officials, and members of the public to meet together and facilitate bilateral regional exchanges of information. Extrapolations from the technological output of the symposia are applicable not only to neighbouring nations but to the whole world. Future symposia are being planned in neighbouring countries.

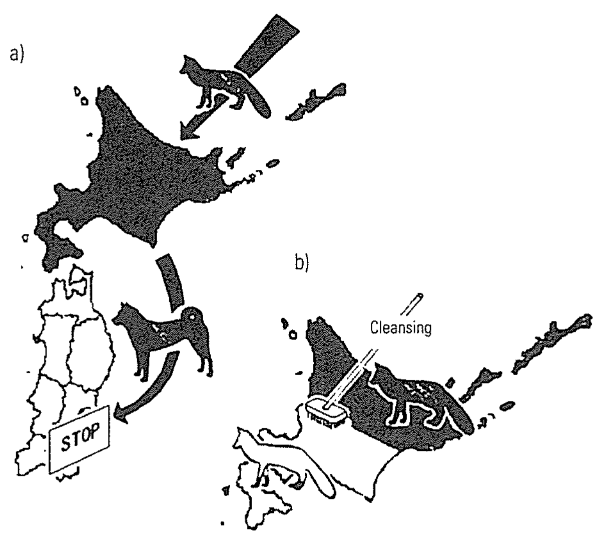


Fig. 7
A two-fold target for the control of echinococcosis in definitive hosts in Japan
a) the immediate prevention of disease spread towards Honshu
b) the 'cleansing' of Hokkaido by means of improved strategic baiting campaigns

Conclusion

The contribution of the OIE Reference Laboratory to controlling echinococcosis in definitive hosts in Japan has been significant and plans for continuing the current activities provide great hope for the future. Over the past few decades, developments in animal modelling and diagnostic methods and other advances in animal research have contributed greatly to efficient baiting strategies. The collaborative schemes of the FEA and cooperation with the international community give cause to believe that echinococcosis can be eliminated from Japan. These schemes, however, necessitate the use of the latest available technologies to maximise efficiency and improve control strategies. Genomics and proteomics are important leaps in science that could assist current strategies. Geographic information system technology is one of the more recent developments that must be exploited to upgrade current approaches.

This paper has described multifaceted strategies for the control of echinococcosis and could serve as an indispensable source of new ideas for Veterinary Services looking for a comprehensive approach to combating zoonotic diseases globally. Moreover, the opportunity for Japan to cooperate more closely with the OIE through the Reference Laboratory for echinococcosis has provided a broader avenue for achieving the much coveted goal of making Japan 'clean' from *Echinococcus* infection in the near future (Fig. 7).

Stratégies actuelles de prophylaxie visant les sources de contamination de l'échinococcose au Japon

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Résumé

Les auteurs décrivent les stratégies actuelles de prophylaxie visant les hôtes définitifs du principal parasite zoonotique au Japon, *Echinococcus multilocularis*. Plusieurs facteurs contribuent à rendre ce parasite particulièrement menaçant pour la santé publique : l'augmentation considérable de la prévalence de l'échinococcose chez les renards à Hokkaido (la deuxième en taille des îles formant l'archipel japonais), la pénétration des populations de renards sauvages dans les zones urbaines, l'infection des chiens errants et domestiques, et enfin le risque d'une propagation de la maladie dans l'île principale du Japon, Honshu. Les résultats des recherches précédentes ainsi que les stratégies appliquées actuellement, notamment les mesures de prophylaxie visant les renards sauvages laissent présager la possibilité d'éliminer l'échinococcose à l'avenir. La mise en œuvre d'un système national de déclaration pour les vétérinaires, le soutien de la coopération internationale et l'ouverture d'un Forum sur l'environnement et les animaux (FEA) offrent des raisons supplémentaires de miser sur cette réussite. Il s'agit de la première description d'une stratégie de prophylaxie multidimensionnelle visant à contrôler l'échinococcose chez les hôtes définitifs du parasite faisant appel à la participation active des habitants locaux. Ce modèle pourrait inspirer les Services vétérinaires du monde entier dans leurs efforts pour venir à bout de zoonoses similaires.

Mots-clés

Appât – Chien – Coopération du secteur privé – Échinococcose – Japon – Renard – Stratégie de prophylaxie – Système national de déclaration – Zoonose.

