Available online at www.sciencedirect.com

SCIENCE DIRECT

Parasitology International 54 (2005) 43–49

www.elsevier.com/locate/parint

Production and characterization of monoclonal antibodies against excretory/secretory products of adult *Echinococcus granulosus*, and their application to coproantigen detection

Cecilia Casaravilla[a], Ramiro Malgor[a], Andrea Rossi[a], Hirofumi Sakai[b], Nariaki Nonaka[b], Masao Kamiya[b], Carlos Carmona[a,*]

[a]*Unidad de Biologia Parasitaria, Facultad de Ciencias, Instituto de Higiene, Universidad de la República, Av. A. Navarro 3051, Montevideo CP11600, Uruguay*

[b]*Laboratory of Parasitology Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan*

Received 16 February 2004; accepted 31 August 2004
Available online 27 October 2004

Abstract

Two IgM murine monoclonal antibodies (MAbs), EgC1 and EgC3, were produced against the excretory/secretory (E/S) products of *Echinococcus granulosus* adult worms. Immunoblotting revealed that both predominantly recognized a 50 kDa antigen in the somatic extract and an 85 kDa component in the E/S products. Immunolocalization showed that both MAbs reacted with the tegument of the parasite, and additionally EgC3 reacted with parenchyma and the tegument lining the external surface of the reproductive organs. A coproantigen capture ELISA was developed using a rabbit polyclonal antibody against E/S products from adult tapeworms as catching antibodies, and each one of MAbs as detecting antibody. The assays detected seven out of eight (EgC1), and eight out of eight (EgC3) experimentally infected dogs (worm burdens ranging from 61 to 57,500), using heat-treated samples obtained at prepatent period, and none (n=8) of helminth-free samples. Time course analysis showed that, after a 12–25 days lag, coproantigen levels rose above cut off O.D. values and typically peaked around 30 days post-infection (DPI) at the end of the experiment. One dog experimentally infected with *Taenia hydatigena* metacestodes was slightly detected as positive at different time points after 30 DPI. Both MAbs showed a similar pattern of recognition, but *T. hydatigena* antigens were undetectable for a longer period, and reached lower O.D. values with EgC1. Interestingly, fecal samples from two experimentally infected dogs with *Echinococcus multilocularis* were not recognized by the EgC1 assay, suggesting a potential value as species-specific diagnostic tool.

Keywords: Coproantigen; *Echinococcus granulosus*; Monoclonal antibodies

1. Introduction

Echinococcus granulosus, the dog/sheep tapeworm, is the causative agent of cystic echinococcosis, an important zoonosis widely distributed throughout the rural areas of the world. Many affected countries have established control programmes predominantly based on regular dosing of dogs, and in some cases a marked reduction in the transmission of the disease has been achieved [1–3]. Accordingly, accurate assessment of *E. granulosus* in dog populations is a critical requirement for evaluating the programme efficacy, and for estimating the potential infection risk for both human and ruminants. The purgation technique with arecoline hydrobromide has been widely used as the standard method for screening dog populations, but the examination of removed material is time-consuming, requires trained personnel, and it is not sensitive enough, as a single dose could detect less than 50% of *E. granulosus* infections [4].

* Corresponding author. Fax: +598 2 4801597.
E-mail address: ccarmona@higiene.edu.uy (C. Carmona).

1383-5769/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.parint.2004.08.006

Detection of parasite antigens in feces has become an important alternative method for the diagnosis of intestinal infections caused either by protozoa or helminths [5,6]. It has the advantage of correlation with current parasitism, as parasite-derived antigens should not be present in the absence of infection. In this sense, different assays have been developed for the diagnosis of *E. granulosus* components in fecal samples using parasite specific polyclonal antibodies [7–10]. Although the sensitivity obtained with these assays has been reported higher than 90%, low parasite burdens with ≤100 worms were responsible for most false negative results [11]. Additionally, in most cases, false-positive reactions caused by infections with related canine tapeworms were observed.

In this context, we initially evaluated a sandwich ELISA system for *E. granulosus* coproantigen detection, using a monoclonal antibody produced against somatic extract of *Echinococcus multilocularis* [12,13]. Although the test showed a very high sensitivity (100%) in naturally and experimentally infected animals, cases of cross-reactivity with *Taenia hydatigena* were also observed.

In the present work, we produced and characterized, for the first time, MAbs against excretory/secretory (E/S) products from *E. granulosus* adult stage, and preliminarily studied their potential as diagnostic reagents for specific coproantigen detection.

