

SHORT COMMUNICATION

Intestinal helminths of dogs in northern Japan

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Humans can become infected by a number of canine intestinal helminths, some of which are considered potential public health problems worldwide. However, intestinal worms of dogs currently receive less attention than their protozoan counterparts. This may partly be due to the decline in endemicity reportedly occurring in developed countries where dogs are likely to receive more attention and anthelmintic treatment from their owners. To illustrate the importance of veterinary care, a longitudinal study in Japan revealed that progress in prophylactic drug administration against dirofilariasis has also decreased the prevalence of several intestinal nematodes in dogs (Asano and others 2004). The low levels of prevalence among canine hosts translate into a reduction in potential zoonotic risk for humans in a given area. However, endemicity has been found to vary markedly from one region to another and is also influenced by aspects of the survey protocols, such as subject choice and the diagnostic techniques that are employed (Robertson and others 2000).

This short communication is part of an ongoing study on the zoonotic helminths of Japan and describes a coprological survey of dogs in Aomori, the northernmost prefecture on the

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 anthelmintics 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 $\mu\text{g}/\text{kg}$ 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.

ACKNOWLEDGEMENTS

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

Helminth species	Hunting dogs		Companion dogs	
	Number (%) of positives	95 per cent CI of positives	Number (%) of positives	95 per cent CI of positives
<i>Toxocara canis</i>	0 (0)	0-2.2	4 (4.7)	1.3-11.5
<i>Trichuris vulpis</i>	52 (38.8)	31.0-47.3	14 (16.3)	10.0-25.5
<i>Dipylidium caninum</i>	0 (0)	0-2.2	2 (2.3)	0.3-8.2
<i>Taenia</i> sp.	1 (0.8)	0.02-4.1	0 (0)	0-3.4
<i>Metagonimus</i> 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	Negative 1	Total 56
	Negative 10	154	164
Total	65	155	220

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.

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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. x 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. x 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. x 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. x 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. x 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. x saginata* (African isolate) after oral inoculation with cysticerci

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Taenia

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 metacystodes

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) (Lightowers *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 from 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 metacystodes

Metacystodes 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 metacystodes from gerbils subcutaneously inoculated with oncospheres and those orally inoculated with eggs of *Taenia saginata asiatica*.

Group	Gerbils		Inoculation		Metacystode recovery	
	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.

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

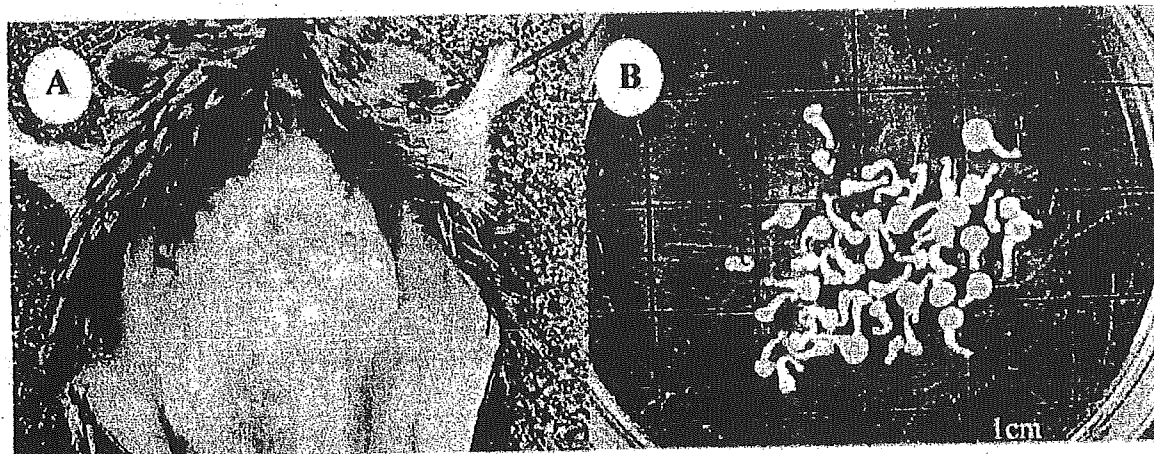


Fig. 1. Cysticerci of *Taenia saginata asiatica* in gerbils at 48 weeks post-infection. (A) Cysticerci seen in the subcutaneous tissue at the inoculated site. (B) The evaginated 48-week-old cysticerci.