Estrategias actuales de lucha específica contra los focos de equinococosis en Japón

M. Kamiya, J.T.G. Lagapa, N. Nonaka, H.K. Ooi, Y. Oku & H. Kamiya

Resumen

Los autores describen las actuales estrategias de control para luchar contra los huéspedes definitivos del parásito zoonótico más importante que está presente en Japón: *Echinococcus multilocularis.* Un espectacular aumento de los niveles de prevalencia de equinococosis en zorros en Hokaido (la segunda isla, por tamaño, del archipiélago japonés); la penetración de zorros salvajes en zonas urbanas; la infección de animales domésticos y perros callejeros; y la posibilidad de que la enfermedad se propague a Honshu, la isla principal del archipiélago; son otras tantas amenazas de importancia que planean sobre la salud pública. Los resultados de las investigaciones realizadas hasta ahora y las estrategias que se aplican actualmente, con medidas como la lucha contra la infección en zorros salvajes, llevan a pensar que en el futuro será posible eliminar la equinococosis. Además, la aplicación de un sistema nacional de notificación para veterinarios, la colaboración internacional y la creación de un Foro sobre Medio Ambiente y Animales (FEA) ofrecen otros tantos motivos de optimismo. Este es el primer informe fruto de una estrategia que aborda la lucha contra la equinococosis en huéspedes definitivos desde varios ángulos a la vez e integra también la colaboración con la población local. Se trata de un modelo que puede brindar ideas novedosas a Servicios Veterinarios de todo el mundo en su lucha contra otras enfermedades zoonóticas conexas.

Palabras clave

Colaboración con el sector privado – Ensayo con cebos – Equinococosis – Estrategia de control – Japón – Perro – Sistema nacional de notificación – Zoonosis – Zorro.

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Echinococcosis Risk among Domestic Definitive Hosts, Japan

To the Editor: Echinococcosis is a serious parasitic zoonosis in the Northern Hemisphere. In Japan, it is characterized by alveolar, hepatic, and cerebral disorders in humans caused by the larval form (metacestode) of the tapeworm *Echinococcus multilocularis*. The life cycle of the parasite is maintained in the wild by gray-backed voles, *Clethrionomys rufocanus*, as intermediate hosts, and by red foxes, *Vulpes vulpes*, as definitive hosts. Humans are infected by ingestion of the parasite eggs, mainly through water contaminated with the feces of wild red foxes, which have an estimated infection prevalence of 54%–56% (*1*).

The echinococcosis-endemic area in Japan is restricted to the northern island of Hokkaido, although sporadic human cases have been reported on other islands (*2*), and infected pigs have been documented on the main island of Honshu (*3*). 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.

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) (Y. Morishima, pers. comm.). 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 (*4*).

Nearly 10,000 pet dogs were estimated to have been transported in 1 year to and from Honshu and Hokkaido by planes and ferries; this presumably included up to 30 *E. multilocularis*–infected pet dogs per year (*5*). 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 3–4 days before traveling would be a helpful preventive measure. As part of an amendment to the Infectious Disease Law in Japan, *E. multilocularis* infection was included among the 4th Category Diseases (*6*). Thus, since October 2004, it has been mandatory for veterinarians who have diagnosed echinococcosis in dogs to report each case to health authorities, the first national reporting system of its kind worldwide.

Our laboratory established the Forum on Environment and Animals (FEA) to meet the demand for accurate and rapid diagnosis of echinococcosis in domestic dogs. FEA is a hub for veterinary practitioners around the country for confirmation of *E. multilocularis* infection in definitive hosts, especially dogs but also cats. Feces submitted are from dogs and cats that are suspected to be infected and that wander or walk in parks and woodlands and likely prey 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 hours at 70°C. Fecal egg examination is conducted by using centrifugal flotation (*7*) with sucrose solution with a specific gravity of 1.27. Sandwich ELISA using a monoclonal antibody EmA9 (*8*) is used for *E. multilocularis* coproantigen detection. Egg- and ELISA-positive fecal samples from dogs are subjected to PCR amplification (mitochondria 12S RNA gene) (*9*).

The Table presents data of samples from both dogs and cats examined by FEA from April 2004 through August 2005. A total of 1,460 domestic dogs were examined, and 4 (0.27%) were confirmed positive to echinococcosis by PCR, all from Hokkaido. Test results from eggs detected in cat feces suggested these animals were infected with *Taenia taeniaeformis*, a cat tapeworm, rather than *E. multilocularis*, because coproantigen ELISA results were negative and an ELISA-positive sample did not contain eggs.

