Introduction

P. pneumotropica is a non-motile, non-hemolytic, facultative anaerobic, Gram-negative coccobacillus, which colonizes on the mucosa of the nasopharynx, trachea, lungs, vagina, uterus, urinary bladder, intestines, and other organs of laboratory animals [5, 12, 15, 18, 20–22]. The pathogenicity of this organism in immunocompetent laboratory mice and rats is known to be extremely weak although it was considered strong in the past. However, this organism can occasionally produce clinical disorders in immunodeficient animals [1, 15].

At present, *P. pneumotropica* is considered a routine test item of microbiologic monitoring of laboratory mice and rats in Japan, the US and Europe [16, 19]. However, many researchers around the world recognize that there is a serious problem concerning identification of this organism. Disagreements of test results between testing laboratories have sometimes been observed, and the same situation is also found in Japan. To minimize confusion caused by discrepancies in test results, a working group was established. The aim of the working group was to propose a new identification procedure that is both practical and reliable for this organism. Reference strains and the isolates provided by group members were checked using the same testing methods and reagents as the ICLAS Monitoring Center.

Materials and Methods

Reference strains and isolated strains

ATCC35149 (biotype Jawetz) and CNP160 (biotype Heyl) were used as reference strains. Sixty-nine isolates from 34 mice, 25 rats, 7 hamsters, 2 rabbits, and 1 guinea pig, which were identified or suspected as having *P. pneumotropica* by biochemical characteristics, were collected from 8 laboratories in Japan.

Examinations

1. Morphological examinations

All strains were plated directly onto 5% horse blood agar plates (Climedia; Sanko Junyaku Co., Tokyo), incubated aerobically at 37°C for 48 h, and examined for colony morphology. Gram-staining (Favor G; Nissui Pharmaceutical Co., Tokyo) was also performed for each isolate.

2. Immunological and biochemical examinations

The slide agglutination test was performed as an immunological examination using rabbit antisera of a mouse isolate ATCC35149 and a rat isolate MaR that were gifts from NIH Japan. Two commercially available identification kits (ID test HN 20 rapid; Nissui Pharmaceutical Co., Tokyo and API20NE; Nihon Biomeriux, Tokyo) were used for biochemical examinations.

3. DNA examination

DNA extraction

All strains were propagated in brain heart infusion broth (Difco Laboratories, MI, U.S.A.) supplemented with 5% horse serum for preparation of DNA extract. Three methods (E.N.Z.A. Bacterial DNA Kit; Omega Bio-tek, GA, U.S.A., MagExtracter Genome; TOYOBO Co., Osaka, and SDS methods) were utilized for bacterial DNA extraction. The methods were selected based on the harvested DNA volume of each strain.

PCR test

Two primer sets reported by Wang *et al.* (Wang's PCR) and Nozu *et al.* (CIEA PCR) were used for PCR tests [17, 20]. Samples(4 μl) containing 60 ng of genomic template DNA were added to 16 μl of PCR mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 mM dNTP mixture, 0.4 μM sense primer,0.4 μM antisense primer, and 5 units of Taq polymerase (Takara Shuzo Co., Kyoto). Each mixture was placed in a programmable thermal cycler (Gene Amp PCR System Model 9700; PE Biosystems, Calif, U.S.A.). PCR was performed according to Wang's or Nozu's procedure. The PCR amplification products were analyzed by gel electrophoresis in 4% agarose.

16S rDNA sequence analysis

Analysis of 16S rDNA sequencing was performed for 35 isolates from 17 mice, 13 rats, 3 hamsters, 1 rabbit, and 1 guinea pig. The five primers used for 16S rDNA sequencing and PCR are listed in Table 1. The templates were amplified by PCR using primers PAS-1 and PAS-2C. Each PCR product was sequenced by an automated sequencer (310 Genetic Analyzer; Applied Biosystems Japan, Tokyo). The data were analyzed using a software package, DNASIS (Hitachi Software Engineering, Tokyo), and compared with the sequences of *P. pneumotropica* biotype Jawetz (M75083) and Heyl (AF012090), *P. dagmatis* (M75051), *P. aerogenes* (M75048), *P. multocida* subsp *multocida* (M35018) and

Table 1. Primers for *P. pneumotropica* complete 16S rDNA sequencing

Primer[a)]	Sequence (5'–3')	Position[b)]
PPSEQ-1	ACTCCTACGGGAGGCAGCAG	331–351
PPSEQ-2C	CACATGAGCGTCAGTACAT	738–756
PAS-1	ATTGAAGAGTTTAGTCATGG	2–21
PAS-2C	TGAATCATACCGTGGTAAAC	1452–1471
16S-1	AACAGGATTAGATACCC	773–788

a) PAS-1 and PAS-2C were used for DNA amplification and sequencing. b) Positions within the *P. pneumotropica* 16S rDNA sequence (M75083) corresponding to the 5' and 3' ends of each primer.

Actinobacillus muris (AF024526) in the Gen Bank database for homology analysis [4, 6, 7, 17].

CLUSTAL W version 1.6 was used for multiple alignments of the data and the sequence of *P. pneumotropica* biotype Jawetz (M75083) and Heyl (AF012090), *P. dagmatis* (M75051) and *A. muris* (AF024526).

Results

Reference strains

The results for the two reference strains are shown in Table 2. Both strains showed smooth colonies 3–5 mm in diameter on blood agar. The colony color of biotype Jawetz was grayish-white and that of biotype Heyl was yellow. They were nearly identical morphologically, Gram-negative short rods or coccobacilli. Both of them were positive in the slide agglutination test, and identified as *P. pneumotropica* by the ID test and API20NE kits. Both strains were detected by the CIEA PCR test. However, biotype Heyl was not detected by Wang's PCR test. The 16S rDNA sequences of biotype Jawetz and Heyl determined in this study were more than 99% identical with those in the Gene Bank database. The 16S rDNA homology between the two biotype strains was under 96%.

Isolates

All 69 isolates showed nearly identical smooth colonies 3–5 mm in diameter on blood agar. Colors were grayish-white or yellow, the same as for the reference strains. Most of them had Gram-negative short rods or coccobacilli, and only three isolates from rat and hamster showed slightly longer shapes.

Thirty-three out of 34 mouse isolates, 11 out of 25 rat isolates, 5 out of 7 hamster isolates, all of 2 rabbit isolates and 1 guinea pig isolate showed agglutination with ATCC35149 antiserum. However, none of the 14 rat isolates agglutinated with ATCC35149 antiserum, but they showed agglutination with MaR antiserum. Sixty-eight of 69 isolates tested with one antiserum were positive by the agglutination test with either antiserum of ATCC35149 and MaR (Table 2). The exception was a mouse isolate.

The ID test identified 61 isolates (30 mouse, 21 rat, 7 hamster, 2 rabbit and 1 guinea pig isolates) as *P. pneumotropica*, but API20 NE identified only 39 isolates (11 mouse, 18 rat, 7 hamster, 2 rabbit and 1 guinea pig isolates) as *P. pneumotropica* (Table 3).

CIEA PCR detected 63 out of 69 isolates and all of 6 isolates which were not detected by CIEA PCR were of rat origin. Wang PCR detected only 27 isolates which consisted of 18 mouse isolates, 7 rat isolates and 2 hamster isolates (Table 3).

Thirty-four out of 35 isolates (16 mice, 13 rats, 3 hamsters, 1 guinea pig and 1 rabbit) showed over 96% homology with the *P. pneumotropica* Jawetz or Heyl 16S rDNA sequence on the database. One isolate classified as *P. pneumotropica* by the agglutination test, API20NE test and CIEA PCR showed 98% homology with *Actinobacillus muris* (Table 2).

The 16S rDNA sequences of 35 isolates, *P. pneumotropica* biotypes Jawetz and Heyl, *P. dagmatis* and *A. muris* could not be classified by cluster analysis of multiple alignments as shown in Fig. 1.

Discussion

First, we will discuss the cause of the discrepancies in identification of *P. pneumotropica* in each testing laboratory. This organism has two biotypes, Jawetz and Heyl. The sequence of 16S rDNA of these biotypes showed that they are different species. *P. pneumotropica* formerly had the Henriksen biotype, which is now classified as a different species, *Pasteurella dagmatis* [14]. Sixty-nine isolates were divided into 10 groups according to biochemical and PCR tests as shown in Table 2. This result shows that *P. pneumotropica* has characteristics too wide for one species. The same conclusion was also drawn from the sequence data of 35 isolates that could not be clearly

Table 2. Characteristics of reference strains and grouping of isolates depending on their characteristics