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

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delete Two human volunteers orally inoculated with cysticerci shed strobila or segments of *T. X 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. The prepatent periods of *T. X 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. Ceerts *et al.* (1981/1982) and Wouters *et al.* (1988) reported that the metacestode of *T. X 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 oncospheres of *T. X 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.

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

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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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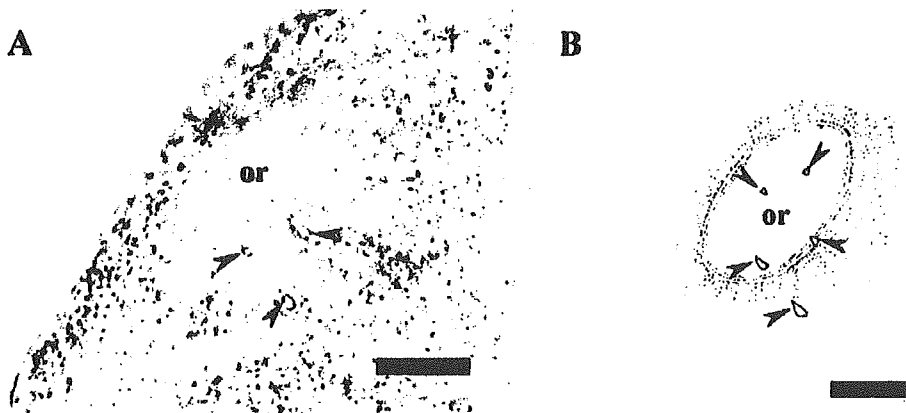


Fig. 2. Rostellar region of *Taenia saginata asiatica* tapeworm from a gerbil. (A) Hooklets (arrowheads). (B) Drawing of the rostellar region. or, ridge of residual rostellar opening.

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

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くつちゃん

広報
2006
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特集「脱・エキノコックス」へ

君は輝いていたートリノ五輪総集編



ニュースあらかると
スーパーバンプス
ニセコカップ
地域イントラ訴訟判決
原告の請求、棄却



寄生虫駆除薬入りのえさ

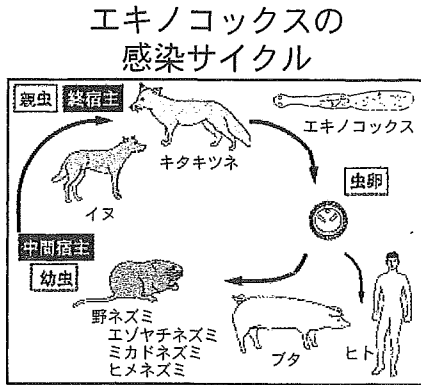
特集

脱「エキノコックス」へ

北海道の風土病ともいわれている「エキノコックス症」。主にキツネに寄生するエキノコックスの卵が何らかの形で私たちの口に入ると、肝臓に幼虫が寄生して障害を起こし、命にかかわる病気ともなります。その元凶であるエキノコックスをなくそうという試みがここくっちゃん、町内の民間団体と大学研究者らによって本格的に始まろうとしています。3月号ではこの計画にかかわる人たちの声を紹介しながら、今後行われる取り組みの内容をお伝えします。

