Mainland, where no such information is currently available. We focussed our analyses on hunting dogs as the way they are managed and their activities in fields makes them more susceptible to acquiring a variety of zoonotic helminths either directly or indirectly from wildlife. Two different diagnostic techniques were employed to make our results more informative, namely a centrifugal-flotation technique (Ito 1980) with a sucrose solution with a specific gravity of 1·27 and a formalin-ethyl acetate sedimentation technique (Young and others 1979). With the exception of *Trichuris* and Taeniidae, all eggs were identified to genus or genus and species on the basis of morphological characters. The eggs of *T vulpis* are morphologically similar to those of other *Trichuris* species (Bundy & Cooper 1989) and parasitic eggs may be excreted after being acquired from other animals through coprophagy (Traub and others 2002). We therefore re-examined a total of 100 randomly selected trichurid eggs from positive faecal specimens according to the dimensions described by Yoshikawa and others (1989). Given that distinguishing between taeniid tapeworm eggs is difficult based on shape and size (Thompson 1995), we differentiated between *Taenia* and *Echinococcus* using nested PCR (Dinkel and others 1998). Pearson's χ^2 test was used to assess differences in the egg prevalence of each helminth between both groups. McNemar's χ^2 test was used to compare the diagnostic efficiency of the coprological techniques. All statistical analyses were performed using a statistical package (S-PLUS 6.1J for Windows; Mathematical Systems, Inc.).

[TABLE 1 will appear here]

Faecal specimens were collected from 134 hunting dogs between December 2003 and March 2005. Sampling of 86 companion dogs was also done. The results of both groups were combined and summarised in Table 1. *Trichuris vulpis* was the most common helminth in both groups, and the prevalence was significantly higher in hunting dogs ($P < 0.001$). Although comparison of results is difficult because a prior study of Yagisawa (1978)

determined prevalence by necropsy, the occurrence of *T vulpis* in dogs in Aomori appears to have decreased and other intestinal worms exhibited an appreciable decline. The reason for the remaining prevalence, particularly in hunting dogs, is uncertain, but it may be related to being reservoir hosts. *Trichuris vulpis* is prevalent among wild canids in Aomori (Sato and others 1999a, b). The hunting dogs may have become infected with this parasite in fields that were contaminated by the sylvatic hosts before then transmitting it to the local dog population. In addition, the choice and/or usage of drugs is particularly important. Several anthelminthics are currently used to prevent canine dirofilariasis. Of these, ivermectin (Cardomec; Merial) is the most commonly used for this purpose in Japan. However, ivermectin has no effect on intestinal trichuriasis at the usual chemoprophylactic dosage level (6 μg/kg bodyweight) and a more than 16-fold increase in concentration is required (Anderson & Robertson 1982). Ivermectin at this concentration showed an effect on other intestinal helminths such as *Toxocara canis*.

[TABLE 2 will appear here]

Regarding the relative sensitivities of each method (Table 2), the sedimentation technique had a significantly higher positive rate of trichurid egg detection than the centrifugal flotation technique ($P = 0.016$). Notably, the centrifugal flotation technique failed to detect instances of low egg density/abundance (<10 eggs per mount in the competitor). Because of the relatively low fecundity of *T vulpis* (Miller 1941), the sedimentation technique therefore appears to be preferable for examination. Despite its apparent zoonotic potential (Dunn *et al.* 2002), *T vulpis* has been somewhat neglected. Consequently, underestimation or underscoring the potential of this parasite is likely to occur if inadequate diagnostic tests are adopted.

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TABLE 1: Numbers and percentages, with 95 per cent confidence intervals (CIs), of intesti helminth egg positives among hunting dogs (*n* = 134) and companion dogs (*n* = 86) in Aon Prefecture, Japan

Helminth species	Hunting dogs		Companion dogs	
	Number (%) of positives	95 per cent CI of positives	Number (%) of positives	95 per cent of positive
Toxocara canis	0 (0)	0-2·2	4 (4·7)	1·3-11·5
Trichuris vulpis	52 (38·8)	31·0-47·3	14 (16·3)	10·0-25·5
Dipylidium caninum	0 (0)	0-2·2	2 (2·3)	0·3-8·2
Taenia sp.	1 (0·8)	0·02-4·1	0 (0)	0-3·4
Metagonimus sp.	2 (1·5)	0·2-5·3	0 (0)	0-3·4

TABLE 2: Comparison of the sensitivity of centrifugal -flotation and formalin-ethyl acetate sedimentation techniques for the detection of *Trichuris vulpis* eggs in 220 canine faecal specimens