2. Materials and methods

2.1. Experimental infections

The infections were performed as previously described by Malgor et al. [13]. Male and female crossbred dogs, aged 6 months to 2 years, were maintained under helminth-free conditions and fed commercial dog food and water ad libitum. One group of dogs was orally infected with 30,000–200,000 protoscoleces from bovine cysts (dogs 1–3), another group was infected with 25,000–65,000 protoscoleces obtained from ovine cysts (dogs 4–6), and a third one was infected with less than 1000 protoscoleces obtained from ovine cysts (dogs 7–8). They were euthanised before patency with an overdose of sodium pentobarbitone on 25 (dogs 7–8), 30 (dog 3), 31 (dogs 1–2), or 35 (dogs 4–6) days post-infection (DPI). The experiments were performed under the control of the Honorary Commission on Animal Experimentation (CHEA) of the University of the Republic in accordance with the Law on the Use of Animals in Experimentation, Teaching and Universitary Research (Ordenanza sobre uso de animales en experimentación, docencia e investigación Universitaria, Diario Oficial No. 25.467, Febrero 21 de 2000, 1440-C a 1444-C, carillas No. 64 a 68).

One dog was experimentally infected with seven *T. hydatigena* metacestodes, and the infection was maintained during the prepatent period (55 DPI), when the dog was treated with praziquantel (10 mg/kg).

2.2. Preparation of parasite extracts

2.2.1. E/S products

E. granulosus adults worms were recovered from the intestine of experimentally infected dogs at 35 DPI. Briefly, the small intestine was divided into three parts, opened and placed over a mesh in a Petri dish with the mucosae surface in Hank's balanced salt solution (HBSS), and incubated for various periods, during which adult worms were released. They were washed in HBSS (pH 7.2) containing gentamicin (200 μg/ml) and then maintained in Medium 199 (Gibco) pH 7.2 supplemented with glucose (4.0 g/l) and gentamicin (200 μg/ml), at 37 °C in a 5% CO_2 incubator. Approximately 7500 worms were cultivated in 150 ml of medium, which was replaced every 6 h during the first 24 h, then collected, and stored at −80 °C until processed. The medium containing the E/S components was concentrated using a YM-10 membrane (Amicon) followed by dialysis with PBS.

2.2.2. Somatic extracts

Adult *E. granulosus* worms obtained as above were washed in Tris–HCl buffer (pH 7.8) containing EDTA (25 μM) and PMSF (200 μM), homogenized, and ultrasonicated at 20 pulses/min (20% power). Sonicated material was centrifuged during 30 min at 10,000×*g* and supernatant was used as somatic extract.

2.3. Preparation of fecal samples

Feces from experimentally infected dogs were daily collected, mixed in a 1:4 ratio (w/w) with 1% formalin, heated at 70 °C for 12 h, centrifuged at 2200×*g* for 10 min, and the supernatant stored at −20 °C until used for coproantigen detection. Positive *E. multilocularis* fecal samples were collected at 45 DPI from two experimentally infected dogs as previously described [14]. Negative fecal samples were obtained on the day prior to the infection either with *E. granulosus* or *E. multilocularis*.

2.4. Monoclonal antibodies (MAbs) production

BALB/c mice were immunized with 100 μg of *E. granulosus* E/S antigen solution in Freund's complete adjuvant. Two weeks after priming, mice were boosted with the same amount of antigen in Freund's incomplete adjuvant. Three days before fusion, a second booster was given in saline. All the immunizations were done by intraperitoneal injection. After three days, mice were sacrificed and the spleen removed. Splenocytes were fused with X63 myeloma cells using a 50% polyethylene glycol 1500 solution in serum free Iscove's modified DMEM medium (IMDM), containing streptomycin sulfate (0.1 g/l)

and penicillin G (10^5 U/l). Fusion and cell-culture procedures were carried out essentially as described by De StGroth and Scheidegger [15]. Cell supernatants were screened for antibody activity using direct ELISA with *E. granulosus* E/S antigen. Hybridoma with suitable growth and higher secretion of antibodies against *E. granulosus* were repeatedly cloned by limited dilution in IMDM with 20% fetal bovine serum (Gibco) and cultured. Thymocytes from BALB/c mice were used as feeder cells. MAbs were recovered from cell culture supernatant.

2.5. ELISA assay

For MAbs screening and isotype determination, direct ELISA was performed as follows: flat-bottomed microtitre plates (Maxisorp, Nunc) were coated with 1 μg/ml antigen (50 μl/well) in 0.05 M $NaHCO_3/Na_2CO_3$ buffer (pH 9.6) and left overnight at 4 °C. Blocking was done with 1% bovine serum albumin (BSA) in PBS (100 μl/well) for 2 h. Hybridoma culture supernatants (50 μl/well) were incubated for 1 h. Bound antibodies were detected after the addition of 50 μl of a horseradish peroxidase conjugated rabbit anti-mouse IgG+A+M (Sigma), diluted at 1/2000 in 0.5% BSA and 0.5% casein in PBS containing 0.05% Tween (PBS-T) for 1 h, and then 0.04% *o*-phenylenediamine, 0.07% H_2O_2 in citrate phosphate buffer (pH 5.5) were added for 10 min at 37 °C (100 μl/well). The reaction was stopped with 50 μl of 4 N H_2SO_4 and the plates were read at 492 nm. All the washes were done with PBS-T. Unless otherwise stated, all procedures were carried out at room temperature (RT). For isotype determination, the same protocol was followed, but horseradish peroxidase conjugated rabbit anti-mouse IgM and IgG (Sigma) were used.

