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Control program against hydatidosis and the decreased prevalence in Uruguay

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Abstract. Cystic hydatidosis/echinococcosis is an important zoonosis caused by the tapeworm *Echinococcus granulosus*. Hydatidosis is a serious parasitic disease in Uruguay, and in 1991 a new national control program was implemented by the national commission against hydatidosis (CHLCH). In 1991 (before the control program), farm and town dogs were examined for the prevalence of the parasite in Tacuarembó, Uruguay, using fecal samples of farm dogs after an arecoline purgation and samples of small intestine of town dogs obtained by necropsy. The prevalence of *E. granulosus* was 23 and 4% in farm and town dogs, respectively. In order to evaluate the impact of the control program, two surveys on ovine hydatidosis were carried out in Tacuarembó before and during the national control program. Sheep were examined in 1991–1992 (before the control program) and 1999 (during the control program). Both prevalence and intensity of *E. granulosus* infection increased with age in both 1991–1992 and 1999. The prevalence of ovine hydatidosis was 41.6 and 8.5% in 1991–1992 and 1999, respectively. The prevalence of fertile cysts in sheep more than 4 years old was 7.3% and 2.3% in 1991–1992 and 1999, respectively. The remarkable decreased prevalence of ovine hydatidosis suggested the successful control program by CHLCH in Uruguay.
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Keywords: *Echinococcus granulosus*; Hydatidosis; Uruguay; Control; Dogs; Sheep

1. Introduction

Cystic hydatidosis/echinococcosis is an important zoonosis caused by the tapeworm *Echinococcus granulosus*. The parasite is distributed world-wide [1], about 2–3 million patients are estimated in the world [2]. The definitive hosts of *E. granulosus* are dogs that harbor adult tapeworms and excrete the parasite eggs with their feces. Main intermediate hosts of the parasite, livestock and human, take the eggs orally, and are infected with larval stage, hydatid cyst in the liver and lungs. The cyst with protoscoleces is called a fertile cyst.

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Older sheep harbor more fertile cysts. When dogs eat the fertile cysts, protoscoleces are released from the cyst, and develop into adult tapeworms in the small intestine of the dogs.

The protoscolex of *Echinococcus* spp. can develop in two directions, one is adult tapeworm in the definitive host and the other is hydatid cyst in the intermediate host. If the protoscoleces are dispersed in the abdominal cavity of the patients during the operation, they cause severe secondary hydatidosis [3].

To control the parasite, treatment of dogs with anthelmintic is the main procedure [8]. Some progress was achieved with this procedure and the prevalence in sheep was reduced significantly in some countries or areas. But after these control campaigns were interrupted, prevalence of the parasite increased. From the endemic status to extinction status, control can be divided into 4 phases; planning, attack, consolidation and maintenance of eradication [4].

2. Control program against hydatidosis in Uruguay

Uruguay is a middle-income developing country, situated between Brazil and Argentina, and is a highly endemic area of *E. granulosus*. The main industry of Uruguay (human population 3.3 million; size of the land 176.215 km^2) is animal husbandry with about 50,000 farms, 20 million sheep and 10 million cattle. Hydatidosis is endemic in Latin America, especially in Uruguay and some regions of Argentina, Brazil, Chile and Peru [1]. In Uruguay cattle are consumed mainly in the urban area, and inspected in large-scale modern slaughterhouses. Thus, many cattle are infected with hydatid cyst, but they are not important intermediate hosts for the transmission of the parasite [5]. In farms, sheep are home-slaughtered routinely. About 20% of the inhabitants carry out home-slaughtering. On such occasions dogs can gain access to viscera of the infected sheep. About 60% of inhabitants own at least one dogs. The prevalence in sheep is one indicator of the endemic situation of *E. granulosus* in Uruguay. Mean life expectancy at birth of the sheep population in Uruguay was estimated at 3.5 and 4.8 years for male and female sheep, respectively [6]. Old sheep are home-slaughtered frequently on farms.

Hydatidosis is a considerable public health problem in Uruguay. The number of patients during the period 1962 to 1971 was estimated at 552 per year (17.4/100,000) [7]. But the seroprevalence of hydatidosis in rural human population was 1.24% [8]. Two surveys using abdominal sonography also showed the prevalence of 1.39% and 1.6% in asymptomatic population [9,10]. The minimum cost (US$25 million/year) was estimated from the condemnation costs of infected offal, production losses of livestock, actual costs of hospital treatment and the reduced income of patients [11].

In Uruguay several efforts have been made since 1965. In 1965, hydatidosis was declared a national pest and all measures aimed at its eradication were declared, and a national commission against hydatidosis (Comission Honoraria de Lucha Contra la Hidatidosis, CHLCH) was organized. A program of Option 2 [4] was operated from 1970–1991. In Option 2, anthelmintic tablets were provided for the owners to treat their dogs periodically. But the prevalence in livestock was not decreased until 1990. The owner may have not given anthelmintic to their dogs properly. In 1991 a new national control program (Option 5 [4]) was started and conducted by CHLCH. The schedules for control included planning phase (1990), diagnosis phase (1991), attack phase (1992–1996) and consolidation phase (1997–

2004) [12]. Option 5 is a fast track approach, and all dogs are treated with praziquantel monthly. In 1997 about 40 *dosificadores* went to farms periodically and gave anthelmintic to dogs throughout Uruguay. Two or three *dosificadores* worked for proper treatment of dogs with praziquantel in the Department of Tacuarembó. CHLCH reported that 92.3% of the entire rural dog population had been dosed in 1997 [12].

3. Materials and methods

We determined the epidemiological status of *E. granulosus* before and during the campaign against Hydatidosis/Echinococcosis, in 1991–1992 and 1999, respectively. In 1991–1992 (before the control program), 79 town dogs and 208 farm dogs in Tacuarembó were examined for the adult stage of cestodes by necropsy and fecal examination, respectively. Stray dogs in the town of Tacuarembó were necropsied and the small intestine examined. Fecal samples were obtained after arecoline purgation in rural area, which were taken by CHLCH.

In Department of Tacuarembó, 639 and 375 sheep were examined in 1991–1992 (before the control program) and 1999 (during the control program), respectively. Ages of the sheep were estimated from their teeth. Viscera (the lungs, heart, liver, kidneys and spleen) of the sheep were obtained from several slaughterhouses, sliced in thickness of about 5 mm and examined for the hydatid cysts. The presence of laminated layer and the number of protoscoleces in each cyst were determined. Fertile cysts and sterile cysts were consequently differentiated. Small lesions (less than 5 mm in size) were examined histologically.