To our knowledge, this survey registered the greatest number of domestic dogs examined recently in Japan

Table. Prevalence of echinococcosis in definitive hosts subjected to fecal egg examination, ELISA coproantigen test, and PCR copro-DNA detection, Japan

Species	No. samples	Positive samples (%)		
		Egg examination	ELISA	PCR
Dogs	1,460	3 (0.20)	6 (0.41)	4 (0.27)
Cats	128	4 (3.12)	1 (0.78)	ND*
Total	1,588	7 (0.44)	7 (0.44)	–

*ND, not done.

for echinococcosis. 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.

A previous report of necropsy examinations of 9,849 dogs from 1966 to 1999 showed a prevalence of 1.0% (*10*). Although necropsy is considered the most reliable method to diagnose *E. multilocularis* in definitive hosts, it is not applicable for live animals such as domestic dogs and cats. Fecal egg examination is generally used; however, infection is difficult to confirm because the morphology of taeniid eggs is indistinguishable from those of *E. multilocularis*, and eggs are excreted intermittently even after the worms mature. Coproantigen detection 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 (*1*). The combined egg examination, ELISA, and PCR methods we used showed an accurate and rapid diagnosis in domestic dogs, which is important for immediate reporting, treatment, and action to safeguard dog owners.

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Comp. Parasitol.
74(1), 2007, pp. 151–153

Research Note

Multiplication of Ovaries in *Ctenotaenia marmotae* (Frölich, 1802) (Cestoda: Anoplocephalidae)

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ABSTRACT: Individuals of the cestode species *Ctenotaenia marmotae* (Frölich, 1802) (Cestoda: Anoplocephalidae) possessing duplicated rudimentary ovaries toward the medial parts of the segments were found in material collected from the Siberian marmot (*Marmota sibirica*) in Mongolia. This tapeworm is characterized by possessing one pair of female genitals per segment. The extra rudimentary ovaries that we found ranged from 1 to 6 in number per segment and were much smaller than the main pairs of ovaries. Although multiplication of ovaries was reported to occur in a species of *Diandrya* (Darrah, 1930) by Rausch (1980), this is the first report of multiplication of ovaries in this species of cestode.

KEY WORDS: Cestoda, Anoplocephalidae, *Ctenotaenia*, *marmotae*, morphology, anatomy, marmot, *Marmota sibirica*, Sciuridae, rodent, Mongolia, Palearctic.

Ctenotaenia marmotae (Frölich, 1802) is a parasite of marmots and ground squirrels in Eurasia. In Mongolia it was first reported from the long-tailed ground squirrel, *Spermophilus undulatus* (Pallas, 1778) in Huvsgul Province (Ganzorig et al., 1988), and Danzan (1978) reported occurrences of *Ctenotaenia citelli* Kirshenblatt, 1933)—which Beveridge (1978) considered to be a synonym of *C. marmotae*—in *S. undulatus*, *Ochotona dauurica* (Pallas, 1776), and *Microtus oeconomus* (Pallas, 1776) in Arhangai Province.

This cestode is characterized by having two sets of reproductive organs per segment, and although there have been many morphological studies of *C. marmotae* (see Spasskii, 1951; Spasskii and Shalayeva, 1961; Tenora, 1976; Beveridge, 1978; Tenora and Murai, 1978; Tenora, 1990; Tenora and Hörning, 1972; Tenora and Hönigova, 1990), Beveridge (1978) was the first to describe the presence of supernumerary vitelline glands in mature segments. Here we report cestodes that we observed to possess extra rudimentary ovaries and compare our findings with what was previously reported for specimens of *C. marmotae*.

The material in this study was collected during a survey of helminths of rodents in Mongolia in 1996. The material was obtained from 2 of 3 Siberian marmots, *Marmota sibirica* (Radde, 1862), Sciuridae, captured in Mt. Ih Bogd uu lin Bayanhongor Province, Mongolia. Of the cestodes collected, 6 complete specimens were used in this study. In addition, 7 specimens of *C. marmotae* from the long-tailed ground squirrel, *Spermophilus undulatus* Pallas, 1779 (Hanh County of Huvsgul Province), deposited at the Department of Zoology of the National University of Mongolia (Ulaanbaatar) were also examined. Cestodes were fixed in 70% alcohol, stained with aceto-carmine, dehydrated in ethanol, cleared in xylene, and mounted on slides under number 1 cover slips in Canada balsam. All measurements were made with the aid of an Olympus video micrometer (Model VM-30). The specimens were deposited in the Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, Japan (Helm. Coll. No. 3000).