2.6. Rabbit anti-E. granulosus E/S products polyclonal antibody

A New Zealand White rabbit was immunized three times subcutaneously with 100 μg of E/S products from adult *E. granulosus*, using Freund's complete adjuvant for the first injection and Freund's incomplete adjuvant for the first booster 2 weeks later. The third injection was in saline. IgG was purified from pooled sera using a Protein A affinity column (BioRad) according to manufacturer's instructions.

2.7. Immunoblotting

Somatic extract and E/S products of adult *E. granulosus* were separated in 10% SDS-PAGE under reducing conditions, and blotted onto a nitrocellulose membrane (Bio-Rad) using a semi-dry horizontal electro-transfer system. Blocking was done with 1% BSA in PBS at 4 °C overnight. The strips were then probed with hybridoma culture supernatant for 1 h at RT, and then incubated with horseradish peroxidase conjugated rabbit anti-mouse IgM (Sigma) diluted at 1/4000 in 0.1% BSA in PBS-T for 1 h at RT. Peroxidase reaction was visualized with 0.06% (w/v) diaminobenzidine tetrahydrochloride in 50 mM Tris–HCl (pH 7.6) and 0.03% (v/v) H_2O_2. The reaction was stopped after 5 min with distilled water.

2.8. Immunofluorescence on histological sections

Adult worms, fixed in 70% alcohol, were dehydrated, cleared, embedded in paraffin wax, and cut 4 μm thick. After dewaxing, sections were incubated with the MAbs (culture supernatant) for 1 h at 37 °C. After washing with PBS-T, a FITC conjugate anti-mouse IgM (Sigma) was added for 1 h at 37 °C. The sections were washed, then mounted and observed in a Zeiss fluorescence microscope.

2.9. Coproantigen detection

Sandwich ELISA for coproantigen detection was performed following the protocol described by Malgor et al. [13]. Flat-bottomed microtitre plates were coated with Protein A purified rabbit anti-*E. granulosus* E/S products antibody (5 μg/ml) in carbonate–bicarbonate buffer (pH 9.6), overnight at 4 °C. The plates were blocked with 1% BSA in PBS for 2 h at RT, and then incubated with fecal supernatant (50 μl/well) or different concentrations of parasite E/S products diluted in negative feces (500 to 10 ng/ml), for 2 h at RT. Then they were loaded with 50 μl of hybridoma culture medium, and after 1 h, 50 μl/well of HRP-conjugated anti-mouse IgM (1:4000) were added for another 1 h. Finally, 100 μl of *o*-phenylenediamine (0.04%) and H_2O_2 (0.07%) in citrate phosphate buffer (pH 5.0) were added for 10 min at 37 °C. The reaction was stopped by adding 50 μl of 4 N H_2SO_4, and plates were read at 492 nm. The cut off values for both MAbs were determined by

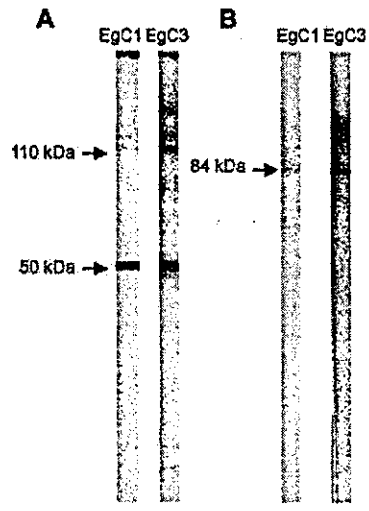

Fig. 1. Western blotting with EgC1 and EgC3 on *E. granulosus* somatic extract (A) and E/S products (B). Apparent molecular weights are shown on the left.

calculating the mean value+3S.D. of the samples collected immediately prior to infection (EgC1=0.088, EgC3=0.164).

3. Results

3.1. Monoclonal antibodies

Two clones of IgM MAbs were produced against the E/S products of adult *E. granulosus*, namely EgC1 and EgC3.

3.2. Immunoblotting

After SDS-PAGE, immunoblotting with both MAbs showed reactivity with a prominent single band of an apparent molecular weight of 50 kDa in the somatic extract, and with a band of 85 kDa in the E/S products (Fig. 1A and B). Additionally, EgC3 reacted with other minor slow migrating bands in the somatic extract (Fig. 1A).