4. Results

4.1. Prevalence in dogs in 1991(before the control program)

Seventy-nine stray dogs in the town of Tacuarembó were necropsied and examined for adult stage of cestodes (Table 1). The prevalence of *E. granulosus* was 4%. Other taeniid cestodes, whose intermediate hosts are livestock, were less prevalent. But the prevalence of *D. caninum*, whose intermediate hosts is the flea, was 38%.

The fecal samples after arecoline purgation were examined for cestodes in the rural area (Table 2). The prevalence of *E. granulosus* in farm dogs was 22.7%. The prevalence of *T. hydatigena* was also higher than in town dogs.

4.2. Prevalence in sheep in 1991–1992 and 1999 (before and during the control program)

In 1991–1992 prevalence of hydatid cysts in sheep was 41.6%. The mean number of hydatid cysts in all examined sheep and in infected sheep were 1.58 (SD 3.97, range 0–

Table 1
Prevalence of cestodes in 79 stray dogs from town of Tacuarembó

Parasite	No. of positive	(prevalence)
Echinococcus granulosus	3	(4%)
Taenia hydatigena	6	(8%)
Multiceps sp.	1	(1%)
Dipylidium caninum	30	(38%)

Table 2
Result of 208 fecal samples from arecoline purgation in rural area

Parasite	No. of positive	(prevalence)
Echinococcus granulosus	47	(23%)
Taenia hydatigena	47	(23%)
Taenia ovis	6	(3%)
Multiceps sp.	8	(4%)
Dipylidium caninum	62	(30%)
Diphyllobithriidae gen. sp.	2	(1%)

47) and 4.00 (SD 5.46), respectively. Frequency distribution of the number of cysts in each sheep was overdispersed. Both intensity and prevalence of *E. granulosus* infection increased with age (Fig. 1). Average increase in the number of cysts per year was about 1. In sheep older than 4 years the prevalence was 49.3% and 18.5% in 1991–1992 and 1999, respectively. Hydatid cysts were found mainly in the lungs (61% of total cysts) and the liver (39% of total cysts) except one case (0.09%) in the kidney and three cases (0.28%) in the spleen. Most of the hydatid cysts were partially regressive (caseificated or calsified and polymorphic or multicystic). In 1999, the prevalence (8.5%) and mean intensity of cyst (mean 0.28, range 0–10, SD 1.17) decreased significantly. The mean numbers of hydatid cysts in infected sheep were 3.28 (SD 2.58). The prevalence in sheep less than 3-years old and more than 4 years old was almost 0 and 18.5%, respectively.

Ratio of number of fertile cysts to number of total cysts was 11.3% (114/1069) and 15.7% (19/121) in 1991-92 and 1999, respectively (Fig. 2). About 84-89% of cysts did not form protoscoleces. In 1991-1992, sheep more than 3 years old harbored fertile cysts. Prevalence of fertile cysts in more than 4-year-old sheep was 7.3% and 2.3% in 1991-1992

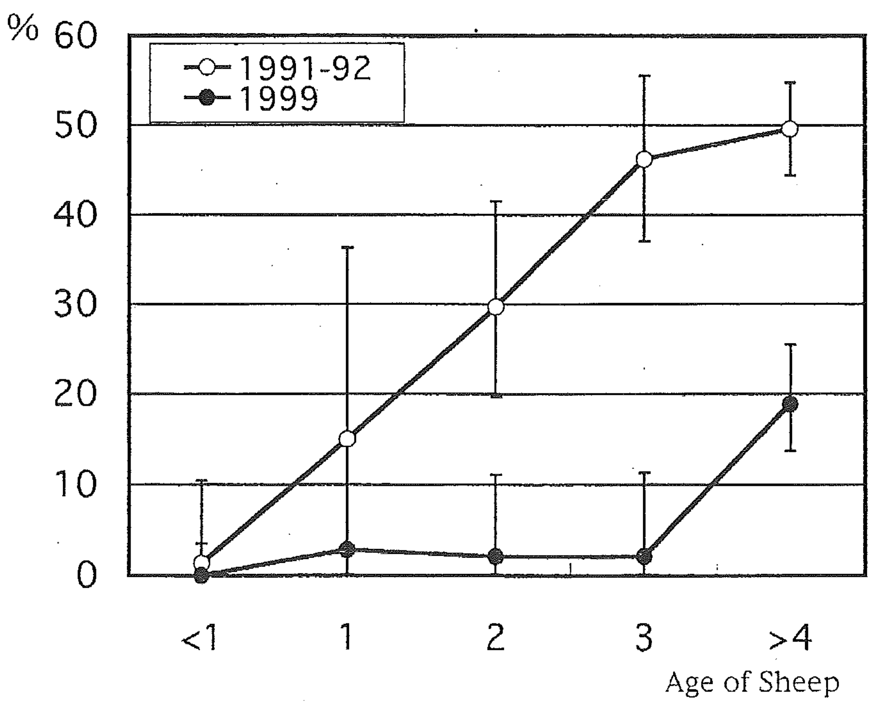

Fig. 1. Prevalence of *E. granulosus* in each age group of sheep in 1991–1992 and 1999 in Tacuarembó, Uruguay Error bars present 95% confidence intervals.

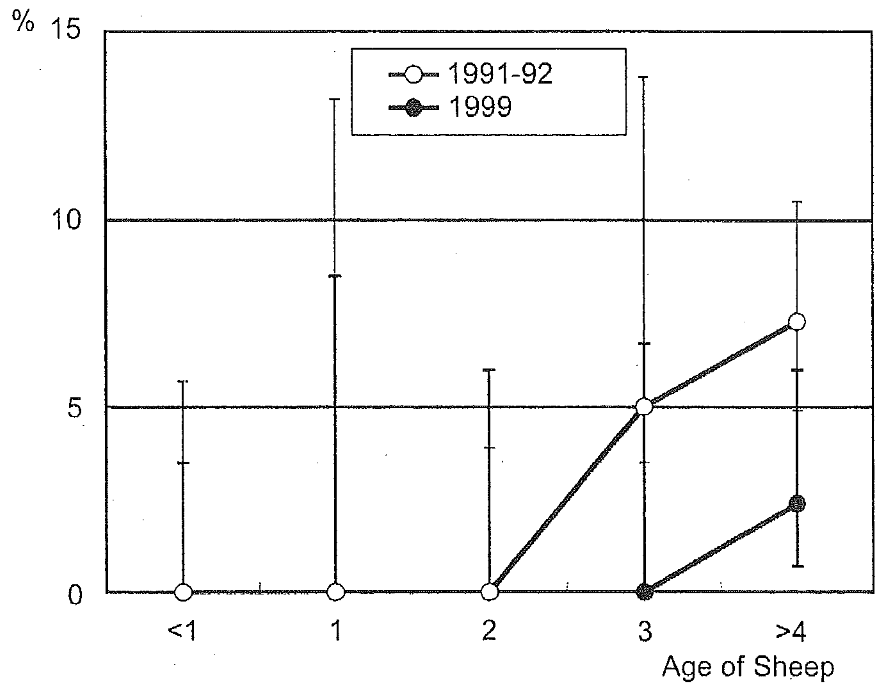

Fig. 2. Prevalence of fertile cysts in each age group of sheep in 1991–1992 and 1999 in Tacuarembó, Uruguay Error bars present 95% confidence intervals.

and 1999, respectively. Mean number of protoscoleces per fertile cyst was 1,437 (n=73) and 5,827 (n=19) in 1991-1992 and 1999, respectively. Most of the fertile cysts contained less than 500 protoscoleces.