Group	Origin	Agg[b]		Biochemical test		PCR tests		Sequence[e]	
		ATCC	MaR	ID test	API20NE	CIEA	Wang	Jawetz	Heyl
Reference strains									
P. pneumotropica									
Jawetz (ATCC35149)		○	○	○[c]	○[d]	○	○	99%	–
Heyl (CNP 160)		○	○	○	○	○	×	–	99%
Isolates									
	Mouse	○		○	○	○	○	99%	–
	Mouse	○	○	○	○	○	○	98%	–
A	Rat	○		○	○	○	○	97%	–
	Hamster	×	○	○	○	○	○	97%	–
	Rat (4)[a]	×	○	○	○	○	○		
	Hamster	×	○	○	○	○	○		
	Mouse (3)	○		○	×	○	○	99%	–
	Rat	○		○	×	○	○	99%	–
B	Mouse	○		○	×	○	○	98%	–
	Mouse (6)	○		○	×	○	○		
	Mouse (3)	○	○	○	×	○	○		
	Mouse	○		○	○	○	×	98%	–
	Rat	×	○	○	○	○	×	96%	–
	Hamster	○		○	○	○	×	–	99%
	Rat (2)	○		○	○	○	×	–	98%
	Rat	×	○	○	○	○	×	–	98%
	Mouse	○	○	○	○	○	×	–	97%
	Hamster	○		○	○	○	×	–	97%
C	Rabbit	○		○	○	○	×	–	97%
	Mouse	○		○	○	○	×	–	96%
	Guinea pig	○		○	○	○	×	–	96%
	Mouse (5)	○		○	○	○	×		
	Rat (2)	×	○	○	○	○	×		
	Rat (3)	○		○	○	○	×		
	Hamster (3)	○		○	○	○	×		
	Rabbit	○		○	○	○	×		
	Mouse (5)	○		○	×	○	×	98%	–
	Mouse	×		○	×	○	×	96%	–
D	Rat	○		○	×	○	×	96%	–
	Mouse	○		○	×	○	×		
	Rat	×	○	○	×	○	×		
E	Rat	○		○	×	×	×	96%	–
	Rat	○		○	×	×	×		
	Mouse	○		×	×	○	○	99%	–
F	Rat	×	○	×	×	○	○	96%	–
	Mouse (2)	○		×	×	○	○		
G	Mouse	○		×	○	○	×	*Actinobacillus muris*[f]	
	Rat	×	○	×	○	○	×	96%	–
H	Rat	×	○	○	○	×	×	97%	–
	Rat	○		○	○	×	×	96%	–
I	Rat	×	○	×	○	×	×	96%	–
J	Rat	×	○	×	×	×	×		

[a] Number of strains showing same characteristics.[b] Slide agglutination test. ATCC is antiserum of ATCC35149. MaR is antiserum of MaR. ○: positive, ×: negative; Blank: not tested. [c] ○: %id 100, ×: %id 0. [d] ○: %id 96–99.9, ×: %id 0–37. [e] 16S rDNA Sequence data were compared with six bacteria data: *P. pneumotropica* biotype Jawetz and Heyl, *P. dagmatis, P. aerogenes, P. multocida* subsp *multocida, A. muris*. –: under 96% homology. Blank: not tested. [f] This isolate showed 98% homology with *A. muris*.

Table 3. Comparison of two biochemical kits and two PCR tests for detection of *P. pneumotropica*

		Isolates from					Total
		Mouse	Rat	Hamster	Rabbit	Guinea pig	
Biochemical test	ID test	30/34[a)]	21/25	7/7	2/2	1/1	61/69
	API 20NE	11/34	18/25	7/7	2/2	1/1	39/69
PCR tests	CIEA PCR	34/34	19/25	7/7	2/2	1/1	63/69
	Wang PCR	18/34	7/25	2/7	0/2	0/1	27/69

[a)] Number positive / number tested.

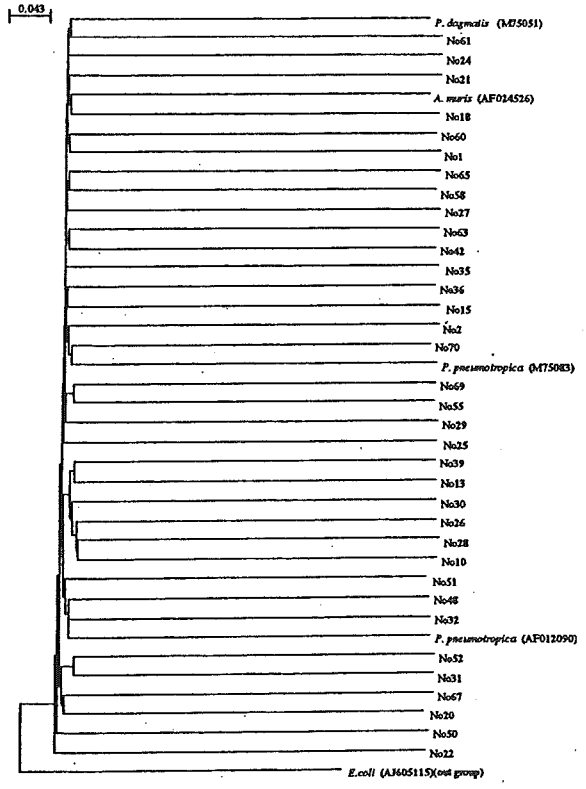


Fig. 1. Phylogenetic tree of tested isolates, *Pasteurella pneumotropica, Pasteurella dagmatis* and *Actinobacillus muris*. The scale bar represents a 0.043 difference in nucleotide sequence, as determined by measuring the lengths of horizontal lines connecting any two species. *P. pneumotropica* (M75083) is biotype Jawetz, *P. pneumotropica* (AF012090) is biotype Heyl.

separated into several clusters (Fig. 1). The taxonomical position of this organism is obscure. Genus Pasteurella is closely related to genus Actinobacillus as shown in Bergey's Manual 9th edition [9]. It may be very difficult to propose a simple identification procedure using a biochemical test or PCR test for this organism.

Second, from the standpoint of practical clinical microbiologists of laboratory animals, we would like to propose an identification procedure by which characters of the two biotypes are met and discrepancies with past identification results are minimized. Positive results for the two biochemical test kits were 61/69 (88%) and 39/69 (57%) in the ID and API 20 NE tests, respectively. The former is a kit for bacteria such as genus Haemophilus and Neisseria, and the latter is a kit for Gram-negative bacteria except enterobacteriaceae. The differences in target bacteria and the nutritional conditions in the two kits may cause the different results. The CIEA PCR test showed a wider detection range than the Wang PCR test, and the results for *P. pneumotropica* identification were basically similar to those of the ID test.

From these results, we recommend the following identification procedure for *P. pneumotropica*: colony observation on 5% horse blood agar plates, Gram-staining, the slide agglutination test with ATCC35149 antiserum for mouse, guinea pig, rabbit, and hamster isolates, and with MaR antiserum for rat isolates and a biochemical test using the ID test (Table 4). With this protocol 60 out of 69 isolates were identified as *P. pneumotropica*. However, one strain in 9 isolates not identified as *P. pneumotropica* showed 99% homology with the 16S rDNA sequence of biotype Jawetz.

There is no standardized identification procedure of *P. pneumotropica* in microbiological monitoring of laboratory animals in the U.S and Europe. Furthermore, the Federation of European Laboratory Animal Science Associations (FELASA) working group described that commercial identification kits do not identify *P. pneumotropica* properly [16]. Therefore,

Table 4. Proposed identification procedure of *P. pneumotropica*

Items	Results
Colony observation on 5% horse blood agar culuture for 48hr at 37°C	3–5 mm in diameter, smooth, low convex, non-hemolytic Jawetz: grayish-white, Heyl: yellow
Gram-stain	Gram-negative short rods or coccobacilli
Slide agglutination test with ATCC35149 anitisera for mouse, guinea pig and, rabbit and hamster isolates, MaR antisera for rat isolates	Agglutinated
Biochemical test with ID test HN 20 rapid	Identification as *P. pneumotropica* with % id 100

we think that our identification procedure of *P. pneumotropica* is useful for standardization between testing laboratories.

P. pneumotropica is considered an opportunistic pathogen in immunocompetent mice and it is recognized only as a factor worsening symptoms by co-infection with other murine pathogens, such as Sendai virus and *Mycoplasma pulmonis* [10]. The ICLAS Monitoring Center checked a total of 17,201 laboratory mice and rats in 2002, and found 508 mice contaminated with *P. pneumotropica*. A gross abnormality, a lung abscess, was found in only one of these contaminated animals and *P. pneumotropica* was isolated from the lung lesion. These results support the above discussion concerning the pathogenicity of *P. pneumotropica*. However, it was reported that *P. pneumotropica* caused orbital abscesses in immunodeficient mice [1,13], dual infection with the organism and *Pneumocystis carinii* caused severe symptoms in B cell deficient mice [11], and that mice with non-functional Toll-like receptor 4, such as C3H/HeJ and C57BL10/ScN, are susceptible to *P. pneumotropica* [2, 3, 8]. These results suggest that *P. pneumotropica* should be eliminated from immunodeficient mice and rats as well as mutant mice with non-functional Toll-like receptor 4.

The ICLAS Monitoring Center has proposed the following categorization of murine pathogens. Category A: Zoonotic and human pathogens carried by mice and/or rats. Category B: Fatal pathogens of mice and/or rats which can cause symptomatic disease and occasional deaths. Category C: Potential pathogens of mice and/or rats which usually cause asymptomatic infections accompanied by alterations of physiological functions. Category D: Opportunistic pathogens. Category E: Microbes as indicators of hygienic status of rearing environments. The ICLAS Monitoring Center has classified *P. pneumotropica* as category C in the categorization of murine pathogens. Based on its pathogenicity and the nature of the species with a wide diversity of characteristics, we propose changing the category of *P. pneumotropica* from C to D, so that the organism is eliminated from routine test items for microbiologic monitoring of immunocompetent mice and rats.

Acknowledgments

This study was supported by a grant (No. 14208097) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors thank Dr. Ken-ichi Yagami of Tsukuba University for providing some isolate strains.