3.3. Immunolocalization

Both MAbs exhibited high intensity staining, predominantly at the tegument of the parasite (Fig. 2A–C). Besides, EgC3 reacted with parenchyma and the tegument lining the external surface of the reproductive organs (Fig. 2A and B).

3.4. Coproantigen detection

Fig. 3 shows the detection of fecal antigens by EgC1 and EgC3 in experimentally infected dogs, harboring from 61 to 57,500 worms. At the last days of prepatent infection (25

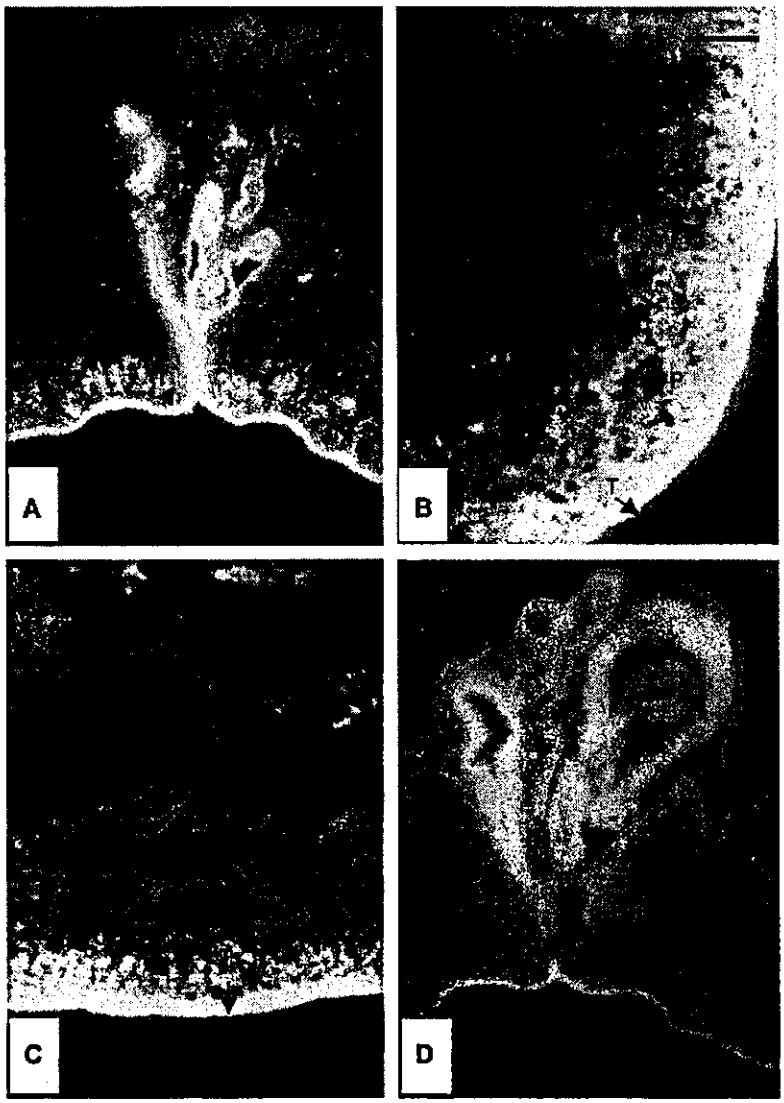

Fig. 2. Immunofluorescence with EgC1 and EgC3 on adult *E. granulosus* sections (posterior proglotid). (A) EgC3 recognition of tegument (T). (B) EgC3 recognition of parenchyma (P). (C) EgC1 recognition of tegument (T). (D) Control without MAb.

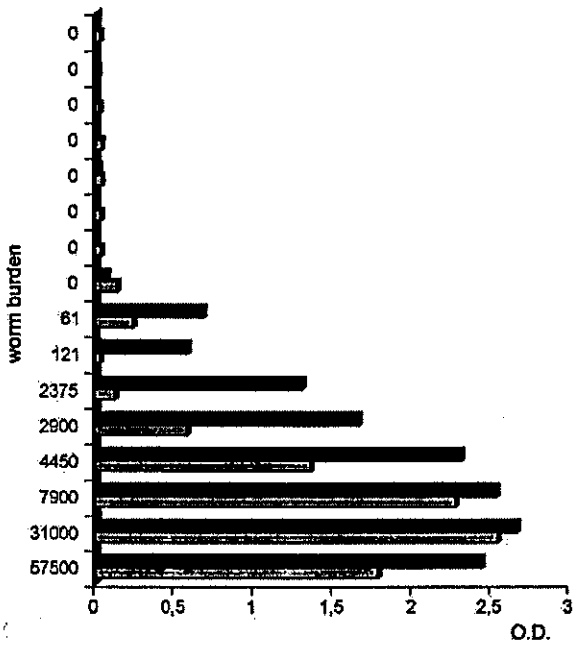

Fig. 3. Detection of *E. granulosus* coproantigens using a sandwich ELISA with a polyclonal antibody developed against *E. granulosus* E/S products as capture and EgC1 and Egc3 as detecting antibodies. Feces from dogs experimentally infected with different worm burdens were tested (samples from dogs harboring from 2375 to 57,500 worms were from 30 DPI, and samples from dogs harboring 61 and 121 worms were from 25 DPI). □, EgC1; ■, EgC3.