5. Discussion

In Uruguay a new national control program started in 1991 [12]. It was applied throughout the country up to the present. CHLCH reported that at the beginning of the program the prevalence in dogs was 10.7% and 29.7% in fecal examination after arecoline purgation in Uruguay and in Tacuarembó, respectively. Our present study in 1991–1992 showed that the prevalence in farm dogs was 22.7% in Tacuarembó. CHLCH and we obtained almost similar results in survey. But in town of Tacuarembó the prevalence in dogs was lower than reported by CHLCH. Our present results suggested that dogs had easy access to the viscera of the infected sheep in rural area. Department of Tacuarembó was a highly endemic area in Uruguay.

Cabrera et al. [13] determined the infection pressure of the parasite in Uruguay, and reported that dogs became reinfected with *E. granulosus* between 2 and 4 months after treatment. CHLCH reported that 18 and 4.43% of dogs ate offal in 1991 and 1997, respectively. The infection pressure decreased by the decreased prevalence in sheep and decreased access to viscera of sheep in Uruguay. According to the data from CHLCH the prevalence in dogs was 0.74% in Uruguay, and 1.51% of farms had dogs infected by the parasite in 1997, when 92.3% of the whole rural dog population in the country had been dosed.

The remarkable decreased prevalence in sheep suggested the successful control program by CHLCH in Uruguay. In our data the prevalence in sheep was 41.6% in

1991–1992. CHLCH reported that the prevalence in sheep was 43.4% in 1991. Our data support their data. In our data the prevalence in more than 4-year-old sheep was 49.3% and 18.5% in 1991–1992 and 1999, respectively. Cabrera et al. [5] reported that the prevalence of hydatidosis was 7.7% in lambs and 18.0% in adults in 1998. Our results and Cabrera's data [5] were similar in adult sheep, but not in lambs. While the difference of both studies was in scale and place of survey, we do not know the cause of the difference.

The prevalence in more than 4 year old sheep was 18.5%. But a few young sheep were also infected with the parasite in 1999. It suggested that sheep were infected before 1997 in Uruguay. Even in the intensive attack phase of control (1992–1996) some sheep were infected. Young sheep, lambs, are usually sent to large-size, modern slaughterhouses and are consumed in the urban area [5]. In the urban area dogs usually have no access to viscera of infected sheep. But in the rural area old sheep with fertile cysts are home-slaughtered. So on the farms a few dogs had the chance to eat the viscera containing fertile cysts during the attack phase.

Although the control program led to the remarkable reduction of prevalence in animal populations in Uruguay, eradication of the parasite has not been achieved. A sustainable control program (less costly consolidation phase) is needed.

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ECHINOCOCCOSIS IN CENTRAL ASIA: PROBLEMS AND SOLUTIONS

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Effective countermeasures against alveolar echinococcosis in red fox population of Hokkaido, Japan

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ABSTRACT

One of the most serious parasitic zoonosis, alveolar echinococcosis, is mainly maintained on the life-cycle between red foxes and voles. The human disease is caused by ingestion of the egg of a taeniid cestode, *Echinococcus multilocularis*, which is found throughout the northern hemisphere. In Japan early human cases were reported from Sendai in mainland Honsyu in 1926 and at Rebun Island Hokkaido in 1937. During the following 3 decades, occurrence of the disease was restricted to Rebun Island. However, in 1966, a case was reported in the eastern region of the main island of Hokkaido. Thereafter, the parasite has gradually been expanding its distribution and presently *E. multilocularis* is found all over Hokkaido Island. In the last decade, the prevalence of the parasite in foxes has dramatically increased in Hokkaido (64% in Sapporo, 2003). Since humans become infected accidentally by ingesting the parasite eggs excreted in fox faeces, effective countermeasures against highly infected foxes are now urgently required. Our group at Hokkaido University has developed a diagnostic method for the definitive hosts. The method detects the parasite antigen in the fox faeces (coproantigen). This means that it is not necessary to autopsy the animal for diagnosis. In 1998, a deworming programme to manage echinococcosis in foxes was initiated in a pilot area (200 km^2) facing the Sea of Okhotsk in Hokkaido. Fish sausage baits containing the anthelmintic praziquantel, were distributed to feed foxes. Using the coproantigen detection and faecal taeniid egg examination methods, it was observed that the number of fox faeces containing the *Echinococcus* eggs was rapidly reduced in the bait distributed area. In contrast, coproantigen positive faeces did not show significant reduction in the first year, indicating that foxes were readily re-infected by ingesting the intermediate hosts, voles, which had been infected before the operation. However, pronounced reduction in the number of coproantigen positive faeces were recognized from the second year, even though frequency of bait distribution was reduced. The results suggested that longer term and broader strategic bait distribution would be effective for the control of *Echinococcus* infection all over Hokkaido Island covering 78,000 km^2. This paper will focus mainly on the control measures against alveolar echinococcosis in the red fox population of Hokkaido, Japan.