References

1. Artwohi, J.E., Frynn, J.C., Bunte, R.M., and Angen,O. 2000. Outbreak of *Pasteurella pneumotropica* in a closed colony of STOCK-Cd28^{tm1Mak} mice. *Contemp.Topics.* 39: 39–41.
2. Branger, J., Knapp, S., Weijer, S., Leemans, J.C., Pater, J.M., Speelman, P., Florquin, S., and van der Poll, Tom. 2004. Role of Toll-like receptor 4 in Gram-positive and Gram-negative pneumonia in mice. *Infect. Immun.* 72: 788–794.
3. Chapes, S. K., Mosier, D.A., Wright, A.D., and Hart, M.L. 2001. MHC II, Tlr4 and Nramp 1 genes control host pulmonary resistance against the opportunistic bacterium *Pasteurella pneumotropica. J. Leukoc. Biol.* 69: 381–386.
4. Dewhirst, F.E., Paster, B.J., Olsen, I., and Fraster, G.J. 1992. Phylogeny of 54 representative strains of species in the family Pasteurellaceae as determined by comparison of 16S rRNA sequences. *J. Bacteriol.* 174: 2002–2013.
5. Goelz, M.F., Thigpen, J.E., Mahler, J., Rogers, W.P.,

Locklear, J., Weigler, B.J., and Forsythe, D.B. 1996. Efficacy of various therapeutic regimens in eliminating *Pasteurella pneumotropica* from the mouse. *Lab. Anim. Sci.* 46: 280–285.
6. Goto, K. and Itoh, T. 1994. Detection of Bacillus piliformis by specific amplification of ribosomal sequences. *Exp. Anim.* 43: 389–394.
7. Goto, K., Nozu, R., Takakura, A., Matsushita, S., and Itoh, T. 1995. Detection of cilia-associated respiratory bacillus in experimentally and naturally infected mice and rats by the polymerase chain reaction. *Exp. Anim.* 44: 333–336.
8. Hart, M.L., Mosier, D.A., and Chapes, S.K. 2003. Toll-like receptor 4-positive macrophages protect mice from *Pasteurella pneumotropica*-induced pneumonia. *Infect. Immun.* 71: 663–670.
9. Holt, J.G. 1994. Bergey's manual of determinative bacteriology, 9th ed., Williams and Wilkins, Baltimore.
10. Jakab, G.J. and Dick, E.C. 1973. Synergistic effect in viral-bacterial infection: combined infection of the murine respiratory tract with Sendai virus and *Pasteurella pneumotropica*. *Infect. Immun.* 8: 762–768.
11. Macy, J.D. Jr., Eleanor C.W., Susan R.C., Mark J., and David g. B. 2000. Dual infection with *Pneumocystis carinii* and *Pasteurella pneumotropica* in B cell-deficient mice: Diagnosis and Therapy. *Lab. Anim. Sci.* 50: 49–55.
12. Mannnig, P.J., DeLong, D., Gunther, R., and Swanson, D. 1991. An enzyme-linked immunosorbent assay for detection of chronic subclinical *Pasteurella pneumotropica*. *Lab. Anim. Sci.* 41: 162–165.
13. Moore, G.J. and Aldred, P. 1978. Treatment of *Pasteurella pneumotropica* abscesses in nude mice (*nu/nu*). *Lab. Anim.* 12: 227–228.
14. Mutters, R., IHM, P., Pohl, S., Frederiksen, W., and Mannhem, W. 1985. Reclassification of the genus Pasteurella Trevisan 1887 on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis*, *Pasteurella canis*, *Pasteurella stomatis*, and *Pasteurella langaa*. *J. Syst. Bacteriol.* 35: 309–322.
15. Needham, J.R. and Cooper, J.E. 1975. An eye infection in laboratory mice associated with *Pasteurella pneumotropica*. *Lab. Anim.* 9: 197–200.
16. Nicklas, W., Baneux, P., Boot, R., Decelle, T., Deenny, A.A., Fumanelli, M., and Illgen-Wilcke, B. 2002. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Lab. Anim.* 36: 20–42.
17. Nozu, R., Goto, K., Ohashi, H., Takakura, A., and Itoh, T. 1999. Evaluation of PCR as a means of identification of *Pasteurella pneumotropica*. *Exp. Anim.* 48: 51–54.
18. Saito, M., Kojima, K., Sano, J., Nakayama, K., and Nakagawa, M. 1981. Carrier state of *Pasteurella pneumotropica* in mice and rats. *Exp. Anim.* 30: 313–316.
19. Waggie, K., Kagiyama, N., Allen, M.A., and Nomura, T. 1994. Manual of microbiologic monitoring of laboratory animals, 2nd ed., NIH Publication, Maryland.
20. Wang, R.-F., Campbell, W., Cao, W.-W., Summage, C., Steele, R.S., and Cerniglia, C.E. 1996. Detection of *Pasteurella pneumotropica* in laboratory mice and rats by polymerase chain reaction. *Lab. Anim. Sci.* 46: 81–85.
21. Ward, G.E., Moffat, R., and Olfert, E. 1978. Abortion in mice associated *Pasteurella pneumotropica*. *J.Clin. Microbiol.* 8: 177–180.
22. Weigler, B.J., Thigpen, J.E., Goelz, M.F., Babineau, C.A., and Forsythe, D.B. 1996. Randomly amplified polymorphic DNA polymerase chain reaction assay for molecular epidemiologic investigation of *Pasteurella pneumotropica* in laboratory rodent colonies. *Lab. Anim. Sci.* 46: 386–392.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
Parasitology International 55 (2006) S259 – S261
www.elsevier.com/locate/parint

Mathematical modeling of *Echinococcus multilocularis* transmission

Hirofumi Ishikawa *

Department of Human Ecology, Graduate School of Environmental Science, Okayama University, Tsushimanaka, Okayama 700-8530, Japan

Available online 15 December 2005

Abstract

A mathematical model for the transmission cycle of *Echinococcus multilocularis* would be useful for estimating its prevalence, and the model simulation can be instrumental in designing various control strategies. This review focuses on the epidemiological factors in the *E. multilocularis* transmission cycle and the recent advances of mathematical models for *E. multilocularis* transmission.

Keywords: *Echinococcus multilocularis*; Fox; Mathematical model; Vole

1. Introduction

Echinococcus multilocularis is distributed in central Europe, North America, and northern and central Eurasia [1]. In Japan, human alveolar *Echinococcus* (HAE) caused by *E. multilocularis* has spread throughout the mainland of Hokkaido [2], making it desirable to design effective control strategies against HAE. It is difficult to elucidate the source of infections due to the long incubation period [3]. A mathematical model for the transmission cycle of *E. multilocularis* would be useful for estimating its prevalence, and the model simulation can be instrumental in designing various control strategies. A few models about *E. multilocularis* transmission have been proposed since 1995 [4–6]. This review focuses on the epidemiological factors in the *E. multilocularis* transmission cycle and the recent advances of mathematical models for *E. multilocularis* transmission.

E. multilocularis carries out its transmission cycle in two hosts; the definitive hosts are canines, while the intermediate hosts are mainly rodents and ungulates [1,7–9]. Individuals are infected by the accidental ingestion of parasite eggs. The intermediate hosts are infected by ingesting parasite eggs voided in the feces of infected definitive hosts, while the definitive hosts are infected by preying on the intermediate hosts that have hydatid cysts. A mathematical model which quantitatively describes the transmission of *E. multilocularis* needs to include the following components [5,10]:

1. dynamics of definitive host population,
2. dynamics of intermediate host population,
3. predator–prey relationship between the definitive hosts (canines) and the intermediate hosts (rodents), and
4. longevity of parasite eggs in the environment.

2. Dynamics of definitive hosts

Foxes mainly maintain the transmission cycle of *E. multilocularis*. The major definitive host is the red fox (*Vulpes vulpes*) for most endemic regions, or the arctic fox (*Alopex lagopus*) for the tundra zone of Eurasia and North America [1,7,9,11]. The dynamics of the fox population show marked seasonal variations because foxes are wild animals. Therefore, a quantitative transmission model needs to include a host population dynamic component [5]. In Hokkaido, Japan, the breeding season of red foxes is generally in the early spring (the last third of March – the first third of April) and newborns after weaning, which might be exposed to *E. multilocularis* infection, emerge from their dens 1 month after birth [12]. Generally, for any wild animal, the death rate of juveniles is significantly higher than that of adults. The death rate of juvenile (under 1 year old) red foxes in Hokkaido was estimated to be 2.5 times higher than that of adults [5]. The seasonal population model of red fox density in Hokkaido is shown in Fig. 1. The arctic fox population is

* Tel.: +81 86 251 8826; fax: +81 86 251 8837.
E-mail address: ishikawa@ems.okayama-u.ac.jp.

1383-5769/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2005.11.038

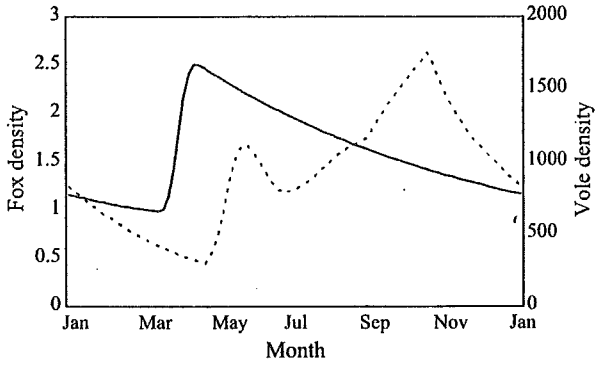


Fig. 1. The seasonal population dynamics models for foxes and voles in Hokkaido. The solid line and the dotted line shows the variations in fox and vole density/km^2, respectively [5].

also influenced by emigration and immigration due to long-distance traveling [11].