DPI for dogs harboring 61 and 121 worms, and 30 DPI for dogs harboring from 2375 to 57,500 worms), all infected dogs were detected as positive for EgC3, and seven out eight for EgC1. None of the helminth-free controls showed false positive reactivity.

The sensitivity of both systems was preliminarily determined evaluating the detection limit for serial dilutions of parasite E/S products in negative feces. The detection limit for EgC1 was below 30 ng/ml and for EgC3 was below 7 ng/ml, equivalent to 120 and 28 ng of parasite components/g of feces, respectively. Fig. 4 expose the individual time course of coproantigen detection in each of the eight dogs experimentally infected with *E. granulosus*. Both MAbs detected released fecal antigens during the prepatent period studied. Using EgC3, fecal samples became positive at 12 DPI in the dogs harboring higher worm burdens (dogs 1 and 2), or later at 15–24 DPI in dogs with less than 10,000 worms (dogs 3 to 6), followed by a rise in O.D. values that remained positive until the end of the experiment. In dogs carrying worm burdens between 61 and 121 (dogs 7 and 8), the coproantigens were detected at the end of the experimental infection (25 DPI), with O.D. values in the same order as those from higher worm burden on 25 DPI. The ELISA assay with EgC1 showed a similar pattern, but antigens were undetectable for a longer period, and reached lower O.D. values.

However, when feces from a *T. hydatigena*-infected dog were assayed, both MAbs showed positive O.D. values in

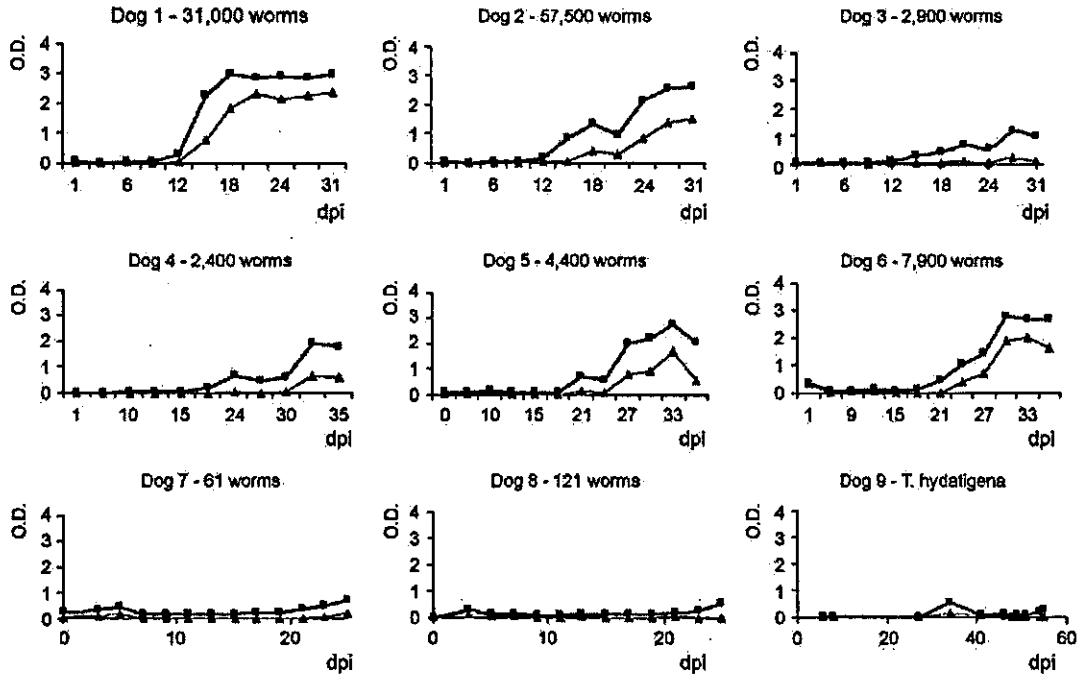

Fig. 4. Time course coproantigen detection in the prepatent period of infection of dogs experimentally infected with different worm burdens of *E. granulosus* or *T. hydatigena*. DPI: days after infection; ▲, EgC1; ■, EgC3.