Эффективные контрмеры против альвеолярного эхинококкоза в популяциях красной лисы в Хоккайдо, Япония

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РЕЗЮМЕ

Один из самых серьезных паразитарных зоонозов - альвеолярный эхинококкоз, главным образом циркулирует между красными лисами и полевками. Заражение людей этой болезнью возникает при попадании в рот яйца цестоды *Echinococcus multilocularis*, который найден всюду в северном полушарии. В Японии о ранних случаях заболевания известны из Сендаи в материке, из островов Хонсу и Ревун в 1926 г. и Хоккайдо в 1937 г. В течение последующих трех десятилетий, возникновение болезни было ограничено ос.Ревун. Однако, в 1966 г., о случае обнаружения болезни сообщили из Хоккайдо, главного острова восточной области. После этого, паразит постепенно расширил свое распространение, и теперь *E. multilocularis* найден на всем протяжении острова Хоккайдо. В прошедшее десятилетие, распространенность паразита у лис в Хоккайдо (64 % в Саппоро, 2003 г.) драматично увеличилась. Так как человек заражается случайно, глотая яйца паразита, выделенных с испражнениями лис, эффективные контрмеры против сильно зараженных лис теперь требует безотлагательного действия. Наша группа в Университете Хоккайдо разработал диагностический метод для установления инвазии у окончательных хозяев. Метод основан на обнаружении антигена паразита в испражнениях лис (копроантиген). Это означает, что нет необходимости проведения вскрытия животных для выявления их зараженности. В 1998 г., кампания по дегельминтизации и по борьбе с эхинококкозом лис была начата на экспериментальной территории (200 km^2) у берега Охотского моря в Хоккайдо. Приманки, рыбные колбасы, содержащие дегельминтизирующее средство - празиквантел, были разбросаны, чтобы покормить лис. Используя копроантиген, обнаруженные в фекалиях и определением яиц тениид, было выявлено, что число лис, испражнения которых содержали яиц *Echinococcus* быстро снизилось на опытной площади, где были разбросаны приманки. Напротив, копроантиген положительные фекалии не показывал существенное сокращение инвазии на первом году, указывая, что лисы повторно часто заражались, поедая промежуточных хозяев, полевок, которые были инвазированы перед началом наших работ. Однако, явное сокращение числа копроантиген положительных фекалий были отмечены со второго года, даже при том, что частота распределения приманки была несколько уменьшена. Результаты позволили предположить, что продолжительное и более широкое стратегическое распространение приманки было бы эффективным для контроля инфекции альвеолярного эхинококкоза на всей территории острова Хоккайдо, закрывающего 78,000 $км^2$. Этот доклад основан главным образом на мерах контроля против альвеолярного эхинококкоза среди популяции красной лисы Хоккайдо в Японии.

INTRODUCTION

In the mid 1990s, WHO declared that we are now being confronted by the risk/crisis of infectious disease spreading on a global scale and no country is safe from that threat. This warning became a reality with the recent appearance of SARS (Severe Acute Respiratory Syndrome). However, echinococcosis has been overlooked because it is a chronic infectious disease. Nevertheless, echinococcosis needs similar challenges to SARS in that it requires a global outlook in risk management toward its control.

In Japan, when pigs in Aomori prefecture in Honshu, on the mainland of Japan were found infected with the larvae of *Echinococcus multilocularis* in 1999, the possibility that the parasite had spread to that area and established itself in Honshu was widely discussed. In addition, a dog that was kept indoors in Sapporo, Hokkaido, was found to be positive (copro-antigen +egg) for *E. multilocularis* infection in December 2002. Moreover a report issued by our group in the fiscal year of 2003 stated as positive 3 cases (copro-antigen +egg) of 1,139 dogs in Hokkaido and 2 (copro-antigen alone) of 69 dogs moving to Honsyu. The Ministry of Health and Labour of the Japanese Government took a serious view of the matter and alerted all local governments to take measures to control the spread of echinococcosis.

It is thus very important to strengthen dog and fox quarantine processes and also to undertake measures aimed at eliminating the source of human infection before the risk of infection increases. The problem of controlling echinococcosis can not be solved naturally, only through human patients being treated by physicians and the echinococcosis infection in dogs being treated by veterinarians. There is an urgent need to address the problem of echinococcosis infection in foxes, which are highly infected with the cestode and pose the greatest biopotential risk. A delay in the implementation of echinococcosis control measure can result in massive economic loss at the same time, as demonstrated by the recent confusion generated in the implementation of control measures against BSE (Bovine Spongiform Encephalopathy) in Japan.

RELEVANT BACKGROUND OF JAPAN

The disease is caused by the cestode, *Echinococcus multilocularis*, and is recognized widely in countries of the northern hemisphere. In Japan early human cases are reported from Sendai at mainland, Honsyu in 1926 and at Rebun Island, Hokkaido in 1937. During the next 3 decades, occurrence of the disease had been restricted in the island, however, in 1966 the case was reported in the eastern region of the main island of Hokkaido. Thereafter, the parasite has gradually been expanding its distribution and at present, *E. multilocularis* is recognized all over Hokkaido Island (Fig 1.).

In the last decade, the prevalence of the parasite in foxes has dramatically increased (64% in Sapporo,1999). Therefore, effective control measures of the parasite in the fox population are now required. Our group at Hokkaido University has developed a diagnostic method for the parasite. The method detects the parasite antigen in the fox faeces (coproantigen), whereby it is not necessary to autopsy the animal for diagnosis. In 1998, a deworming program to manage echinococcosis in foxes was initiated in a pilot area (200 km^2) facing the Sea of Okhotsk in Hokkaido. Fish sausage baits containing the anthelmintic praziquantel were distributed to foxes. Using the coproantigen detection and faecal egg