		Sedimentation		
		Positive	Negative	Total
Centrifugal-flotation	Positive	55	1	56
	Negative	10	154	164
Total		65	155	220

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Development of *Taenia saginata asiatica* cysticerci to infective stage and adult stage in Mongolian gerbils

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Abstract

The development of metacestodes and adult worms of *Taenia saginata asiatica* in Mongolian gerbils (*Meriones unguiculatus*) were observed. Cysticerci were recovered from gerbils subcutaneously injected with hatched oncospheres. The recovery rate ranged from 0.1 to 3.2%. No cysticerci were recovered from the orally inoculated gerbils. The infectivity of the cysticerci recovered at 48 weeks post-infection was evaluated. Tapeworms were recovered on day 14 post-infection from the small intestine of 5 of 11 gerbils, with a recovery rate of 27% (6 worms recovered/22 worms inoculated). Three and four adult worms were recovered from two human volunteers who ingested five cysticerci after 4 months post-infection. In worms recovered from gerbils, segmentation and genital primordia in the posterior proglottids and hooklets in the residual rostellum were observed. The results indicate that gerbils can serve as an alternative intermediate host and that partial development of the adult worm stage occurs in gerbils.

Introduction

Human taeniasis caused by *Taenia saginata asiatica* (Bowles & McManus, 1994; Fan *et al.*, 1995) has been reported in Taiwan, China, Korea, Indonesia, Thailand, Philippines, Malaysia and Myanmar (Fan *et al.*, 1990a, 1992; Bowles & McManus, 1994; Simanjuntak *et al.*, 1997; Zhang *et al.*, 1999; Fan, 2000; Eom & Rim, 2001; Ito *et al.*, 2003). Adult worms of *T. asiatica* develop only in the human intestine but its metacestodes can develop in a wide range of intermediate hosts, such as pigs, wild boars, cattle, goats and monkeys (Fan *et al.*, 1990c). Although *T. asiatica* has been proposed to be a new species, ~~namely, *T. asiatica*~~ (Eom *et al.*, 2002; Ito *et al.*, 2003), ~~it is considered as a subspecies of *T. saginata* in this paper until more complete evidence has been presented to confirm its status as a new species.~~

Recently, *T. asiatica* metacestodes have been reported to develop in SCID mice (Ito *et al.*, 1997; Ito & Ito, 1999). Compared to SCID mice, Mongolian gerbils do not require any special facility, and they have been used as animal model for many parasites. For human *Taenia* species, only *T. saginata* metacestodes (Belgian isolate and African isolate) had been reported to develop in gerbils subcutaneously inoculated with hatched oncospheres (Geerts *et al.*, 1981/1982; Wouters *et al.*, 1988), and no study has been done with *T. asiatica*. Thus, in the first part of this study, Mongolian gerbils were orally inoculated with eggs or subcutaneously injected with hatched oncospheres to examine whether the animal can serve as an alternative intermediate host.

Mongolian gerbils have been reported to serve as alternative definitive hosts for *T. solium* and *T. saginata* (African isolate) after oral inoculation with cysticerci

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derived from their natural intermediate hosts (Kamiya *et al.*, 1990; Maravilla *et al.*, 1998). Moreover, golden hamsters have been reported to serve as alternative definitive hosts for *T. solium* after oral inoculation with cysticerci derived from SCID mice (Wang *et al.*, 1999). However, gerbils have not been reported to be alternative definitive hosts for human *Taenia* by inoculation with the cysticerci derived from rodent alternative intermediate hosts. Thus, in the present study, the development of *T. asiatica* cysticerci in gerbils and the infectivity of cysticerci and the partial development of the adult stage in gerbils were examined.

Materials and methods

Eggs of *T. asiatica* were collected from gravid segments that were excreted in the faeces of Taiwanese aborigine patients after deworming with atabrine (Quinacrine) (Fan *et al.*, 1990b).