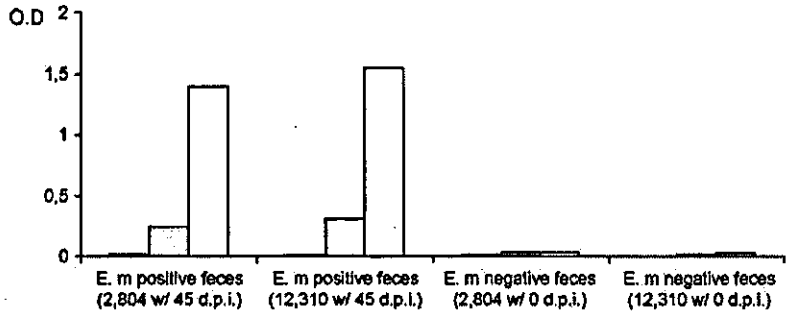

Fig. 5. Coproantigen detection by EgC1 ■, EgC3 ▣ and EmA9 □ in feces from dogs experimentally infected with *E. multilocularis*.

samples at different time points from 30 DPI until the end of the experiment on 55 DPI.

An indication of specie-specificity of EgC1 is shown in Fig. 5. EgC1 did not recognize either *E. multilocularis* somatic extract (not shown) or positive feces from infected dogs. The results were compared with EmA9, an MAb prepared against somatic extract of *E. multilocularis* [16].

4. Discussion

It is increasingly recognized that an accurate measurement of the prevalence of canine infection is a critical requirement in order to establish the epidemiological status of cystic echinococcosis in a given situation. However, the use of the standard purgation method with arecoline hydrobromide is highly problematic mainly due to its low sensitivity and operational difficulties, making it unsuitable for the screening of large dog populations. In this context, the immunodetection of soluble released antigens in fecal supernatants has gained increasing support as an alternative method capable of overcoming these difficulties [10]. Aimed at developing a highly specific assay, we produced two MAbs, EgC1 and EgC3.

The accuracy of coproantigen detection in canine echinococcosis is critically dependent on the parasite burden, as initially observed by Deplazes et al. [8], who detected only one in eight dogs infected with less than 100 worms, using polyclonal antibodies produced against E/S products from adult tapeworms. Another study using the same assay showed that 92% of dogs harboring more than 100 worms were positive, while detection capacity dropped to 30% in those animals with less than 100 parasites [11]. Similarly, a burden-related effect was observed in another field study that employed a coproantigen capture ELISA with affinity-purified polyclonal IgG anti-*E. granulosus* somatic homogenate. In this case, false negative coproantigen samples were from dogs with less than 20 worms detected at purge. It has been shown that the average worm burden of *E. granulosus* is about 200/dog in endemic areas for cystic echinococcosis [17].

For the coproantigen detection ELISA utilizing EgC1 and EgC3, time course profiles of coproantigen detection during experimental infections were very similar. Fecal samples became positive during the prepatent period after a lag phase of 12 to 25 DPI, being later for dogs with lower worm counts. After detection, coproantigen levels showed a steady rise that peaked at about 30 DPI. Within the range of parasite counts, EgC3 showed higher values than EgC1, and also a trend of positive correlation between OD values and worm burden (not observed with EgC1). Dogs infected with less than 121 worms were detected by EgC3 near day 25 (EgC1 only detected dog 7), when the experimental infection was finished. The O.D. values for these samples (25 DPI) were similar to those of dogs harboring higher worm burdens.

These findings suggest that the detection limit of the coproantigen assay is related to the biomass and the antigen production capacity of growing parasites. Alternatively, it is possible that some *E. granulosus* antigens released to the intestinal lumen during the early phase of development were stage-specific and hence, not recognized by EgC1 and EgC3, produced against E/S products from older prepatent worms.

Unlike previous reports that utilized either polyclonal or monoclonal-based assays, none of the studied dogs exhibited strong fluctuations in coproantigen excretion levels [7,13,14] indicating an even distribution of released parasite antigens in the feces.

Cross-reactivity with *Taenia* spp. constitutes another major hurdle for the development of a highly specific coproantigen detection method for echinococcosis. The cross-reaction has been reported for all the developed coproantigen tests [7,8,13] and it can be undoubtedly a problem in countries like Uruguay, where *T. hydatigena* is in hyperendemic steady state [18]. Recently, a major field study conducted by Christofi et al. [10] in Cyprus revealed that ECHINOTEST, a commercial coproantigen kit based in the polyclonal-based assay developed by Allan et al. [7], had a sensitivity of 83% and a specificity that ranged from 80% to 98%, depending on the presence of *Taenia* spp. infection in the group under evaluation.

Our results showed that MAbs reacted with fecal supernatants from a dog infected with seven worms of *T. hydatigena* at different prepatent time points starting on 35 DPI until the end of the experiment on 55DPI. However,

extensive field studies in naturally infected animals with *Taenia* are necessary to assess assay specificity, particularly using EgC3.