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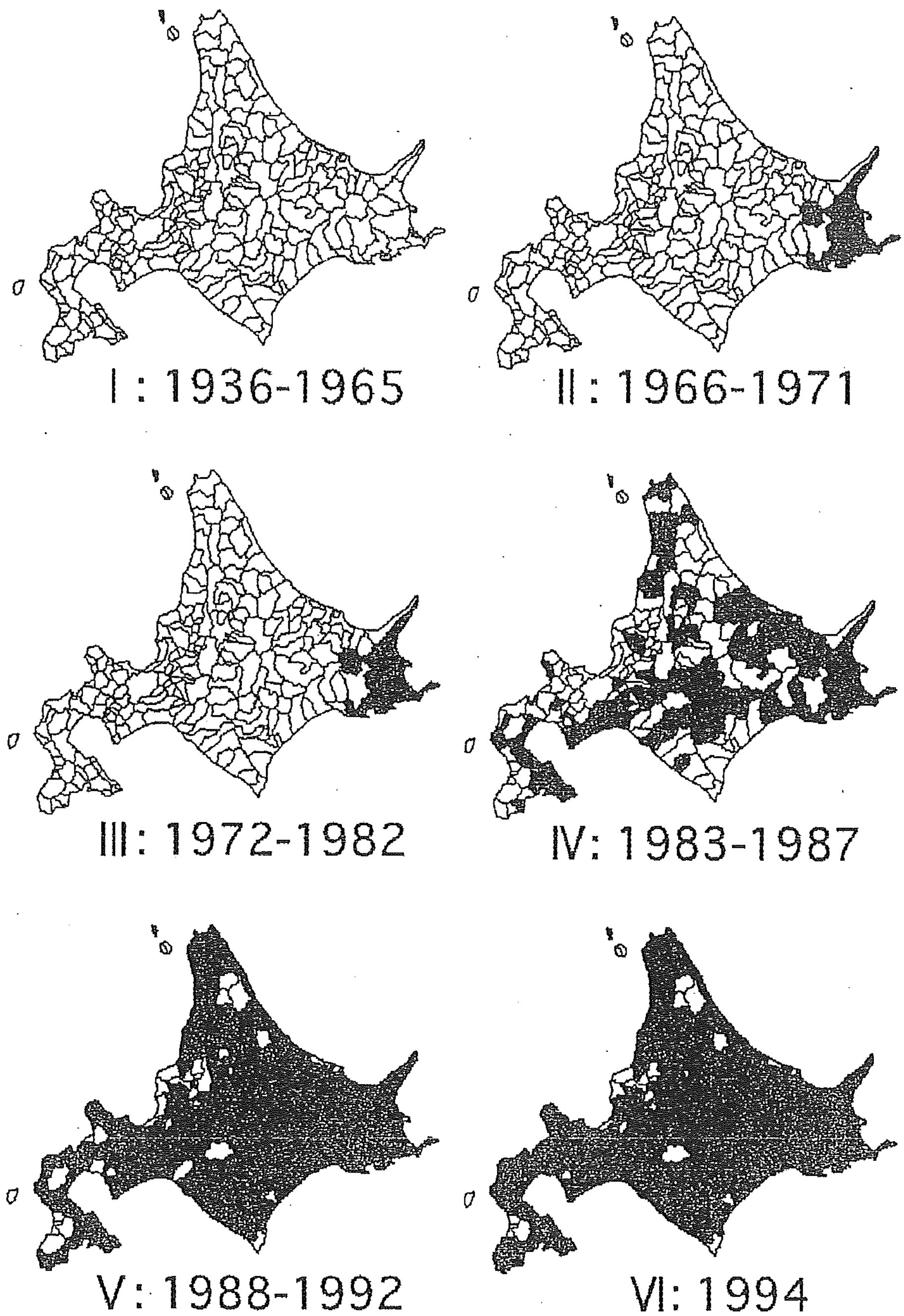

Fig 1. Expansion of the endemic area of *Echinococcus multilocularis* in Hokkaido. I 1936-1965 - The occurrence of the disease was restricted to Rebun Island. II 1966-1971 - Patients and infected animals were found in the eastern part of Hokkaido. III 1972-1982 - The endemic area was considered restricted in the eastern part of Hokkaido. IV 1983-1987 - Patients and infected animals were found in various regions. V 1988-1992 - The endemic area was further expanding and *E. multilocularis* was considered present throughout Hokkaido in 1992. VI The prevalence in foxes has an increasing trend.

examination methods, it was observed that the number of fox faeces containing the *Echinococcus* eggs was rapidly reduced in the bait distributed area. In contrast, coproantigen positive faeces did not show much reduction in the first year, indicating that foxes were readily re-infected by ingesting the intermediate hosts, voles, which had been infected before the start of the programme. However, obvious reduction in the number of coproantigen positive faeces was recognized from the second year, even though frequency of bait distribution was reduced. The results suggested that longer term strategic bait distribution would be effective for the control of *Echinococcus* infection in foxes.

To monitor the change in prevalence, the Hokkaido Government has been performing necropsy surveys in foxes captured in winter at various sites throughout Hokkaido; this showed that the overall prevalence was 17.8% in 22,268 foxes surveyed during 1966-1999. However, in the last decade, the prevalence of the parasite in foxes has dramatically increased (58% in 1998) (Fig. 2). Our necropsy surveys conducted in the suburbs of Sapporo City demonstrated a similar high prevalence in foxes: 54.9% in 1997-1998 (Morishima *et al* 1999a) and 56.7% in 1999 (Yimam *et al* 2002). Since the definitive hosts excrete the parasite eggs infective to humans, effective countermeasures to control the parasite in foxes are now urgently required because of the high prevalence rates.

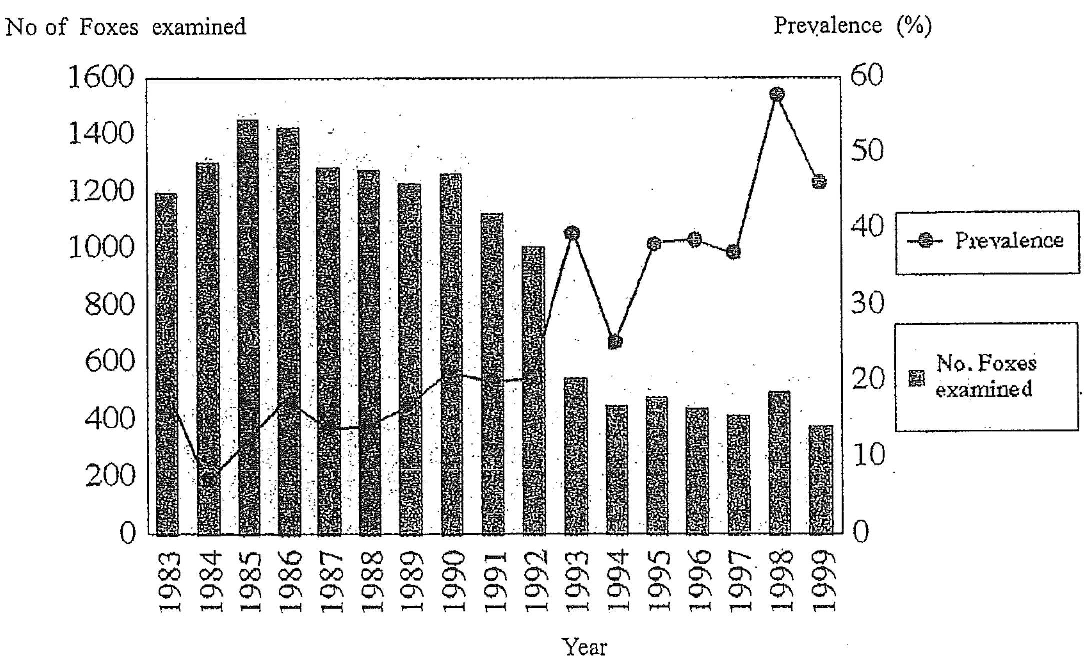

Fig 2. Change in the prevalence in winter captured foxes in Hokkaido during 1983-1999 (data from Hokkaido Government).