Development of metacestodes

Embryophores were removed by 20 min incubation of eggs in sodium hypochlorite solution, which was a 10 times dilution with physiological saline of stock solution (containing at least 5% of active chlorite, Kanto, Tokyo, Japan) (Lightowlers *et al.*, 1984). The released oncospheres were washed 5 times with sterile physiological saline containing antibiotics (penicillin G: 400 IU s ml^{-1}, streptomycin: 1 mg ml^{-1}). Fourteen female Mongolian gerbils, 7–13 weeks old, raised in our laboratory were used. Five were orally inoculated with eggs, nine were subcutaneously inoculated with oncospheres. Gerbils were fed commercial pellet food (CLEA, Tokyo, Japan) and given water *ad libitum*. Gerbils were then divided into three groups, namely, G1, G2 and G3 (Table 1). Each animal of group G1 and G2 was subcutaneously injected with 2.5 mg prednisolone acetate (Tong Yong Pharmaceutical, Shanghai, China) once a week froRm 4 days before inoculation to 38 days post-inoculation (DPI) (a total of seven times). Each animal of group G3 was subcutaneously injected with 2.5 mg prednisolone acetate once a week from 7 days before inoculation to 21 DPI (a total of five times). Animals of group G1 and G2 were all killed at 9 weeks after inoculation. Animals of group G3 were killed at 45 to 48 weeks after inoculation. For recovering the parasite, the inoculation sites, livers and peritoneal cavities of the gerbils were examined at necropsy. For morphological observations, cysticerci were relaxed in physiological saline at 4°C overnight after evagination, fixed in 70% alcohol and cleared in glycerin. The animal experiments in this study complied with the Guidelines for Animal Experiments of the Graduate School of Veterinary Medicine in Hokkaido University according to Japanese law.

Development of adult worms in gerbils and humans

Eleven male gerbils, 11–14 weeks old, raised in our laboratory were used in this study. Each gerbil was orally inoculated with two 48-week-old cysticerci recovered from the group G3 gerbils, fed with commercial pellet food (CLEA, Tokyo, Japan) and given water *ad libitum*. Each gerbil was subcutaneously injected with 2.5 – 5 mg prednisolone acetate once a week from 5 days before inoculation to 9 DPI with the cysticerci (a total of three times). All gerbils were killed under ethyl ether anaesthesia at 14 DPI and adult worms were recovered from their small intestine. The worms were relaxed in distilled water, fixed in 70% ethanol followed by observation under light microscope after staining with acid carmine. In addition, two human volunteers (volunteer A: female, 24 years old; volunteer B: male, 42 years old) ingested five of the 48-week-old cysticerci individually. After ingesting the cysticerci, the human volunteers checked their faeces daily for the presence of proglottids.

Statistical analysis

The morphometrics of the 9 and 45–48-week-old cysticerci were assessed using a non-parametric Mann-Whitney's U test. Statistical analyses were performed using the Stat View 4.0 for Macintosh.

Results

Development of metacestodes

Metacestodes were recovered from the inoculation sites of all nine gerbils (group G2 and G3) subcutaneously inoculated with oncospheres, but not from the liver or peritoneal cavity. The recovery rate of cysticerci in gerbils of group G2 ranged from 1.2 to 3.2%, while those in gerbils of group G3 ranged from 0.1 to 0.8%. None were found in five gerbils (group G1) orally inoculated with intact eggs (fig. 1 and table 1).

Table 1. Recovery of metacestodes from gerbils subcutaneously inoculated with oncospheres and those orally inoculated with eggs of *Taenia ~~saginata~~ asiatica*.

Gerbils			Inoculation		Metacestode recovery	
Group	Number	Weeks post-infection	Inoculation route	Inoculation dose*	Number	Rate (%)
G1	5	9	Oral	12,000	0	0
G2	5	9	Subcutaneous	10,000	122–315	1.2–3.2
G3	4	45–48	Subcutaneous	24,000	30–183	0.1–0.8

* Inoculation of group G1 was done by intact eggs and those of other groups by hatched oncospheres.