「虫下し」による除去

しっかりとした「縄張り」を持つとされるキツネの習性を生かしたのが、今回の取り組みです。いわば、キツネを味方につける方法です。具体的には、寄生虫の駆除薬(虫下し)を入れた魚肉の塊(ベイト)をキツネに食べさせ、エキノコックスを持たなくした上で、そのキツネによって他地域からのキツネの侵入を阻んでもらおうというものです。



昨年9～11月まで3回の予備調査では、町内全域を対象にキツネのふんを採取し、感染率を調べました。その結果、ふん268標本のうち、感染していたのは24%で、道内平均(平成15年調査)の43%を大きく下回っていることが分かりました。この結果を踏まえ、本調査として今年5～11月まで毎月ベイトを散布し、9月にはふんを採取して駆除の効果を調べる作業を今後5年間継続していく計画です。

「安心・安全」のくつちゃんへ一歩

観光・農業地域のニセコ地域で、エキノコックスがいけないということは安心・安全を与え、もてなしにつながります。



白木美恵子さん
NPO法人ニセコ・羊蹄
再発見の会WAO
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私たちも安心して暮らせます。地域に人材があり、そして大学の協力で環境を整っていたことも今回の調査に結びつきました。私たちは共有財産であるこの地域の資源を再発見し、「私たちの問題は私たちの手で解決しよう」という思いで活動しています。今回の調査は町全体の理解が必要です。これから理解の輪、活動の輪を広げていければと考えています。

酪農学園大の神谷正男教授、北海道大の奥祐三郎助教授らが平成10年から5年間、網走管内清水町で実験的に行った際、40%あったキツネの感染率がほぼゼロになりました。ヨーロッパでも成果を上げているといえます。

くつちゃんではNPO(特定非営利活動団体)法人「ニセコ・羊蹄再発見の会WAO」(事務局・倶知安町、古谷和之理事長)など地域住民が中心となり、大学研究者が協力する初めての体制で注目されています。

人と動物が共生できる環境づくりが可能に



神谷正男さん
(酪農学園大学教授)

(1)なぜ倶知安町で調査をすることになったのか？

研究からこの感染源対策を始めたのが清水町です。リスクを除去する効果が実証されました。倶知安では、感染率が道内平均より低いうちに調査を始めることが必要で、より効果的です。

札幌圏と道南圏を結ぶ地理的な要素もあります。今回、住民主体でこの事業のお手伝いを始めることになりましたが、これはこの地域の皆さんの環境を守ろうとする意識が高かったからです。

(2)この調査の意義は？

動物、この場合は主にキツネを生きたまま調査・監視できることで、従来だと分からなかったエキノコックスの自然界の営みや増え方、弱点などが分かるようになり、キツネの生態も知ることができます。このことで、環境を元に戻す(エキノコックスのいない環境)仕組みが明らかになります。

さらに(倶知安のケースのように)地域の力や資源、人材、文化を引き出し、人と動物が共生できる環境をつくるのが可能になるのでしょうか。

(3)今後期待される効果は？

安心して農業、観光業ができるようになるでしょう。北海道の農産物への評価は高まり、ブランド化がさらに期待できるでしょう。

(4)土壌、人体などへの影響は？

この問題解決型の事業は「キツネの感染調査+虫下し散布+効果判定」から成り立つと考えます。「虫下し」は50年前に開発されて世界中で使用され、現在も犬への虫下し(条虫IIサナダムシ)や人の住血吸虫症に使われていますが、問題はありません。これまで土壌への蓄積、人体などへのマイナスの影響は私の知る範囲では聞いていません。

環境システム学部環境動物学教授。北大獣医学部卒、東大大学院農学研究科修士課程修了。北大獣医学部家畜寄生虫病学講座、同大学院疾病制御学講座教授を経て現職に。専門は環境動物学。生物リスク、特にエキノコックスによる汚染環境の修復に関する研究を国内外で実施。平成17年から酪農学園大がOIE(国際獣疫事務局、本部・パリ)エキノコックス症研究対策の専門機関に指定され、現在そのディレクターを勤めている。