This false positive reaction with *Taenia* positive samples contrasts with the lack of reactivity showed by EgC1 with patent feces from *E. multilocularis* experimentally infected dogs, harboring >1000 worms. Such species-specificity might be useful in epidemiological settings where both *Echinococcus* species coexist, as in parts of Central Europe and China [19].

The capacity of both MAbs for spotting animals for treatment before eggs can contaminate the environment is a valuable feature for control campaigns where rates of reinfection in dogs are being monitored. Additionally, both MAbs detect heat-resistant epitopes, possibly carbohydrate moieties, allowing the sterilization of fecal samples by heating, thus rendering them safe for the personnel involved.

Acknowledgements

This work was supported by grants from the Japan Health Sciences Foundation and the International Foundation for Science.

References

[1] Gemmel MA, Roberts MG. Modelling *Echinococcus* life cycles. In: Thompson RCA, Limberly AJ, editors. *Echinococcus* and Hydatid disease. Wallingford, UK: CAB International; 1995. p. 333–54.

[2] Cabrera PA, Lloyd S, Haran G, Pineyro P, Parietti S, Gemmel MA, et al. Control of *Echinococcus granulosus* in Uruguay: evaluation of different treatment intervals for dogs. Vet Parasitol 2002;103:333–40.

[3] Jimenez S, Perez A, Gil H, Schantz PM, Ramalle E, Juste RA. Progress in control of cystic echinococcis in La Rioja, Spain: decline in infection prevalences in human and animal hosts and economic costs and benefits. Acta Trop 2002;83:213–21.

[4] Wachira TM, Macpherson CNL, Gathuma JM. Hydatid disease in the Turkana district of Kenya: VII. Analysis of the infection pressure between definitive and intermediate hosts of *Echinococcus granulosus*, 1979–1988. Ann Trop Med Parasitol 1990;84:361–8.

[5] Vinayak VK, Dutt P, Puri M. An immunoenzymatic dot-ELISA for the detection of *Giardia lamblia* antigen in stool eluates of clinical cases of giardasis. J Immunol Methods 1991;137:245–51.

[6] Fraser A, Craig PS. Detection of gastrointestinal helminth infection using coproantigen and molecular diagnostic approaches. J Helminthol 1997;71:103–7.

[7] Allan JC, Craig PS, Garcia-Noval J, Mencos F, Liu D, Wang Y, et al. Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology 1992;104:347–55.

[8] Deplazes P, Gottstein B, Eckert J, Jenkins DJ, Ewald D, Jimenez-Palcios S. Detection of *Echinococcus* coproantigens by enzyme-linked immunosorbent assay in dogs, dingoes and foxes. Parasitol Res 1992;78:303–8.

[9] Craig PS, Gasser RB, Parada L, Cabrera P, Parietti S, Borgues C, et al. Diagnosis of canine echinococcosis: comparison of coproantigen and serum antibody tests with arecoline purgation in Uruguay. Vet Parasitol 1995;56:293–301.

[10] Christofi G, Deplazes P, Christofi N, Tanner I, Economides P, Eckert J. Screening of dogs for *Echinococcus granulosus* coproantigen in a low endemic situation in Cyprus. Vet Parasitol 2002;104:299–306.

[11] Deplazes P, Jimenez-Palacios S, Gottstein B, Skagg J, Eckert J. Detection of *Echinicoccus* coproantigen in stray dogs of northern Spain. Appl Parasitol 1994;35:297–301.

[12] Sakai H, Malgor R, Basmadjian I, Gallardo R, Carmona C, Sato H, et al. An enzyme-linked immunosorbent assay (ELISA) for the detection of *Echinococcus granulosus* coproantigens in dogs. Jpn J Parasitol 1995;44:453–61.

[13] Malgor R, Nonaka N, Basmadjian I, Sakai H, Carambula B, Oku Y, et al. Coproantigen detection in dogs experimentally and naturally infected with *Echinococcus granulosus* by a monoclonal antibody-based enzyme-linked immunosorbent assay. Int J Parasitol 1997;27:1605–12.

[14] Nonaka N, Iida M, Yagi K, Ito T, Ooi HK, Oku Y, et al. Time course of coproantigen excretion in *Echinococcus multilocularis* infections in foxes and an alternative definitive host, golden hamsters. Int J Parasitol 1996;26:1271–8.

[15] De StGroth SF, Scheidegger D. Production of monoclonal antibodies: strategy and tactics. J Immunol Methods 1980;35:1–21.

[16] Khono H, Sakai H, Okamoto M, Ito M, Oku Y, Kamiya M. Development and characterization on murine monoclonal antibodies to *Echinococcus multilocularis* adult worms and its use for the coproantigen detection. Jpn J Parasitol 1995;44:404–12.

[17] Gemmel MA, Lawson JR, Roberts MG. Towards global control of cystic and alveolar hydatid diseases. Parasitol Today 1987;3:144–51.