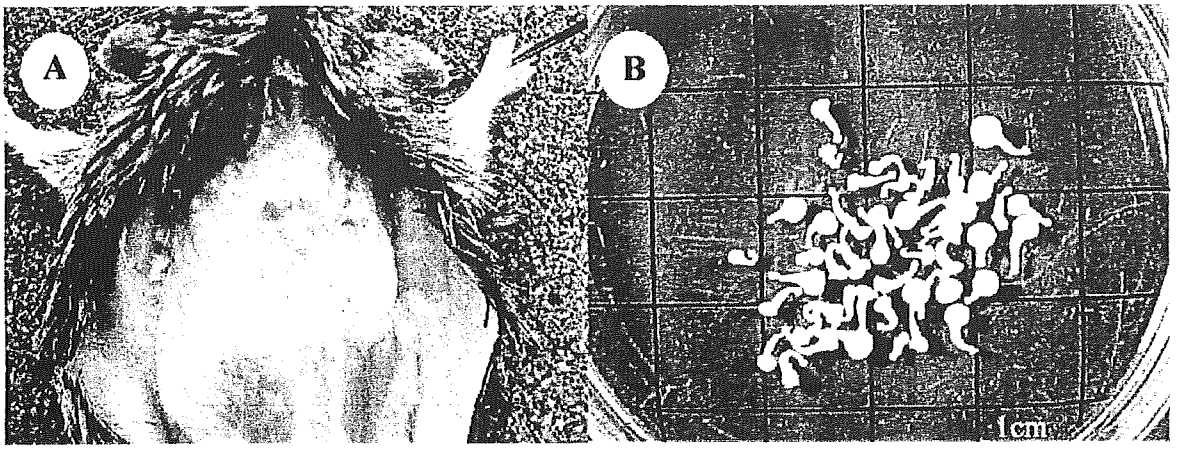

Fig. 1. Cysticerci of *Taenia saginata asiatica* in gerbils at 48 weeks post-infection. (A) Cysticerci seen in the subcutaneous tissue at the inoculated site. (B) The evaginated 48-week-old cysticerci.

The total length, width of the scolex and diameter of the suckers of the 9- and 45-48-week-old cysticerci after evagination are shown in Table 2. The 45-48-week-old cysticerci were significantly larger than the 9-week-old cysticerci in the total length, width of the scolex and diameter of the suckers (Mann-Whitney's U test, $P < 0.0001$).

Microscopic observations revealed that calcareous corpuscles were few in 9 week-old cysticerci but abundant in 45–48 week-old cysticerci. Many small-granules aggregates could be seen at the rostellar region of 9-week-old cysticerci, but only a few aggregates were observed in the 45–48 week-old cysticerci.

Development of adult worms

delete Six adult worms of *T. ✕ asiatica* were recovered from 5 of 11 inoculated gerbils (designated gerbil A, B, C, D and E). All tapeworms were recovered from the anterior part of the small intestine. The length of the tapeworms varied from 3 to 40 mm. Genital primordia were observed in the posterior segments of two worms (worm A1 and C1). Hooklets were observed in the rostellum of two worms (worm A1 and E1) (table 3, fig. 2).

delete Two human volunteers orally inoculated with cysticerci shed strobila or segments of *T. ✕ asiatica* in their faeces. Gravid proglottid excretion of volunteer A was found from 95 to 122 (number of proglottids excreted in a day ranging from 0 to 3) and that of volunteer B was found from 105 to 125 (ranging from 0 to 35 proglottids) DPI.

delete The prepatent periods of *T. ✕ asiatica* infection in the two human volunteers were 95 and 105 days, respectively. The two human volunteers were dewormed using atabrine at 122 (expulsion of three worms) and 125 (expulsion of four worms) DPI, respectively.

Discussion

Mongolian gerbils have been used as a rodent models for the development of human taeniids. Geerts *et al.* (1981/1982) and Wouters *et al.* (1988) reported that the metacestode of *T. ✕ saginata* could be recovered from Mongolian gerbils which were subcutaneously inoculated with hatched oncospheres but not from gerbils intraperitoneally inoculated. Although we did not inoculate the hatched oncopheres of *T. ✕ asiatica* intraperitoneally into gerbils, the present result concurred with the finding that metacestodes could be recovered from gerbils subcutaneously inoculated with hatched oncospheres. This indicates that the subcutaneous tissue of the Mongolian gerbil may present a suitable site for oncosphere development.

delete delete

Morphological observations of 45-week-old cysticerci recovered from gerbils showed a similar development to those from SCID mice; the width of scolex of the cysticerci after evagination were similar to those from SCID mice, but the total worm length and the diameter of the suckers were smaller than those from SCID mice (Chang *et al.*, 2005). In addition, calcareous corpuscles were abundant in the neck region and few small granules aggregated in the rostellum of the cysticerci from the gerbils. These morphological features are considered as important

delete Table 2. Morphometrics of *Taenia saginata asiatica* cysticerci recovered from gerbils subcutaneously inoculated with oncospheres.

Age of cysticerci (weeks)	Number of gerbils examined	Total length (μm) (Mean ± SD)	Width of scolex (μm) (Mean ± SD)	Diameter of sucker (μm) (Mean ± SD)	No. of calcareous corpuscles
9	$n = 34$	2,100 ± 410	530 ± 80	170 ± 40	Few
45–48	$n = 44$	4,860 ± 750	660 ± 60	280 ± 40	Abundant

Table 3. Morphometrics of the six adult worms of *Taenia ~~saginata~~ asiatica* recovered from gerbils.