3. Dynamics of intermediate hosts

Rodents mainly maintain the transmission cycle of *E. multilocularis* as the intermediate hosts, and the species that are involved in the cycle vary in different endemic regions [1,9]. In Hokkaido, the major intermediate host is the gray-sided vole (*Clethrionomys rufocanus*) [7]. The gray-sided vole breeds in three seasons of the year (all seasons except winter) [13,14]. The survival rate of voles depends on the season and age, with that for the first month of life being lower than that of >1 month [13,14], while the survival rate in winter is higher than that in summer [15]. Besides the season variation, the dynamics of the vole population vary on a large scale annually and have certain geographical characteristics [16]. There is no necessity to consider emigration or immigration in the dynamics of the vole population because of the small size of home ranges [13].

4. Transmission processes of *E. multilocularis*

The definitive host is infected with *E. multilocularis* by preying on rodents which harbor multilocular *Echinococcus* with infectious protoscoleces. Therefore, the prevalence of *E. multilocularis* is affected by the average number (NVF) of voles ingested by a fox each day, which depends on the density of the vole population and on the depth of the snow factors [17, 18], which were introduced into the transmission model [5].

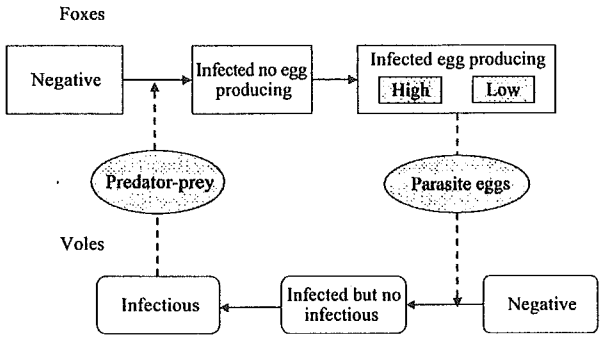


Fig. 2. The basic scheme for the model of the *Echinococcus multilocularis* transmission cycle between foxes (the major definitive host) and voles (the major intermediate host).

The intermediate host is infected by ingesting *E. multilocularis* eggs voided in the feces of infected definitive hosts. The duration of the egg's infectious ability is mainly affected by temperature and humidity. The tenacity of eggs is sensitive to elevated temperature, to very low temperature and to desiccation [19]. The experimental formula for the longevity (d for days) of eggs at temperature (t at °C) was established as $d=\exp[-0.135(t-43.7)]$ [20].

5. Mathematical models of *E. multilocularis* transmission

A deterministic model for the transmission of a parasite essentially describes its transmission cycle as a set of differential equations. Roberts and Aubert [4] constructed a simple deterministic *E. multilocularis* transmission model to evaluate the effect of control by addition of praziquantel in France. Ishikawa et al. [5] proposed a model that took into account the influence of the dynamics of both the definitive and the intermediate host populations and the seasonal effects on the longevity of *E. multilocularis* eggs and NVF to describe the mechanism of seasonal transmission in Hokkaido quantitatively. Hansen et al. [6] tried to develop a stochastic transmission model from the Roberts and Aubert model to devise a hypothesis that would fit well with the prevalence data during the pre- and post-control periods in the northern Germany. In these models [4–6], each host population is broadly divided into three epidemiology classes. Moreover, in the quantitative model shown in Fig. 2 [5], the infected egg-producing class in foxes is subdivided into two subclasses according to whether egg production is abundant or not.

The basic reproductive rate (R_0) is the theoretically maximum number of secondary infections. R_0 was estimated from the Roberts and Aubert model [4] or the

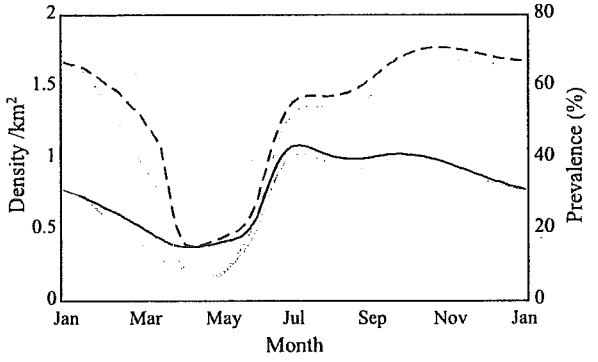


Fig. 3. Seasonal variations in the density/km^2 of foxes infected with *E. multilocularis* (solid line) and the prevalence of *E. multilocularis* in the fox population (broken line). The black and gray lines show the Nemuro and Abashiri situations, respectively [5].

model of Ishikawa et al. excluding seasonal factors [5] as follows:

$$R_0 = \frac{\lambda_f \lambda_v \overline{N_f}}{\delta_v(1+\delta_v\tau_v)(1+\delta_f\tau_f)(1+\delta_f\eta_t)}\eta_t$$

$$R_0 = \frac{s_f \overline{\mathrm{NVF}}\ \lambda_v \overline{N_f}}{\delta_v(1+\delta_v\tau_v)(1+\delta_f\tau_f)(1+\delta_f\eta_h)}\left(\rho\eta_h + \frac{\eta_l}{1+\delta_f\eta_l}\right)$$

The symbols λ_a, δ_a, τ_a, s_f, $\overline{\mathrm{NVF}}$, $\overline{N_f}$, η_t, η_h, η_l, and ρ represent the infectious contact rate ($a=f$, v), the death rate ($a=f$, v), the period of no egg production ($a=f$) or for acquiring infectious protoscoleces ($a=v$) expressed as days after infection, the conditional probability of maturity of worms (f), the average NVF, the average of density (f), the durations of total, high and low egg production, and the multiplicative factor caused by high egg production, with the suffixes f and v standing for fox and vole, respectively.

The seasonal variations of the prevalence and the density of infected foxes were simulated for the two endemic regions in Hokkaido, Japan: Nemuro and Abashiri, where the average prevalence rates (1995–2000) were 53% and 48%, respectively. There is a great difference between the two regions in terms of snowfall. Comparison of two regions using the model simulation shows that the winter density of the infected foxes is maintained at a certain level in Nemuro, while it falls to a low level in Abashiri, which leads to the difference of the winter prevalence between Nemuro and Abashiri (Fig. 3) [5].

6. Risk of HAE

The risk to the human population of being infected with HAE has a close relation to the amount of *E. multilocularis* eggs that maintain infectious ability in the environment. A comparative study on the risk of HAE between Sapporo, the capital of Hokkaido, and Nemuro was carried out by simulating the seasonal fluctuation in *E. multilocularis* egg dispersion in the environment based on the model [5].

7. Prospects

Recent advances in mathematical modeling of *E. multilocularis* transmission were summarized here. There has been steady progress in mathematical modeling of *E. multilocularis* transmission into consideration taking seasonal factors. Further follow-up studies based on field data will be needed to precisely estimate the effects of control strategies against *E. multilocularis* using model simulations.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant No. 16540105) and by grants from the Ministry of Health, Labour and Welfare, Japan for "The control of emerging and reemerging diseases in Japan" (Principal investigator: Prof. M. Kamiya).

References

[1] Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS. WHO/OIE Manual on Echinococcosis in Human and Animals: A Public Health Problem of Global Concern. Paris: World Organisation for Animal Health; 2001.

[2] Annual reports of the council for alveolar *Echinococcus* in Hokkaido 1984–1994. Department of Health and Welfare, Hokkaido Government, Sapporo, 1995.

[3] Doi R, Nakao M, Nihei N, Kutsumi H. Epidemiology of alveolar hydatid disease (AHD) and estimation of infected period of AHD in Rebun Island, Hokkaido. Jpn J Public Health 2000;47:145–52 [in Japanese with English abstract].

[4] Roberts MG, Aubert MFA. A model for the control of *Echinococcus multilocularis* in France. Vet Parasitol 1995;56:67–74.

[5] Ishikawa H, Ohga Y, Doi R. A model for the transmission of *Echinococcus multilocularis* in Hokkaido, Japan. Parasitol Res 2003;91: 444–51.

[6] Hansen F, Tackmann K, Jeltsch F, Wissel C, Thulke H-H. Controlling *Echinococcus multilocularis* – ecological implications of field trails. Prev Vet Med 2003;60:91–105.

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

[8] Petavy AF, Deblock S, Walbaum S. Life cycles of *Echinococcus multilocularis* in relation to human infection. J Parasitol 1991;77:133–7.

[9] Rausch RL. Life cycle patterns and geographic distribution of *Echinococcus* species. In: Thompson RCA, Lymbery AJ, editors. *Echinococcus* and hydatid diseases. Wallingford Oxon: CAB International, 1995. p. 88–134.

[10] Saitoh T, Takahashi K. The role of vole populations in prevalence of the parasite (*Echinococcus multilocularis*) in foxes. Res Popul Ecol 1998;40:97–105.

[11] Fay FH, Rausch RL. Dynamics of the arctic fox population on St. Lawrence Island, Bering Sea. Arctic 1992;45:393–7.

[12] Uraguchi K, Takahashi K. Ecology of the red fox in Hokkaido. In: Tsuduki T, editor. Alveolar *Echinococcus* in Hokkaido. Sapporo: Hokkaido Institute of Public Health, 1999. p. 39–48 (in Japanese).

[13] Ota K. Study on wild murid rodents in Hokkaido. Sapporo: Hokkaido University Press; 1984 (in Japanese).