DEWORMING TRIAL OF FOXES

Our group at Hokkaido University has developed a sandwich ELISA based diagnostic method for the definitive hosts of *Echinococcus* species (Kohno *et al* 1995, Nonaka *et al* 1996). The method detects the parasite excretory/secretory antigen in the fox (or dog) faeces (coproantigen) (Fig 3.) and it is not necessary to autopsy the animal for diagnosis. Using this

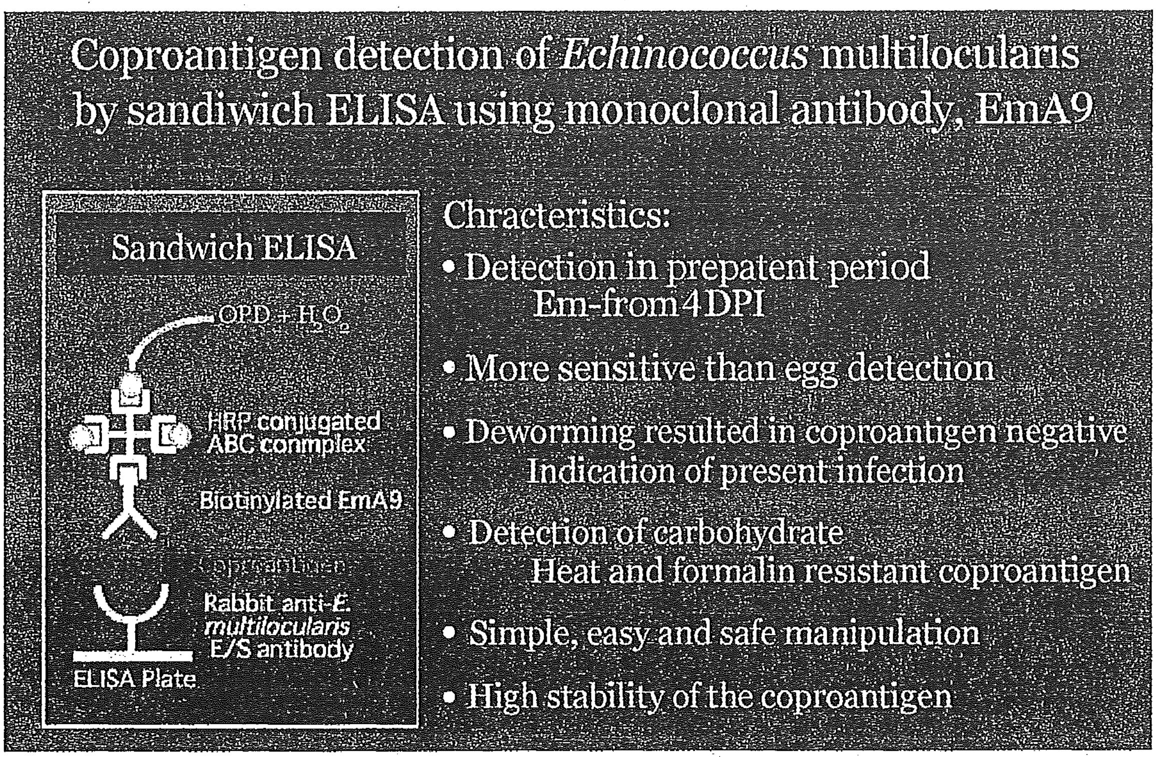

Fig 3. Coproantigen detection of *Echinococcus multilocularis* by a sandiwich ELISA using monoclonal antibody, EmA9, directed against adult *E. multilocularis* somatic antigen

method, a survey was conducted in a pilot area (200 km^2) of Koshimizu facing the Sea of Okhotsk in Hokkaido during 1997 to 1998 (Morishima *et al* 1999b). Fox faeces were collected around 36 fox breeding dens found at the beginning of this survey and the seasonal variation of *E. multilocularis* prevalence in foxes was evaluated by the coproantigen detection method and faecal egg examination. This survey provided baseline fox prevalence data for the next deworming trial (see below), showing that the prevalence of coproatigen positive faeces was relatively high with no distinct seasonal fluctuations (51.6?66.7%). However, the prevalence of egg positive faeces varied, with higher prevalences found in summer and winter (31.1% and 38.7%) than spring and autumn (13.3% and 13.5%). The observed difference between coproantigen-based and parasite-egg based prevalences was estimated due to the difference of the sensitivities between the two methods and due to the difference of the seasonal intensities of the parasite in the fox population. Since higher intensities were found both in coproantigen (ELISA OD values) and egg detection (egg count) in juvenile foxes compared to adult foxes, it was indicated that juveniles played a more important role in the environmental contamination with the parasite eggs. In Sapporo, another survey based on coproantigen detection was conducted on the foxes having their den sites in the parks or woodlands of urban areas (Tsukada *et al* 2000). Infected foxes were found in the Sapporo urban area and the suitable intermediate hosts, arvicolid rodents, were captured at the urban fringe although not all the rodents captured were infected with *E. multilocularis*. This survey suggested that the urban fringe offers a potential condition for the maintenance of *E. multilocularis* life-cycle. In France, the level of endemicity in different study sites was evaluated using coproantigen detection methods in fox faeces. The study concluded that coproantigen detection in field faeces could serve for large-scale surveillance as an alternative to necropsy (Raoul 2001). Those surveys suggested that the coproantigen detection in field fox faeces could provide reliable information for diagnosis of the assessment of changes in the fox prevalence and in the assessment of the regional endemicity of foxes.

The first deworming trial against *E. multilocularis* in wild foxes was conducted in Germany in a study area of 566km^2 (Schelling *et al* 1997). Baits containing 50 mg of the anthelmintic praziquantel were evenly and repeatedly distributed in the study area either by hand or dropped from aircraft. After 6 baiting campaigns over a period of 14 months, the prevalence of *E. multilocularis* in foxes had fallen from 32% to 4%. The effect was most pronounced in the central part of the baiting area, where no positive foxes were found in the last 2 months. However, in the marginal part of the baiting area, the reduction of the prevalence was moderate and the prevalence fluctuated between 5% to 20 % after the baiting campaigns started. The observed difference in the baiting effects at central and marginal parts were attributed to the evaluation method of fox prevalence. In this campaign, an average 2.2 foxes/km^2 were captured and necropsied to evaluate the change of fox prevalence. Because the hunting pressure for foxes in the baiting area was high, those in the surrounding area tended to move into the area to fill any vacant niche created. The observed higher prevalence in the marginal part was, therefore, due to the effect of those migrating foxes. This dewoming trial showed that a bait distribution is effective for reducing the fox prevalence, however it also suggested that the scale of operation must be large enough to enable the true effect of bait distribution to be evaluated.

THE TRIAL IN HOKKAIDO

In 1998, our deworming programme to manage echinococcosis in foxes was initiated at Koshimizu. This Koshimizu trial has several unique features. First, since faeces were used for the evaluation of fox prevalence with coproantigen detection as an alternative to necropsy, a survey can be performed with a minimal ecological disturbance. Second, because of the fox-family based study, precise evaluation of bait consumption by foxes and even collection of faeces in the study area can be achieved. The study area (200 km^2) was divided into two parts (Fig. 4), one (resided by 18 fox families) with bait distribution and the other (20 fox families) without bait distribution.