Gerbils	Tapeworms	Length (mm)	No. of proglottids	Genital Primordia	Hooklets
A	A1	40	154	+	+
	A2	6	0*	–	–
B	B1	3	0		
C	C1	35	147	+	–
D	D1	4	0	–	–
E	E1	5	0	–	+

*Proglottids were undetectable.

criteria for determining the maturity of cysticerci (Chang *et al.*, 2005). Thus, 45–48-week-old cysticerci recovered from gerbils in the present study were well developed and infective to humans.

Adult worms of *T. ~~saginata~~* and *T. solium* had been reportedly recovered from immunosuppressed gerbils after oral inoculation with cysticerci derived from cattle and pigs, respectively (Kamiya *et al.*, 1990; Maravilla *et al.*, 1998). However, the adult stage of *T. solium* could not be recovered from Mongolian gerbils orally inoculated with cysticerci derived from SCID mice (Wang *et al.*, 1999). These reports suggested that the infectivity of cysticerci from alternative intermediate hosts might be much lower than those derived from the natural intermediate hosts. However, the adult stage of *T. ~~asiatica~~* was recovered from gerbils and gravid proglottids were observed in the faeces of human volunteers after oral inoculation with cysticerci derived from gerbils in the present study. Since Wang *et al.* (1999) did not state the maturity of the cysticerci used, the failure to establish experimental infections in this case might be due to immaturity of the cysticerci. Thus, the present study is the first demonstration of the infectivity of human-infecting *Taenia* cysticerci derived from gerbils to gerbils and humans.

Immature proglottids have been observed in the adult stage of *T. solium* and *T. ~~saginata~~* recovered from Mongolian gerbils on day 14 and 23 after oral inoculation with cysticerci (Kamiya *et al.*, 1990; Maravilla *et al.*, 1998). In the present study, immature segments with genital primordia were observed in the posterior segments on day 14 after oral inoculation. This indicates that the development of *T. ~~asiatica~~* in gerbils is as good as those of the other two human taeniid cestodes. Morphological features of *T. ~~asiatica~~* were described completely by Fan *et al.* (1995). Generally, no hooklets could be observed in the rostellum of *T. ~~asiatica~~* (Fan, 1988; Eom & Rim, 1993). However, rudimentary hooklets were observed in two specimens of the tapeworms in the present study. Thus, rudimentary hooklets might still be present in the rostellum of *T. ~~asiatica~~* during the early phase of infection.

In conclusion, the present results show that full mature infective cysticerci can be obtained and maintained for at least 48 weeks in gerbils under laboratory conditions. Moreover, these cysticerci can develop into the adult stage not only in humans but also in rodent alternative definitive hosts, suggesting that this experimental model might be useful for studying *T. ~~asiatica~~* in the laboratory.

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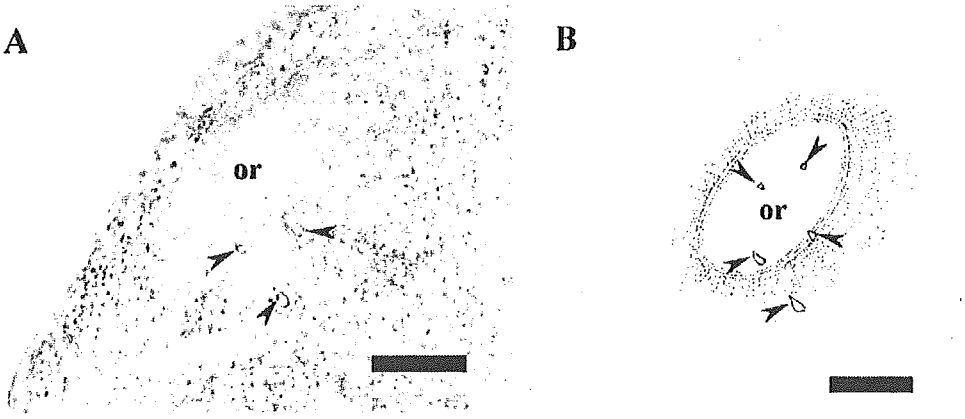

Fig. 2. Rostellar region of *Taenia ~~saginata~~ asiatica* tapeworm from a gerbil. (A) Hooklets (arrowheads). (B) Drawing of the rostellar region. or, ridge of residual rostellar opening.