[14] Yoccoz NG, Nakata K, Stenseth NC, Saitoh T. The demography of *Clethrionomys rufocanus*: from mathematical and statistical models to further field studies. Res Popul Ecol 1998;40:107–21.

[15] Dewa H. Seasonal variation of the daily activity rhythms in snow season. Res Bull College Exp Forests Hokkaido Univ 1975;22:105–20 (in Japanese with English summary).

[16] Saitoh T, Stenseth NC, Bjornstad ON. The population dynamics of the vole *Clethrionomys rufocanus* in Hokkaido, Japan. Res Popul Ecol 1998;40:61–76.

[17] Abe H. Winter Food of red fox, *Vulpes vulpes schrencki* Kishida, in Hokkaido, with special reference to vole populations. Appl Entomol Zool 1975;10:40–51.

[18] Yoneda M. Influence of red fox predation upon a local population of small rodents: III. Seasonal changes in predation pressure, prey preference and predation effect. Appl Entomol Zool 1983;18:1–10.

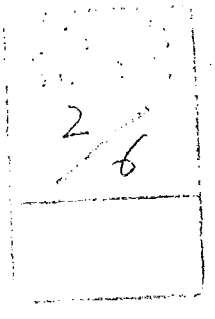
[19] Veit P, Bilger B, Schad V, Schafer J, Frank W, Lucius R. Influence of environmental factors on the infectivity of *Echinococcus multilocularis* eggs. Parasitology 1995;110:79–86.

[20] Ishige M, Itoh T, Yagi K. Biological characters of *Echinococcus multilocularis* on temperature effects on life span of eggs. Rep Hokkaido Inst Public Health 1993;43:49–51 [in Japanese with English summary].

A stochastic model of Echinococcus multilocularis focusing on protoscoleces

Tomohiko NISHINA, CHEN Tian Tian, Kazutoshi FUJITA and Hirofumi ISHIKAWA

Journal of
The Faculty of Environmental Science and Technology
Okayama University
Volume 11, Number 1, February 2006



ournal of the Faculty of Environmental Science and Technology,
)kayama University
Vol.11, No.1, pp.9-14, February 2006

A stochastic model of *Echinococcus multilocularis* focusing on protoscoleces

Tomohiko NISHINA [1], CHEN Tian Tian[1], Kazutoshi FUJITA [2] and Hirofumi ISHIKAWA[1]

(Received November 30, 2005)

The red fox (*Vulpes vulepes*) and the vole (*Clethrionomys refocanus*) are principal hosts of *Echinococcus multilocularis* in Hokkaido, Japan. How protoscoleces increases in voles and the level of immunity in foxes remain unknown because of the lack of survey data, so that it is important to clarify these mechanisms in order to develop control strategies against *E. multilocularis*. In this study, the growth of protoscoleces in the infected voles was approximated as the logistic curve, the level of immunity in the fox was assumed to depend on the experience of the infection with *E. multilocularis*, and the worm burden in the fox was assumed to be governed by the amount of protoscoleces in the vole. Our model showed that the population densities of the hosts and the level of immunity influenced the prevalence of the *E. multilocularis*.

Key words: *Echinococcus multilocularis, protoscoleces, immunity, worm burden, stochastic model*

1. INTRODUCTION

Echinococcus multilocularis, a type of zoonoses, is now prevalent in Hokkaido, Japan. The life-cycle of *E. multilocularis* takes place in the definitive hosts and the intermediate hosts. The principal definitive host and the principal intermediate host in Hokkaido are recognized as the red fox (*Vulpes vulepes*) and the grey-sided vole (*Clethrionomys refocanus*), respectively. The prevalence of *E. multilocularis* in the foxes was estimated as 45% in 1998, and has remained high until now (The Department of Health and Welfare, the Hokkaido Government). Moreover, domestic dogs may also be infected with *E. multilocularis* as the definitive hosts. In 1965, the first case of human alveolar echinococcusis was reported (Yamamoto et al., 1966). In the future, 1000 new patients are predicted to be infected over the next ten years, so that immediate control strategies against of *E. multilocularis* are needed (Doi, 1995).

Humans and voles become infected with *E. multilocularis* when they ingest free living parasite eggs discharged by the definitive hosts orally. The parasite eggs start to make protoscoleces in the vole about 40 days after infection (Yagi and Ito, 1998). When a fox preys upon an infected vole which has an adequate amount of protoscoleces, *E. multilocularis* will be transmitted from the vole to the fox. It is important to consider the population dynamics of both the definitive and the intermediate hosts because the variances of the population of the both hosts influence the transmission of *E. multilocularis* greatly. Both fox and vole populations have a seasonal fluctuation so that the time-course of *E. multilocularis* infections depend on the season.

Mathematical models have been developed to explain observations from field studies. Recently, Ohga et al. (2002) investigated the seasonal differences of the food habits of foxes, and Ishikawa et al. (2003) the seasonal fluctuations of both host populations, which had a large effect on the time-course of *E. multilocularis* population levels. Hansen et al. (2003, 2004) introduced a spatial model which included heterogeneity of the dispersing hosts.

Moreover, Kato et al. (2005) reported that foxes had an immune response, although the relation between the level of immunity and worm burden was not well known.

Department of Human Ecology, Graduate School of Environmental Science, Okayama University, 700-8530, Japan[1]
Department of Environmental Synthesis and Analysis, Graduate School of Natural Science and Technology, Okayama University, 700-8530, Japan[2]

In the model, we supposed that the level of immunity in the fox depends on the experience of the infection of *E. multilocularis*.

There have been few critical studies on the formation of protoscoleces in the infected voles. Yagi and Ito (1998) carried out experimental infections to investigate how protoscoleces increase in the infected vole. It was uncertain how to protoscoleces increase up to numbers in the millions in voles and how many protoscoleces are attached to the intestine of a fox when the fox preys on an infected vole. In the model, the growth of protoscoleces in the infected voles was approximated as a logistic curve.

The model simulates the infection from voles to foxes stochastically under the condition that the worm burden in the fox is governed by the amount of protoscoleces in the vole. Additionally, the model takes into consideration the seasonal fluctuation of the hosts through the population dynamics for the definitive and the intermediate hosts.

We carried out 1000 repeated trials of the model to study the seasonal fluctuation of the prevalence and also to consider mechanism of the immunity of foxes. We observed that the prevalence was influenced by the population densities of the hosts. Moreover, from the results of the simulations, it was plausible that a high level of immunity would be acquired in the several first exposures to infection.

2. Materials and Methods

2-1 Population dynamics of hosts

Regarding the life cycle of *E. multilocularis*, the population dynamics of definitive and intermediate hosts which have a seasonal fluctuation play an important role in the transmission of *E. multilocularis*. In Hokkaido, the major definitive hosts are foxes and the major intermediate hosts are voles.

The breeding season of foxes in Hokkaido begins in spring. The mortality of juvenile foxes (less than 1 year old) is considerably higher than that of adult foxes (more than 1 year old) (Uraguchi and Takahashi, 1991, 1998). Although foxes eat voles by preference, the deeper the snow falls, the less foxes feed on voles (Kondo et al., 1986).

The breeding season of voles is recognized as occurring in three seasons every year. The main breeding seasons are spring and autumn, since the breeding rate in summer is much less than that in the other breeding seasons (Kaneko et al., 1998). The females born in spring reach maturity and account for a large part of pregnant voles in autumn. On the other hand, only the females born in autumn breed in the spring. When the density of the vole population is fairly high, their pregnancy rate will tend to decrease because of the tendency of a slowdown in the rate of maturity. In contrast, they will mature faster and become active for breeding, resulting in their population becoming large, when the density of the vole population is low (Ota, 1984).

In this report, the fox population dynamics will be modeled stochastically with survival, infection and experimental status for every fox, which leads to various situations regarding the prevalence of *E. multilocularis* in every trial. On the other hand, the vole population dynamics will be modeled deterministically. The parameter values in both the host population models change according to the season and densities to take into account the seasonal fluctuation.

2-2 Transmission of *E. multilocularis* from foxes to voles

If a vole ingests a free living egg contained in the feces of a fox orally, it becomes infected with *E. multilocularis*. The environmental conditions such as temperature and humidity can influence the longevity of *E. multilocularis* eggs. Yagi and Ito (1991) gave the experimental formula on the relationship between the longevity (d days) and the temperature (t℃) for experimental infections:

$$d = \exp(-0.135(t - 45.37))$$

In Nemuro, Hokkaido, Japan, the longevity of eggs in summer at the average temperature 19.9℃ (observed by the Japan Meteorological Agency in August, 2005) is about 31 days according to the experimental formula. We assume that the longevity of eggs cannot exceed 100 days due to the experimental report that the infectivity was maintained only for 125 days after infection (Yagi and Ito, 1998), as the low temperature in winter will surpass the limits of the formula. It is very difficult to survey how many eggs exist in the environment, how often voles come into contact with and ingest infectious eggs, and additionally the number of eggs required to infect a vole.

Nevertheless, it is natural to think that the risk of infection for voles may depend on the number of free living parasite eggs. Then, the infection risk depends

on the number of active eggs and the number of contacts. Therefore, we use the infection risk as the transfer rate from the susceptible class to the infected class.