The specimens from the hosts, *M. sibirica* and *S. undulatus*, were morphologically identical, and their dimensions fit the ranges given by Beveridge (1978). Rudimentary ovaries were found in some mature and pregravid segments in all 6 specimens from *M. sibirica*, but not in those from *S. undulatus*. The extra ovarian tissue is present near the posterior margin of the segment, between the fully developed ovary and the midline, posterior to the uniformly arranged linear testes and closely associated with additional vitelline glands (Figs. 1–3) (see Beveridge, 1978). In our specimens, the number of rudimentary

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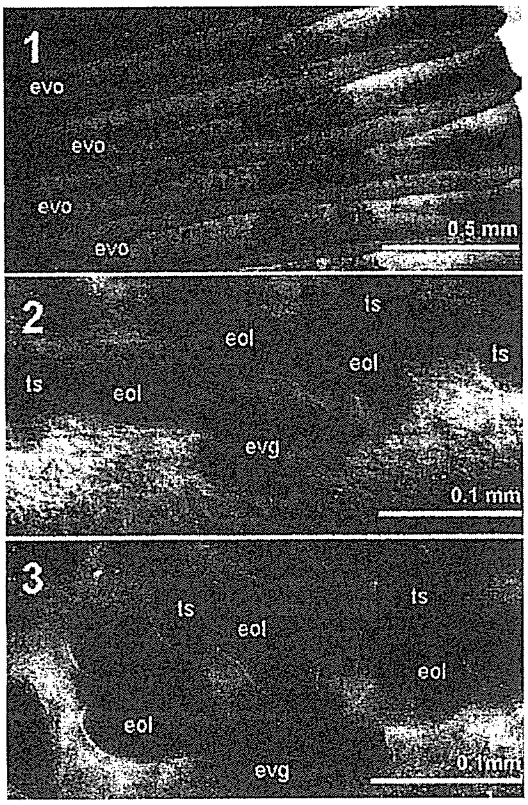

Figures 1–3. *Ctenotaenia marmotae* from *Marmota sibirica*. **1.** Placement of organs in mature segments, ventral view. **2, 3.** Details of rudimentary ovary, ventral view. (evo, extra vitelline gland and ovary; eol, extra ovary lobule; evg, extra vitelline gland; ts, testes).

ovaries ranged from 1 to 6 (mean, 4.1) per segment, and they were much smaller than the main ovary. Rudimentary ovaries possessed from 1 to 7 lobules (mean, 5), whereas the main ovary consisted of 44 to 86 lobules (mean, 62.6). One to 9 (mean, 3.1) additional vitelline glands of various shapes and sizes were present in all specimens from both host species and were located posterior to the testes in most premature, mature, and pregravid segments. The rudimentary ovaries measured 0.079–0.133 mm by 0.063–0.132 mm, about half the size of the fully developed ovaries (0.191–0.282 mm by 0.099–0.169 mm). Posteriad, the rudimentary ovaries persisted in the developing and maturing segments much longer than normal ovarian tissues, vitelline glands, and testes and were present in segments already filled by the developing uterus. In the present study, rudimentary ovaries were not found in specimens from *S. undulatus*, although sections were made and examined carefully.

The results are of special interest with respect to the evolution of the uterus in this family of cestodes, with variation ranging from very simple to highly differentiated and complex. The presence of additional ovaries has not been reported in any previous descriptions of this species; however, a similar structure was described by Rausch (1980) in *Diandrya composita* Darrah, 1930 from Nearctic marmots. He found that rudiments of the supernumerary female genital organs were taken to be "interproglottidal glands" reported in earlier descriptions of this species. The rudimentary female genital organs we found in *C. marmotae* show close similarity in location, comparative size, and number with those present in *D. composita*. In both species of parasites, the rudimentary organs persist longer than normal organs.

Cestodes with paired genitalia are present in nearly half of all genera of the family Anoplocephalidae. Beveridge (1978) suggested that duplication of genitalia occurs invariably within the same host group and is clearly a common phenomenon within this family. The duplication of genitalia in these cestodes is suggested to be apomorphic, whereas single genitalia are considered a primitive or plesiomorphic state (Beveridge, 1994). The multiplication of female genital organs in both *Ctenotaenia* and *Diandrya* and the historical geography of the host group support this hypotheses. The genus *Diandrya* was suggested by Rausch (1980) to be a derivative of *Andrya* and perhaps arose in Nearctic marmots during the early Pleistocene, after the trans-Beringian dispersal of *Marmota* to the Palearctic (Rausch, 1980).