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Echinococcosis/Hydatidosis

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Summary of general activities related to the disease

1a) Types of test(s) in use/or available, purpose of testing (diagnosis, surveillance, etc.) and approximate number performed for each purpose

Test	Diagnosis			Surveillance		Total
	Dog	Cat	Other	Fox	Raccoon dog	
ELISA (coproantigen detection)	2,117	175	4	410	55	2,821
Postmortem examination	0	0	53	46	1	100
Faecal examination	1,765	175	2	431	55	2,428

2. Production, testing and distribution of diagnostic reagents

A new rapid diagnostic method based on EmA9 monoclonal antibody for the coproantigen detection was tested in the Kazakhstan, Institute of Zoology, Academy of Sciences of the Republic Kazakhstan.

3. Research especially related to development of diagnostic methods and vaccines

Experimental infections of dogs and Mongolian gerbils with *Echinococcus multilocularis* have been conducted for the study of systemic and cellular immunity to *E. multilocularis*.

In 2003, the PCR tests have been used for the diagnosis of echinococcosis from the fox and dog faeces. The parasite DNA has been extracted from the host faeces and amplified by PCR. The PCR products were sequenced and compared with the sequences from other *Echinococcus* and *Taenia* species.

Experimental thermotherapy of alveolar echinococcosis were done using cotton rats.

Activities specifically related to the mandate of OIE Reference Laboratories

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

For the purpose of the standardisation of diagnostic testing the coproantigen detection of the *E. multilocularis* using EmA9-ELISA was performed on fox and dog faeces collected from different regions in Japan and Kazakhstan.

Activities specifically related to the mandate of OIE Reference Laboratories

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

For the purpose of the standardisation of diagnostic testing the coproantigen detection of the *E. multilocularis* using EmA9-ELISA was performed on fox and dog faeces collected from different regions in Japan and Kazakhstan.

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

None.

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

Deworming program in wild (free ranging) red foxes in two areas (Koshimizu and Otaru, Japan) were conducted to reduce the prevalence of *E. multilocularis*. The originally produced baits containing 50 mg praziquantel were repeatedly distributed in both pilot areas. The effect of bait distribution has been evaluated by change in the positive rate of coproantigen and taeniid egg in fox faeces collected in pilot and control area of Koshimizu. In environments of Otaru, the success rate has been evaluated by change in the rates of coproantigen, adult worm prevalence and taeniid egg rates in foxes. In Otaru, the raccoon dog a post-mortem and coproantigen examination was done, also.

A diagnostic service for the domestic dogs and cats in Hokkaido has been continued in the 2003, also. That work has been done with the cooperation of the Hokkaido Small Animal Veterinary Association.

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

Professor M. Kamiya visited Kazakhstan to provide consultant expertise. Dr. H. Sakai dispatched to Uruguay, Dr. Y. Oku dispatched to Uruguay and Mexico.

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

In 2003, several foreign scientist including, two visiting scientists from China, two from Kazakhstan, and one from France came to the laboratory to study about current diagnostic and control methods for the disease.

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

On September 27, 2003, an international symposium entitled "Fight against echinococcosis" was organised in Sapporo, Japan by Dr. Masao Kamiya. In the symposium, the distinguished researchers from China, Kazakhstan, Taiwan and Japan exchanged the update information of echinococcosis.

7. Participation in international scientific collaborative studies

The laboratory has on-going collaborating projects on the control of echinococcosis/hydatidosis with Dr. J. J. Chai and Dr. J. Wei, National Hydatid Research Center in Urunqi (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. Giraudoux 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 HSF.

8. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences)

■ *Presentations at international conferences and meetings*

Kamiya M. (2003). – Introduction – An urgent need for control measures against Echinococcosis. COE International Symposium "Fight against echinococcosis", Sapporo, Japan.

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Echinococcosis/Hydatidosis

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Summary of general activities related to the disease

1. Types of test(s) in use/or available, purpose of testing (diagnosis, surveillance, etc.) and approximate number performed for each purpose

Test	Diagnosis			Surveillance		Total
	Dog	Cat	Others	Fox	Others	
ELISA (coproantigen detection)	1095	272	81	74	300	1822
Post-mortem examination	0	0	0	0	250	250
Faecal examination	1095	272	81	472	300	2220

2. Production, testing and distribution of diagnostic reagents

None.

3. Research especially related to development of diagnostic methods and vaccines

Mucosal immune responses to *Echinococcus multilocularis* for basic research in vaccine development were carried out using the rodent alternative defintive hosts. *In vitro* lymphocyte proliferation assay using rodent model (Mongolian gerbil) and dogs has been performed. Specific antibody detection by ELISA and its reactivity with parasitic antigens by immunoblotting are also examined.