2-3 Growth of protoscoleces in voles

An ingested egg starts to develop protoscoleces of *E. multilocularis* in the vole about 40 days after infection. If a fox preys on a vole having an adequate amount of protoscoleces, it can be infected with *E. multilocularis*, so the amount of protoscoleces in voles plays a fundamental role in the transmission of *E. multilocularis*. However, there are few studies about the growth of protoscoleces in voles. Yagi and Ito (1991) reported in experimental infections that 11 voles produced 200 eggs, with one of the necrospied voles starting to make protoscoleces 44 days after infection, and another vole having 3.300.000 protoscoleces 142 days after infection. We assume that the number of protoscoleces in voles increases exponentially after the latent period, that the rate of increase declines slowly due to environmental factors, and that the number of protoscoleces is finally saturated. In the model, we approximate the growth of protoscoleces ($P(t)$, say, protoscoleces-day model) voles as the logistic curve (Fig.1):

$$\frac{dP}{dt} = r\left(1 - \frac{P(t)}{P\ \max}\right) P(t)$$

with P_{max}, r being the maximum number of protoscoleces and the growth rate, respectively.

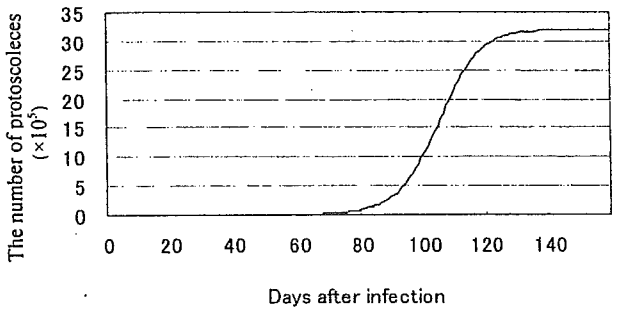


Fig.1 The time-course of prevalence of infection in voles. The curve was obtained using the logistic differential equation.

2-4 Transmission of *E. multilocularis* from voles to foxes

A fox becomes infected with *E. multilocularis* after ingestion of an infected vole which has a sufficient amount of protoscoleces. *E. multilocularis* in the fox starts to produce eggs about 30 days after infection, and egg production lasts for about 80 days after infection (Yagi and Ito, 1998). The more protoscoleces in the vole eaten by a fox, the larger worm burden the fox has. In addition, the total number of eggs discharged depends on the number of worms in the fox. We propose a model scheme from preying on voles to discharging eggs as below:

1. The number of voles preyed on by a fox per day follows the food habit function (Ohga et al., 2002)
2. If a fox preys on infected voles which have an adequate amount of protoscoleces, the fox will be infected. The probability that foxes will become infected follows from the vole prevalence. Precisely, the probability follows the binominal distribution function $f(k)$, where n, k and p stand for the number of voles ingested by a fox per day, the number of infected voles ingested and prevalence rate of voles:

$$f(k) = \binom{n}{k} p^k (1-p)^{n-k}$$

   Then, the sum of $f(k)$ over $k \geqq 1$ is the probability that a fox will become infected per day.
3. For an infected fox, we decide the age of infected voles preyed on by the fox following from the age distribution (the number of protoscoleces in the vole following the protoscoleces-day model).
4. The worm burden in the fox is determined by both the protoscoleces-day model and the immune response, which will be discussed in the next section. The infected fox discharges parasite eggs constantly, depending on its worm burden after the latent period (30 days) during a 50 days period (from the end of latent period).

2-5 Immune responses against *E. multilocularis* in foxes

Various studies concluded that foxes might have immunity (Kato et al., 2005a; 2005b), but it is not well known such immunity would affect the worm burden. Hofer et al. reported (2000) that there were remarkable differences of worm burden between

Table 1 Range and mean worm burden of foxes collected in the city of Zurich in winter [a]

	Number of examined infected foxes	Worm burden range	Mean worm burden
juvenile	68	1-56970	4995
adult	65	1-19344	907

[a] Derived from Hofer et al. (1999)

males and females, and also between juveniles and adults. Especially, the mean worm burden in juveniles was five-seven times higher than that in adults (Table 1). Then we assume that the immunity of *E. multilocularis* in foxes can be strengthened in proportion to the number of infection experiences, and adopt the following formula for the level of immunity:

$$I_{fox} = 1 - 0.8^n$$

with n being the number of infection experiences.

3. Results

Each fox structure consists of 8 characteristics, which we listed in Table 2.

We carried out simulations of the model 1000 times under the immune assumptions argued in the "immune response against *E. multilocularis* in foxes" subsection.

Prevalence of infection of host population

The mean prevalence of infection of the fox population in 1000 trials varied within the range of 12~48%, and the yearly mean prevalence ranged over

Table 2 Structure of characteristics in fox in the model

litter size (only female)
alive or dead
sex
age (year)
number of infection experiences
day after infection
number of worms
pregnancy or not (only female)

6.0~57%, and the average was estimated as 33%, which agreed with the recent reports of prevalence (30~50%) in Hokkaido. The prevalence falls remarkably after the breeding season (May), and quickly rises to the peak thereafter (Fig.2). The prevalence falls slightly from autumn to winter, but the prevalence in winter always fluctuates on a large scale. For the case of high prevalence in the breeding season, the prevalence in the next winter tends to remain at a low level, and diminishes 20~30% compared to the prevalence in summer, while for the case of low prevalence in the breeding season, the prevalence in the next winter tends to remain fairly high, and diminishes only 10% compared with the prevalence in summer.

The mean prevalence of the vole population in 1000 trials varied within the range of 1.8~6.2%, and the yearly mean prevalence ranged over 1.26~8.77%, and the average was estimated as 3.8%. The prevalence in spring has a tendency to fall with a small fluctuation (Fig.3), and the prevalence in voles in spring remained low in all the trials because the variance was fairly small. In contrast, the prevalence in voles increases

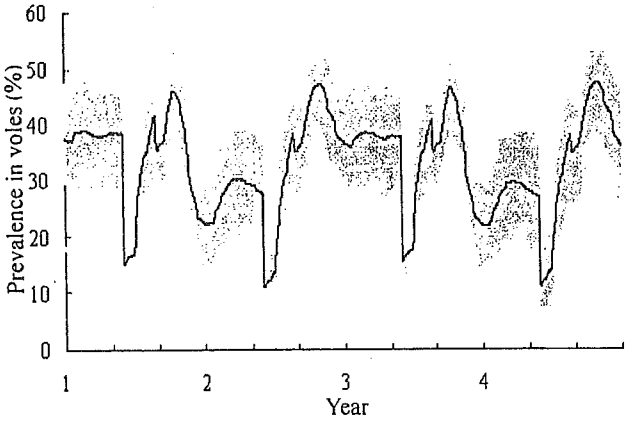


Fig. 2 Variation in the prevalence of infection in the fox population obtained by simulation. The black line shows the average prevalence in 1000 trials. The grey zone shows the prevalence in 100 trials.

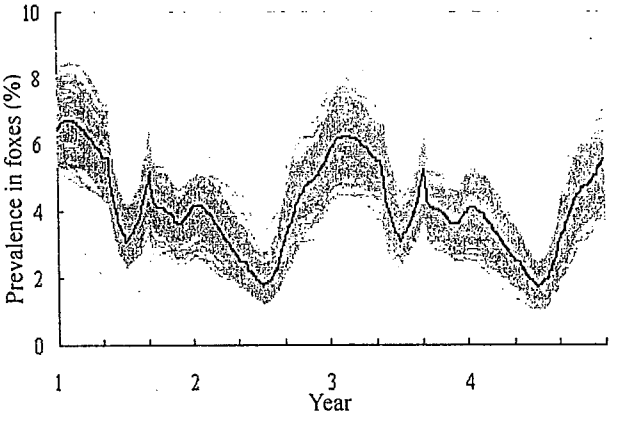


Fig. 3 Variation in the prevalence of infection in the vole population obtained by simulation. The black line shows the average prevalence in 1000 trials. The grey zone shows the prevalence for 100 trials.

Table 3 Range and mean worm burden of foxes in the results of simulations

	Number of examined infected foxes	Range of worm burden	Mean worm burden
juvenile	35	28-99300	22270
adult	65	1-25670	3300

together with a large scale of fluctuation in summer. The peak of prevalence occurs variably between autumn and winter depending on the year in repeated trials.

Worm burden in foxes

It followed from the simulations that the worm burden in juvenile and adult foxes infected with *E. multilocularis* ranged from 28~99,300 and from 1~25,670, and that it averaged 22,220 and 3,300, respectively (Table 3), which would lead to the overgrowth of the worm burden, especially in juveniles, because Hofer (2000) estimated the average worm burden as 4,995 and 907 based on actual surveys (Table 1). Fig.4 shows the graphs of the distributions of worm burden and logarithmic worm burden. Since the latter curve was almost a straight line, the worm burden would disperse exponentially. The stochastic system of foxes preying on voles taking into consideration the age structure and the growth of protoscoleces resulted in a reasonable distribution of worm burden.

4. Discussion

Although little is known about how protoscoleces increase in infected voles due to the lack of survey data, our approximate protoscoleces-day model could reproduce the distribution of the worm burden in foxes. Actually, stochastic simulation achieved a reasonable distribution of worm burden in foxes.

The average worm burden obtained from simulations went beyond the bounds of the field survey (Hofer, 2000), although the range of worm burden in foxes agreed with the field survey. We assume that the immunity is strengthened in proportion to the number of infections experienced by a fox. However, it is plausible that a high level of immunity would be acquired in the several first experiences. In the model, we take no account of the reduction of immunity or shortening of the period of discharging eggs, which must be addressed in further studies.