Interestingly, marmots in the Holarctic region serve as hosts for species of *Paranaplocephala*, whereas the origination of species of *Ctenotaenia* in marmots in the Palearctic region seems to parallel the simultaneous but vicariant origin of species of *Diandrya* in marmots of the Nearctic region. This curious geographic vicariance seems worthy of additional study, and perhaps some light can be shed on this by work now being conducted by the field teams of the Beringian Coevolution Project in both the Nearctic and the Palearctic regions.

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Echinococcosis/Hydatidosis

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Summary of general activities related to the disease

1. Test(s) in use/or available for the specified disease at your laboratory

Test	Diagnosis			Surveillance		Total
	Dog	Cat	Others	Fox	Others	
ELISA (coproantigen detection)	459	39	-	321	37*	856
PCR	2	1	-	92	5	100
Faecal Examination	459	39	-	321	37*	856

*Animal species of the source of faeces were identified with Multiplex PCR (11 Weasel, 1 Racoon dog, 25 unidentified)

2. Production and distribution of diagnostic reagents

None.

Activities specifically related to the mandate of OIE Reference Laboratories

3. International harmonisation and standardisation of methods for diagnostic testing or the production and testing of vaccines

To standardise diagnostic tests for surveillance of definitive hosts, in-house tests for detection of coproantigen using immunochromatography was used for examination of samples from China.

4. Preparation and supply of international reference standards for diagnostic tests or vaccines

Under preparation.

5. Research and development of new procedures for diagnosis and control

In-house kits are currently processed for patenting in the diagnosis of echinococcosis in definitive hosts, such as rapid ELISA kit and immunochromatography. Evaluation for their use in surveillance to monitor prevalence rates in dogs and foxes is ongoing.

6. Collection, analysis and dissemination of epizootiological data relevant to international disease control

6.1 Follow-up survey of *Echinococcus* prevalence in wild (free ranging) red foxes in Koshimizu town, Hokkaido, Japan covering 200 km^2 to determine the effect of deworming program implemented in cooperation with local resident volunteers. In the year 2006, our baiting strategy was proven successful in the control of echinococcosis in wild foxes as indicated by the zero prevalence.

6.2 Another follow-up survey was made in Kutchan Town (Hokkaido, Japan). Surveillance study showed that there was a sharp decrease in the prevalence of echinococcosis among wild foxes in the area resulting from the intervention conducted by this laboratory by strategic distribution of praziquantel-laced baits.

6.3 Diagnostic service for the domestic dogs and cats has been provided with the cooperation of the Forum on Environment and Animals (FEA), Hokkaido Small Animal Veterinary Association, Tohoku Small Animal Veterinary Association and government as well as private animal clinics/hospitals.

7. Provision of consultant expertise to OIE or to OIE Member Countries

Consultant expertise has been provided to Franche-Conte University, France and Institute of Zoology of the Ministry of Education and Science, Republic of Kazakhstan.

8. Provision of scientific and technical training to personnel from other OIE Member Countries

Training on coproantigen and copro-DNA diagnostic methods was provided for veterinary personnel from China.

9. Provision of diagnostic testing facilities to other OIE Member Countries

ELISA test kits for the diagnosis of echinococcosis in definitive hosts were provided to Uruguay.

10. Organisation of international scientific meetings on behalf of OIE or other international bodies

None.

11. Participation in international scientific collaborative studies

This laboratory participated in international collaborative studies and projects on the control of echinococcosis/hydatidosis with Dr. J.J. Chai and Dr. J. Wei, National Hydatid Research Center in Urumqi (China), Prof. H.K. Ooi, National Chung Hsing University in Taichung (ROC), Dr. C. Carmona, the Parasite Biology Unit, Institute of Hygiene (Uruguay), Prof. B. Shaikenov, Institute of Zoology, Academy of Sciences (Kazakhstan), Prof. P. Giradoux and Prof. D.A. Vuitton, Institute of Environmental Science and Technology, WHO Collaborating Center for Prevention and Treatment of Human Echinococcosis, University of Franche-Comte (France) and Prof. B. Gottstein, Institute of Parasitology, University of Bern (Switzerland). The Projects have been supported by the Japanese Ministry of Education, Science and Culture, and by the Ministry of Health, Labour and Welfare associated with Human Science Foundation (HSF).