Baits used in this study had a fish sausage base. The manufactured fish sausages (90g, 2cm diameter x 12cm long) were cut into pieces 1.5 cm long and a half Droncit® tablet (50mg praziquantel per tablet; Bayer Co.) was embedded in each piece of fish sausage. To distribute baits, 5 bait holes (15 cm diameter x 30 cm depth) were made within an area 100 m apart from each fox breeding den in the bait-distributed area and the 2 pieces of baits were put inside each hole (2 baits/hole). To check the bait uptake by foxes, a smooth slope of about 30cm long and 20cm wide was prepared in front of each bait hole. Consumption of baits by foxes was checked by fox footmarks left on the slope. Bait was distributed every month in the first year and spring and autumn in the second year and only spring in the third year, with 4 consecutive days per month. To evaluate the change in the fox prevalence during the campaign using the coproantigen detection and fecal egg examination methods (Morishima *et al* 1999a), even number (3-5) of fox faeces were collected every one or two months within an area of 500 m from each fox breeding den both in bait-distributed and bait-nondistributed areas. It was observed that the number of fox faeces containing the *Echinococcus* eggs was rapidly reduced in the bait-distributed area. In contrast, coproantigen positive faeces did not show dramatic reduction in the first year, indicating that foxes were readily re-infected by ingesting the intermediate hosts, voles, which had been infected before the operation. However, an obvious reduction in the number of coproantigen positive faeces were recognized from the second year, even though frequency of bait distribution was reduced. The results suggested that: 1) deworming of foxes was achieved by the distribution of baits containing praziquantel; 2) environmental contamination with *Echinococcus* eggs was reduced by the bait distribution; 3) short term bait distribution was not enough for controlling re-infection and 4) longer term strategic bait distribution would be required for the efficient control of *Echinococcus* infection in foxes.

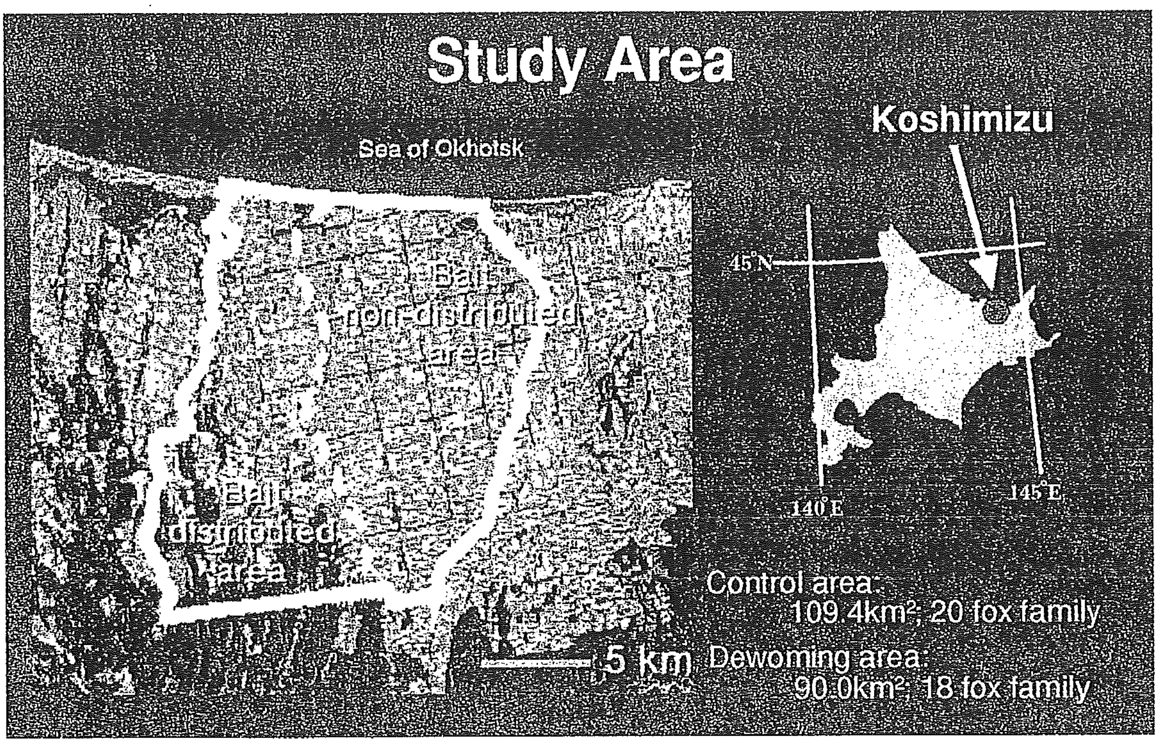

Fig 4. Study area (Koshimizu, Hokkaido) of a deworming trial against foxes

Based on these results, a new deworming programme started in 2001 for covering larger areas in Koshimizu and Otaru. In the new programme, machine-made baits were produced from the fishery waste and distributed along all the roads in the study area. The data are now under analysis in our laboratory to clarify the efficiency of the new baits and the bait distribution method in the management of echinococcosis in the fox populations.

As represented by the appearance of urban foxes (Tsukada *et al* 2000), the habitats of humans and foxes overlap in certain regions in the world. In this context, Hokkaido is not an exception, and human-red fox contacts at various places such as farm lands, tourist locations and large parks in urban areas have been frequently observed. Considering the high prevalence of red foxes with *E. multilocularis*, management of the disease in wildlife is now required. At present, a large-scale efficient control measure for alveolar echinococcosis has not been established and even transmission routes to humans have not been completely clarified. However, risk control of individuals, by targeting foxes coming into contact with each individual, could be started immediately by modifying our deworming trial. In Zurich, Switzerland, 50 pieces of bait each containing 50 mg praziquantel were distributed per km^2 ; and distributed monthly in six areas of one km^2, and one area of $6km^2$ from April 2000 to October 2001. Its efficacy was then determined by the coproantigen analysis. Significant decreases in the one km^2 bait areas (38.6% to 5.5%) and in the 6- km^2 bait area(66.7% to 1.8%) were observed (Hegglin *et al* 2003).

It is now paramount to clarify the individual potentialy high risk factors and to immediately establish and apply small scale efficient management measures (Nonaka *et al* 1998).

From these trials the baiting strategy using the coproantigen detection system for evaluation might be feasible to eliminate the source of infection from fox population on Hokkaido Island (78,000 km^2) if a larger scale operation with a proper combination of machine–made bait, distribution method and evaluation system could be applied (Kamiya 2004). For such an operation we have designed a scheme called Forum on Environmental Animals (FEA) which has been established since 1999 (Fig 5).