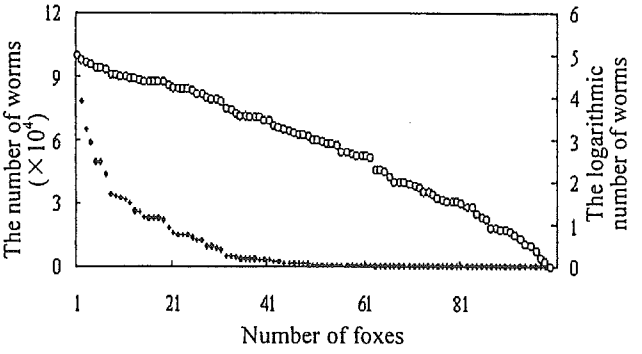


Fig. 4 Distribution of the worm burden in the infected foxes obtained by simulation. The number of worms (worm burden) and the log number of worm burden are shown by dots and circles, respectively.

The prevalence of infection in the fox population varies according to the season. It decreases remarkably after the breeding season (May) because of newborns, afterwards, newborns become infected so that the prevalence quickly rises to a peak. Actually, most newborns experience infection with *E. multilocularis* by summer. It was shown that the prevalence of infection in foxes decreases slightly from the peak, and the prevalence in winter varies on a large scale from year to year. The dangerous term for the infection of individuals with *E. multilocularis* comes after the fox breeding season because that is the time with the highest density of foxes, which is supported by the fact that the prevalence of infection in the vole population increases from summer to winter. When the fox population is large in a certain trial, the prevalence tends to hold at a high level compared with the prevalence in a small fox population in other trials. A large fox population and a high prevalence lead to a large number of infected foxes and free living parasite eggs, and may increase the prevalence in voles, too. If the fox population increases by some chance, the prevalence of *E. multilocularis* becomes high in both the fox and vole populations.

The low prevalence in voles from June to July is due to the short longevity of the parasite eggs and the fact that most juvenile foxes stay in the latent period. In winter, the prevalence in vole population increases along with prevalence in the fox population, reaches a peak and varies widely, which means that the prevalence in voles strongly depends on the population dynamics of the fox.

In the model, the infection risk in the vole population depends only on active parasite eggs

because the contact rate is set at a constant value. The human risk of infection with *E. multilocularis* can be thought of as the same as the vole risk because both humans and voles are infected by ingesting parasite eggs orally.

In the model, the longevity of the parasite eggs is assumed to depend on only temperature, but actually it also depends on humidity. Moreover, it was reported that there were some hot spots because voles are distributed heterogeneously. It is conceivable that under natural circumstances the rate of contact of voles with parasite eggs is influenced by some environmental factors such as the densities of both the definitive and the intermediate hosts and the habits of the hosts. Further improvement of the model, including consideration of the effect of control measures against *E. multilocularis,* is needed to forecast future prevalence precisely.

ACKNOWLEDGEMENTS: This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant No. 16540105) and by grants from the Ministry of Health, Labour and Welfare, Japan for "Research for emerging and re-emerging infections" (Principal investigator: Prof. M. Kamiya).

REFERENCES

Doi R (1995) A critical situation of the prevalence of echinococcos is (alveolar hydatid disease) – necessity immediate action for the prevention (in Japanese). Japanese Jounal of Public Health **42**:63–68

Hansen F, Tackmann K, Jeltsch F, Wissel C, Thulke HH. (2003). Controlling *Echinococcus multilocularis* ecological implications of field trials. Preventive Veterinary Medicine **60**:91–105

Hansen F, Jeltschb F, Tackmannc K, Staubachd C, Thulke HH (2004): Processes leading to a spatial aggregation of *Echinococcus multilocularis* in its natural intermediate host *Microtus arvalis*. International Journal for Parasitology **34**:37–44

Hofer S, Gloor S, Muller U, Mathis A, Hegglin D, Deplazes P (2000): High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zuerich, Switzerland. Parasitology **120**:135–142

Ishikawa H, Ohga Y, Doi R (2003): A model for transmission of *Echinococcus multilocularis* in Hokkaido, Japan. Parasitol Reserch **91**:444-451

Kaneko Y, Nakata K, Saito T, Stenseth NC, Bjornstad ON (1998): The biology of the vole *Clethrionomys rufocans*: a review. Researches on Population Ecologies **40**:21-37

Kato N, Nonaka N, Oku Y, Kamiya M (2005): Modified cellular immune responses in dogs infected with *Echinococcus multilocularis*. Parasitol Reserch **95**:339–345

Kato N, Nonaka N, Oku Y, Kamiya M (2005): Immune responses to oral infection with *Echinococcus multilocularis* protoscoleces in gerbils: modified lymphocyte responses due to the parasite antigen. Parasitol Reserch **96**:12-17

Kondo N, Takahashi K, Yagi K, (1986):Winter food of the red fox, *Vulpes vulpes schrenki* Kishida, in the endemic area of multilocular Echinococcosis. *Bulleten of Preparative Office of Nemuro Municipal Museum* **1**:23-31

Ohga Y, Ishikawa H, and Doi R (2002): Simulations of Prevalenceof *Echinococcus multilocularis* in Hokkaido on the Basis of Vole Population Dynamics. Jounal of Faculty of Enviromental science and technology, Okayama University **7**:1-5

Ota K(1984) Study on wild murid rodents in Hokkaido, Hokkaido Univercity press, Sapporo, (in Japanese)

Uraguchi K, Takahashi K (1991) Wildlife conservation. In: Maruyama N, Bobek B, Ono Y, Regelin W, Bartos L, Ratcliffe PR (eds) Proceedings of the International Symposium on Wildlife Conservation in Tsukuba and Yokohama, Japan 1990, p228

Uraguchi K, Takahashi K (1998) Ecology of the red fox in Hokkaido. In: Tsuduki T, Yano S, Furuya K, Takahashi K, Kawase S, Yagi K, Sudo S (eds) Alveolar echinococcus in Hokkaido (in Japanese). Hokkaido Institute of Public Health, Sapporo, pp 39–48

Yagi K, Itoh T (1998) Biological characteristics of *Echinococcus multilocularis* clarified in experimental infections (in Japanese).In: Tsuduki T, Yano S, Furuya K, Takahashi K, Kawase S, Yagi K, Sudo S (eds) Alveolar echinococcus in Hokkaido.Hokkaido Institute of Public Health, Sapporo, pp 51–63

Yagi K, Itoh T (1999) Influence of low temperature and desiccation on the infectivity of *Echinococcus multilocularis* eggs (in Japanese). Rep Hokkaido Institute Public Health **49**:167–168

Yamamoto K, Tanaka T, Yamada J, Sasaki H, Ohira S, Suzuki R, Iida H, Danjyo T (1966): A child case of alveolar hydatid disease in Nemuro, Hokkaido(in Japanese). Rinsho Shoni Igaku/J Clinical Pediatrics, Sapporo **14**:60-64

多包条虫疫学調査への応用を目的とした野外採取糞便の排泄動物鑑別法の検討

野中成晃[1]、井上貴史[1]、佐野隆史[1]、片倉　賢[1]、福井大祐[2]、奥祐三郎[1]
[1]北海道大学大学院獣医学研究科寄生虫学教室、[2]旭川市旭山動物園

Idenitification of animals excreting feces collected in field for epizootiological studies of echinococcosis

Nariaki Nonaka[1], Takashi Inoue[1], Takafumi Sano[1], Ken Katakura[1], Daisuke Fukui[2], Yuzaburo Oku[1]
[1]Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University,[2]Asahiyama Zoo

これまで我々は野外で採取した糞便を材料として多包条虫の動物疫学調査を実施してきたが、糞便の排泄動物の判定は、大きさ、形、内容物や匂い等に頼っていた。今回、糞便排泄動物の区別をより明確にするため、糞便内DNAを利用した糞便排泄動物鑑別法の開発を試みた。まず、糞便DNA抽出法の改善として、糞便中に含まれるPCR阻害物質の混入を最小限にし、かつ、糞便表面に付着する腸粘膜細胞を効率的に回収するために、凍結糞便表面の洗浄液を抽出材料として、QIAmp DNA Stool Mini KitによりDNA抽出を行った。次に、北海道において多包条虫の終宿主となるキツネ、タヌキ、イヌ、ネコおよびこれらの動物と類似の糞便を排泄するアライグマ、イタチ類のミトコンドリアDNA D-loop領域について、増幅産物の大きさの違いで鑑別が可能となるようにそれぞれ特異的な6種類のプライマーを設計しmultiplex-PCRを行ったところ、本法は食肉目間で交差反応を起こさず、糞便排泄動物の鑑別が可能であることが示された（図1）。糞便に含まれる餌動物の影響についても検討したが、北海道に生息する野鼠のDNAとの交差反応は認められなかった。実験的に屋外環境で8週間放置したキツネ糞便からも10個全てのサンプルで明瞭な増幅バンドが認められ、本法の安定性が示された。2004年5～8月に小樽市および余市町で採取した147個の糞便に本法を適用したところ、140個（95%）について糞便排泄動物を鑑別することができた。以上の結果、野外採取糞便を材料とした多包条虫の動物疫学調査において、本法を併用することにより、宿主動物種を区別したより精度の高い感染率調査が実施可能となることが示された。