Activities specifically related to the mandate of OIE Reference Laboratories

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

Echinococcus coproantigen detection using EmA9-ELISA was performed on dog faeces collected in a different endemicity area in Kazakhstan. The results indicated that the method could be useful for large-scale surveillance.

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

None.

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

Control measures for the definitive host of *E. multilocularis* were conducted in Koshimizu and Otaru, Hokkaido to reduce the prevalence of *E. multilocularis* in red foxes. The pilot area in Koshimizu is located in north-eastern Hokkaido facing the sea of Okhotsk, covering 200 km^2. The baits containing 50 mg praziquantel were made using fish meat and distributed in the pilot area (40/km^2) from April to November (8 times). The effect of bait distribution has been evaluated by change in the positive rate of coproantigen and taeniid eggs in fox faeces. The data from coproantigen positive cases were analysed using GIS (Geographical Information System).

The control trials in Otaru (located in western Hokkaido facing the sea of Japan) were conducted to reduce the prevalence of *E. multilocularis* in red foxes by the distribution of baits containing 50 mg praziquantel and 160 mg tetracycline. The consumption of the baits by foxes have been evaluated by the detection of tetracycline deposit in teeth of foxes.

A diagnostic service for domestic dogs and cats in Hokkaido was driven by the cooperation of Hokkaido Small Animal Veterinary Association using the coproantigen detection method and fecal examination. Epidemiological data was also collected by questionnaire for the determination of the risk factors for the infection.

Also, almost 600 domestic dogs and more than 100 cats specimens collected from Honshyu island (supposed non-endemic area) were examined.

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

Professor M. Kamiya and Dr. S. Ganzorig visited Kazakhstan and Xinjiang (China) to provide consultant expertise; conduct a field survey, and presentation at the international seminars. Dr. Y. Oku dispatched to Xinjiang of China and Dr. N. Nonaka dispatched to Switzerland, Germany and France through the Japanese Ministry of Education, Science and Culture. Also, Dr. H. Sakai dispatched to Brazil through Japan International Cooperation Agency (JICA).

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

One visiting researcher from National Chung Hsing University in Taichung (ROC) came to the laboratory to study about current diagnostic methods for the disease.

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

None.

7. Participation in international scientific collaborative studies

This laboratory has on-going collaborating projects on the control of echinococcosis/hydatidosis with Dr. J. J. Chai, 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 Science (Kazakhstan), Prof. P. Deplazes, Institute of Parasitology, University of Zurich (Switzerland) and Prof. P. Giraudoux and Prof. D. A. Vuitton, Institute of Environmental Science and Technology, University of Franche-Comté (France). 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).

8. Publication and dissemination of information relevant to the work of OIE (including list of scientific publications, internet publishing activities, presentations at international conferences).

- *Presentations at international conferences and meetings*

Kamiya, M. (2002): Current status of alveolar echinococcosis and its control in Japan. The International seminar on "Echinococcosis: understanding of the epidemiology and transmission dynamics and recomendations for surveillance and control in Central and Northern Asia", Almaty, Kazakhstan.

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Oku, Y., Yamanouchi, T., Matsuda, K., Abella, JAC., Ooi, HK., Ohtsubo, R., Goto, Y., Kamiya, M. (2002): Retarded gastric acid secretion in rats infected with larval *Taenia taeniaeformis*. *Parasitology Research*, **88**, 872-873.

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8. Schistosomiasis Mansoni

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(1) Introduction

In volume 1-5 of "Progress in Medical Parasitology in Japan", published in 1965, there was no chapter on schistosomiasis mansoni. A search in the database of the Japanese Journal of Parasitology, volume 1-42, showed that there were only 17 original scientific papers and 103 meeting abstracts related to schistosomiasis mansoni being published in the journal. In Japan, from the historical perspective, the mainstream research had been focussed on schistosomiasis japonica all along. However, from a global perspective, schistosomiasis mansoni is an important parasitic disease and just like schistosomiasis japonica in Japan, had been the subject of active and intense research.

A search in the aforementioned database also showed that the first conference report related to schistosomiasis mansoni in Japan was presented by Sakumoto *et al.* (1960) in which they described the egg shell structure of *Schistosoma mansoni*. From then on, many researches on schistosomiasis mansoni had been carried out in Japan and publications had also accumulated. This article will focus on the pathology, immune protection and immunodiagnosis of schistosomiasis mansoni. To avoid repetition of information, the biology of this trematode will not be included in this article.