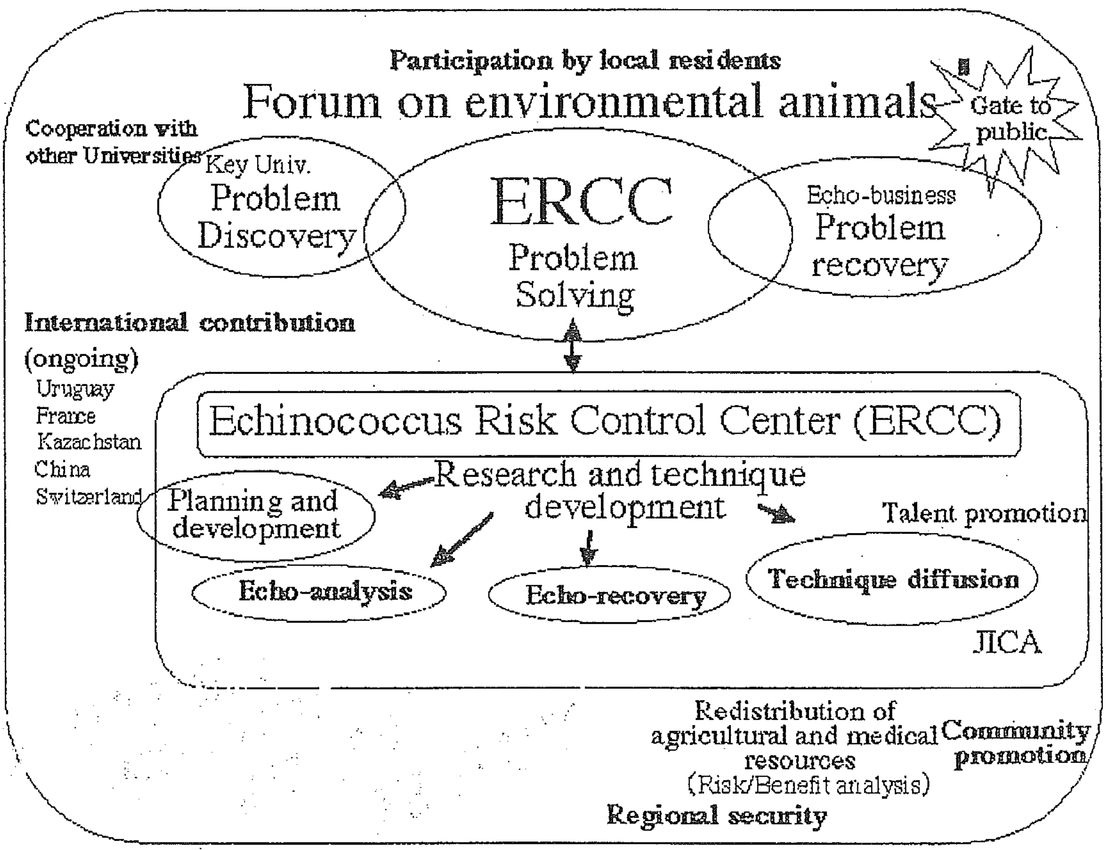

Fig 5. Composition of Forum on Environmental Animals (FEA)

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WORLD ORGANISATION FOR ANIMAL HEALTH

MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS (mammals, birds and bees)

Volume I

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CHAPTER 2.2.3.

ECHINOCOCCOSIS/HYDATIDOSIS

SUMMARY

Diagnosis of echinococcosis in dogs or other susceptible carnivores depends on the demonstration of adult cestodes of the Echinococcus *genus in their faeces or small intestine. In intermediate hosts, this depends on detection of the larval cyst form that can infect almost any organ, particularly the liver and lungs.*

Identification of the agent: *At present, four species of the genus* Echinococcus *are regarded as taxonomically valid. These are* E. granulosus, E. multilocularis, E. oligarthrus *and* E. vogeli. *The latter two species occur less frequently than the others. These four species are morphologically distinct in both adult and larval stages. A number of intraspecific variants have been described for* E. granulosus, *which exhibit morphological and biological characteristics, and these can reliably be differentiated by DNA analysis.*

Larval forms of Echinococcus *can usually be visually detected in organs. Special care has to be taken for a specific diagnosis of echinococcosis in instances where* Taenia hydatigena *in sheep is also a problem. Histological examination may confirm the diagnosis after formalin-fixed material is processed by conventional staining methods. The presence of a periodic-acid-Schiff positive, acellular laminated layer with or without an internal cellular, nucleated germinal membrane can be regarded as a specific characteristic of metacestodes of* Echinococcus. *The identification of larval* E. multilocularis *in rodents and other hosts is possible by macroscopic or microscopic examination and by DNA detection using the polymerase chain reaction (PCR).*

The small intestine is required at necropsy for the detection of adult Echinococcus *spp. in wild or in domestic carnivores. The technique of carrying out* surveys *with the use of arecoline has been generally adopted for determining the prevalence of* Echinococcus granulosus *in dogs. Handling infected material presents a risk to the operator of contracting a potentially fatal disease. Significant progress is being made in the development of immunological tests for the diagnosis of intestinal* Echinococcus *infections by use of coproantigen detection. The technique is currently used in surveys for* E. multilocularis *in populations of foxes, dogs and cats. Coproantigen detection is possible in faecal samples collected from dead or living animals or from the environment.*

PCR/DNA methods for the detection of E. multilocularis *and more recently* E. granulosus *in definitive hosts have now been established in specialised laboratories as diagnostic techniques.*

Serological tests: *Antibodies directed against oncosphere, cyst fluid and protoscolex antigens can be detected in the serum of infected dogs and sheep, but this approach is presently of limited practical use as it does not distinguish between current and previous infections. Cross-reactivity between* Echinococcus *and* Taenia *species also may occur.*

Requirements for vaccines and diagnostic biologicals: *Excellent progress is being made in the development of a vaccine against the larval stage of* E. granulosus in sheep and cattle.

A. INTRODUCTION

At present, four species of the genus *Echinococcus* are accepted taxonomically, namely *E. granulosus, E. multilocularis, E. oligarthrus,* and *E. vogeli.* These are morphologically distinct both in their adult and larval stages. A number of interspecific and intraspecific variants have been described for *E. granulosus.* Some genotypes of *E. granulosus* exhibit characteristic features that would justify the recognition as separate species according to some authors. Recently other species and genotypes of *Echinoccocus* have been proposed (21). Further studies are needed to define the full range of genetic diversity (12, 15, 16, 20). *Echinococcus granulosus*