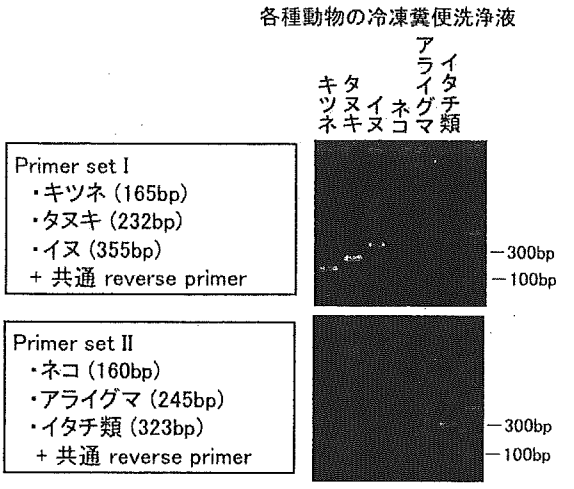


図1　Multiplex PCRの糞便由来DNAへの適用

Keywords: *Echinococcus*, feces, PCR, epizootiology

連絡先責任者：野中成晃、北海道大学大学院獣医学研究科寄生虫学教室、〒060-0818　札幌市北区北18条西9丁目
E-mail: nnonaka@vetmed.hokudai.ac.jp
Correspondance: N. Nonaka, Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, Kita-ku Kita 18, Nishi 9, Sapporo 060-0818, Japan

駆虫を組み合わせたプレパテント期における多包条虫感染のcopro-DNA診断

神廣創太[1]、野中成晃[1]、片倉賢[1]、八木欣平[2]、奥祐三郎[1]
[1]北海道大学大学院獣医学研究科寄生虫学教室,[2]北海道立衛生研究

Copro-DNA diagnosis of prepatent infection with *Echinococcus multilocularis* in combination with anthelmintic treatment

Sota Kamihiro[1], Nariaki Nonaka[1], Ken Katakura[1], Kinpei Yagi[2], Yuzaburo Oku[1]
[1]Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University,
[2]Hokkaido Institute of Public Health

Prevalence of *Echinococcus multilocularis* in foxes in Hokkaido has been around 40%, increasing the risk of infections to domestic dogs and cats. At present, standard diagnostic procedure for dogs and cats is detection of coproantigen and fecal taeniid eggs, followed by the confirmative DNA detection from the eggs. However, during the prepatent period, eggs cannot be detected while coproantigen is already positive. Unfortunately, positive results of coproantigen alone can not be definitive indication of the infection because of its cross reactivity with other *Taenia* infection and occasional false positive result. To correctly evaluate the risk of *E. multilocularis* and to take effective preventive measures, it is essential to detect DNA from the feces even during the prepatent period.

Five dogs were orally given 1,000 (1 dog), 150,000 (3) and 1,000,000 (1) protoscoleces of *E. multilocularis*. Feces were collected daily until 21 days after infection and DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen) and tested by specific PCR [1]. As a result, DNA was detected only sporadically, indicating that it is difficult to detect DNA during the prepatent period.

In the next experiment, two dogs were orally given 10,000 and 100,000 protoscoleces respectively. Fourteen days after infection, they were treated with praziquantel.

Feces were collected until 21 days post infection and DNA was extracted and tested by the PCR. Before the praziquantel treatment, only one sample was positive for the PCR. But after the treatment, the PCR was positive in feces of both dogs. Detected DNA is probably derived from the worms that were killed and excreted together with feces. These results suggest that DNA detection from feces in combination with the praziquantel treatment can be a new option for the diagnosis of *E. multilocularis* infection in the definitive hosts.

Key words: *Echinococcus multilocularis*, Diagnosis, copro-DNA

Reference

[1] van der Giessen JWB, et al. (1999): Detection of *Echinococcus multilocularis* in foxes in the Netherlands. *Vet. Parasitol.*, 82, 49-57

連絡先責任者：野中成晃、北海道大学獣医学研究科寄生虫学教室、〒060-0818　北海道札幌市北区北18条西9丁目
E-mail: nnonaka@vetmed.hokudai.ac.jp
Correspondence: N. Nonaka, Laboratory of Parasitology, Graduate School of Veterinary Medicine, Hokkaido University, 060-0818 Sapporo, Japan

SCIDマウスとスナネズミにおけるアジア条虫の幼虫の発育と人および代替終宿主に対するその感染能

張　秀玲、野中　成晃、黄　鴻堅、神谷正男、奥　祐三郎
北海道大学大学院獣医学研究科寄生虫学教室

Larval development of *Taenia asiatica* in scid mice and gerbils and their infectibity to humans and alternative definitive hosts

Shiou Ling CHANG, Nariaki NONAKA, Hong Kean OOI, Masao KAMITA, Yuzaburo OKU
Laboratory of Parasitology, Graduate School of Veterinary Medicine

【目的】アジア条虫 *Taenia asiatica* は無鉤条虫に近縁な条虫で主な中間宿主は豚で、その肝臓に嚢虫が寄生するがその寿命は短い事が特徴である。アジアで多くの人体寄生例が報告され、有鉤条虫や無鉤条虫とならんで公衆衛生上重要な条虫である。我々はSCIDマウスとスナネズミおける幼虫の発育と、感染後10から48週後に得られた嚢虫の感染能力を調べた。

【方法・結果】虫卵の皮下接種後SCIDマウスの皮下から10、24、45週後に嚢虫を回収した（表1）。嚢虫には少数の遺残的小鉤が額嘴領域に認められ、10週齢ではこの領域周囲に小型顆粒が多数認められたが、24と45週齢では減少していた。一方、10週齢では石灰小体の数が少なかったが、24と45週齢では増加していた。スナネズミにおける発育も同様であった。SCIDマウスから回収した10週齢嚢虫を経口投与したスナネズミでは全例陰性(0/21例）だったのに対し、20から45週齢の嚢虫を経口投与した場合、虫体陽性率（陽性ネズミ数/全投与ネズミ数）は6％から18％で（表2）、ハムスターの虫体陽性率もほぼ同様であった。虫体回収率は0.5-6％であった。また、スナネズミから回収した48週齢の嚢虫を経口投与したスナネズミの虫体陽性率は45％（5/11）で、虫体回収率は27％であった。これらのハムスターおよびスナネズミとも部分的な虫体の発育が観察された。一方、SCIDマウスとスナネズミから回収されたそれぞれ45と48週齢の嚢虫を経口投与したボランティアの人では、それぞれ2/3、2/2に感染し、受胎片節を排泄し、駆虫による虫体回収率は約50％であった（表3）。

表1 アジア条虫の六鉤幼虫を投与したSCIDマウスにおける幼虫の発育

六鉤幼虫*			SCIDマウス		回収幼虫
	投与経路	投与数	頭数	剖検(週数)	回収率(範囲)
A	皮下	20,000	5	12,20 週	0.1-1.1%
	腹腔	18,600	5	12,20 週	0-0.3%
B	皮下	40,000	3	24,62 週	0.003-1.1%
	皮下	20,000	5	10-45 週	0.9-6.4%

* 幼虫被殻を除去した六鉤幼虫を感染に使用した。

A　2003年12月台湾で受胎片節採取　2004年1月感染実験に使用
B　2004年4月台湾で受胎片節採取　2004年5月感染実験に使用

表2 SCIDマウスから得た各種週齢の嚢虫の感染能の検討 -代替終宿主を用いて-

動物	嚢虫		陽性動物/全使用動物	虫体回収率*
	週齢	投与数	(1-4週に剖検)	
スナネズミ				
A	10	20,40	0/21(0 %)	0/640(0 %)
B	20	6	2/32(6 %)	2/192(1.0 %)
C	21	25-40	2/11(18 %)	2/403(0.5 %)
D	45	2	2/17(11 %)	2/34(5.8 %)
ハムスター				
A	20	6	3/41(7 %)	3/246(1.2 %)
B	24	15	3/14(21 %)	3/210(1.4 %)

* 回収虫体数/全投与虫体数(%)

表3 SCIDマウス(45週齢)およびスナネズミ(48週齢)の嚢虫を用いた人への感染結果

人	由来動物	週齢	投与数	駆虫後の回収虫体数
A	SCIDマウス	45	5	0
B	SCIDマウス	45	5	1
C	SCIDマウス	45	5	2
D	スナネズミ	48	5	3
E	スナネズミ	48	5	4

* A-Cの駆虫は感染後7ヶ月、DとEの駆虫は感染後4ヶ月
虫体陽性率は4/5(80%)、虫体回収率は10/25(40%)

【総括】アジア条虫の嚢虫の額嘴領域の小型顆粒と石灰小体の数は成熟の指標として評価できることが示唆され、SCIDマウスとスナネズミにおいて感染能を有する嚢虫まで発育することを示した。

Keywords, *Taenia asiatica*, alternative host

連絡先責任者：奥祐三郎　北海道大学大学院獣医学研究科 寄生虫学教室、〒060-0818　札幌市北区北18条西9丁目
E-mail: oku@vetmed.hokudai.ac.jp
Correspondence: Y.OKU, Laboratory of Parasitology, Graduate School of Veterinary Medicine, Kita-18, Nishi-9, Kita-ku, Sapporo, 060-0818, Japan

The Veterinary Record

ART NO. C4210

SHORT COMMUNICATION

Intestinal helminths of dogs in northern Japan

Y. MORISHIMA, H. SUGIYAMA, K. ARAKAWA, M. KAWANAKA

Y. Morishima, DVM, PhD, **H. Sugiyama**, DVM, PhD, **K. Arakawa**, DVM, **M. Kawanaka**, DVM, PhD, Department of Parasitology, National Institute of Infectious Diseases, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

Correspondence to Dr Morishima

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