(2) Research on the pathology of schistosomiasis mansoni

Compared with schistosomiasis japonica, not much research had been done on the pathology of schistosomiasis mansoni in Japan. The major pathological lesion of schistosomiasis mansoni is the egg granuloma complex of the worm and its cause was proposed by Tanabe *et al.* (1983) to be due to increased activity of β-glucuronidase. This enzyme was observed to increase significantly in the spleen and serum following infection. Tanabe and colleagues worked further along this line of research and applied the kinetics of laminin and beta-subunit prolyl 4-hydroxylase as parameters in the diagnosis of hepatic fibrosis in schistosomiasis mansoni patients in Brazil (Tanabe *et al.*, 1989b). Furthermore, they also observed that when mice were either infected with *S. mansoni* or intraperitoneally transplanted with egg granuloma, their blood urea level as well as their hepatic ornithine carbamoyl transferase and carbamoyl phosphate synthetase activities were found to be suppressed. They suggested that the egg granuloma might be secreting a factor that led to suppression of the enzyme activities (Tanabe *et al.*, 1989a). In addition, the concentration of L-proline released from the liver was observed to be highest at 7 weeks postinfection when the host response to the schistosome egg granuloma was greatest and the amino acid continued to be released after 8-9 weeks postinfection (Tanabe *et al.*, 1991). Thus, from the results of their biochemical approach in an attempt to elucidate the mechanism of the schistosome egg granuloma formation, they had contributed to clarifying the mecha-

nism of the pathogenesis of the disease. From similar perspective, it also became clear that extracellular matrix molecules such as fibronectin and heparan sulfate proteoglycan were found abundantly around the schistosome egg at 11 weeks postinfection. These molecules, together with the activated macrophage, were deeply associated with the inflammatory response of the egg granuloma (Nishimura *et al.*, 1985). Moreover, in *S. mansoni* infected mice administered with vitamin D3, the activity of the angiotensin converting enzyme (ACE) in the hepatic egg granuloma was seen to increase and this phenomenon had been proposed to be the same as sarcoidosis. The vitamin D3 produced by macrophage was thought not only to be involved in the metabolism of Ca^{2+} but also enhanced the production of ACE by macrophage (Nishimura *et al.*, 1991). IL-2 had been implicated in the formation of schistosome egg granuloma (Yamashita and Boros, 1990) and that IL-2 production was controlled by IL-4 (Yamashita and Boros, 1992). In addition, it was also thought that the egg granuloma formation was regulated by the immune response because of the presence of cross-reactive idiotypes anti-soluble egg antigen antibodies in different animal hosts. The antibodies were reportedly found to be useful as a probe in further research along this line of thought (Amano *et al.*, 1996).

As stated above, the mechanism of egg granuloma formation had been studied mostly from the viewpoint of pathogenesis. There were also other interesting reports relating to schistosomiasis mansoni. Fujiwara *et al.* (1988) observed the development of severe glomerulonephritis in chronically *S. mansoni* infected BxSB strain of mice. Furthermore, Amano *et al.* (1990) reported a decrease in pregnancy rate, parturition and live birth among *S. mansoni* infected mice. Similar effect could be produced by repeated administration of antibodies to schistosome egg antigen. Thus, this novel observation must be taken into consideration when evaluating the effects of schistosomiasis mansoni infection on the inhabitants in the endemic areas.

In addition, there were also reports on the mechanism of cerebral schistosomiasis mansoni (Kamiya *et al.*,1994) and hepatic cancer associated with schistosomiasis mansoni in chimpanzee (Abe *et al.*, 1993).

(3) Studies on immune protection against *S. mansoni*

Schistosome uses a variety of different "strategy" to evade the attack by the host immune response. The mechanisms of these immune evasion were thought to include mimicry of host antigen such as the blood group determinant, turnover of the worm surface antigen and the interference of the host immune system by the schistosome. In addition, Tanaka *et al.* (1989) and Iwamura *et al.* (1991) reported that *S. mansoni* were able to incorporate the DNA of the host retrovirus genome, and subsequently expressed the retrovirus associated antigen on its surface. This resulted in deceiving the host immune elements from being able to recognize the worm as being "non-self". This has been suggested to be a new mechanism in immune evasion employed by the schistosome. Detailed reviews on immune evasion by schistosome had been published by Smithers and Doenhoff (1982), McLaren (1980; 1984), Damian (1984) and Yoshimura (1980). However, once an animal had been infected with *S. mansoni*, there will always be some form of immune protection expressed by the host against subsequent infection. With 250 million people estimated to be infected with schistosomiasis mansoni worldwide, study on the immune protection against the disease had been deemed a priority.