[18] Cabrera PA, Haran G, Benavidez U, Valledor S, Perera G, Lloyd S, et al. Transmission dynamics of *Echinococcus granulosus*, *Taenia hydatigena* and *Taenia ovis* in sheep in Uruguay. Int J Parasitol 1995;27:807–13.

[19] Schantz PM, Chai J, Craig PS, Eckert J, Jenkins DJ, MacPherson CNL, et al. Epidemiology and control of hydatid disease. In: Thompson RCA, Limbery AJ, editors. Echinococcus and Hydatid disease. Wallingford, UK: CAB International; 1995. p. 233–331.

□寄生虫学□ Full paper

Analyses of Regional Environmental Factors on the Prevalence of *Echinococcus multilocularis* in Foxes in Hokkaido, Japan

Yasushi YOKOHATA[1] and Masao KAMIYA[2]

1) Laboratory of Environmental Biology, Faculty of Education, Toyama University, Gofuku 3190, Toyama City, Toyama 930-8555, Japan
2) Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan (Present address:Center for Advanced Science and Technology, Hokkaido University, Sapporo 001-0021, Japan)
(2003.12.11 受付, 2004.3.31 受理)

北海道におけるキツネの多包条虫 *Echinococcus multilocularis* 感染率に対する地域的環境因子の分析

横畑泰志[1], 神谷正男[2]

1) 富山大学教育学部環境生物学研究室 〒930-8555 富山県富山市五福3190
2) 北海道大学大学院獣医学研究科動物疾病制御学講座寄生虫学教室 〒060-0818 北海道札幌市北18条西9丁目
(現.北海道大学先端科学技術共同研究センター 〒001-0021 北海道札幌市北区北21条西11丁目)

ABSTRACT. Relationships between the prevalence of alveolar hydatid (*Echinococcus multilocularis*) in red foxes (*Vulpes vulpes*) captured in 74 regions of Hokkaido, Japan from 1985 to 1990 and some regional environmental factors (including population density of voles, temperature, snowfall depth, mean degree of slope, mean altitude, human population density, etc.) were examined using simple and multiple regression analysis methods. Eight explanatory variables were selected from 15 types of candidate variables belonging to eight respective categories of the regional environmental factors, based on the simple regression analysis. In the multiple regression analysis, only two of these eight explanatory variables were selected with a stepwise process, and the following model was obtained: $Y = 0.00979X_1 - 0.00037X_2 + 0.23833$ (Y: arcsin-root transformed prevalence of *E. multilocularis* in foxes, X_1: captive number of voles in September, X_2: number of days with snowfall deeper than 50 cm, $r=0.32180$, $P=0.0001$). The higher density of the voles is supposed to make the establishment of the life cycle of this cestode species more successful. The negative influence of deeper snowfall on the prevalence is attributable to the lower predation pressure on the voles by the foxes in deeper snowfall, which suppresses the hunting behavior of the foxes.

Key words : *Clethrionomys* spp., *Echinococcus multilocularis*, environmental factor, multiple regression analysis, *Vulpes vulpes*

Jpn. J. Zoo Wildl. Med. 9(2) : 91-96, 2004

INTRODUCTION

The alveolar hydatid (*Echinococcus multilocularis* (Leuckart 1863)) has a very wide host range and worldwide distribution, and the alveolar hydatid disease (AHD), which is caused by this cestode, causes substantial public health problems in many areas in the world [1]. In Japan, this disease is the most important zoonosis on the main island of Hokkaido, where the major intermediate and final hosts of *E. multilocularis* are red-backed voles of genus *Clethrionomys* and red foxes (*Vulpes vulpes* ; Linnaeus 1758), respectively. More than 200 patients and numerous infected host animals have been found in most areas of Hokkaido since 1937 [2-4]. Intensive monitoring has been performed on the prevalence of *E. multilocularis* on wild, feral and domestic animals in many regions by Hokkaido's Prefectural Government, but this data has never been used for epidemiological study on the AHD, except for Takahashi [5] and Saitoh and Takahashi [6]. Especially, Saitoh and Takahashi [6] examined annual fluctuation of the prevalence of this cestode species in the fox in three areas in eastern Hokkaido by means of multivariate statistical comparison with density of the voles and with that of the cestode in the preceding years with the aspect of delayed density-dependence.

In the present study, an attempt has been made to analyze

Table 1. Simple correlation coefficients (r) between the prevalence of *Echinococcus multilocularis* in foxes *Vulpes vulpes* and each of the 15 variables on eight (1 ~ 8) candidate environmental factors

Categories (units) and/or their variables	r
1. Population density of voles (/0.5 ha/3 nights)	
Number of voles captured in July	0.116
Number of voles captured in August	0.384**
Number of voles captured in September	0.417**
Mean number among three month	0.397**
2. Temperature (℃)	
Total mean temperature	−0.268*
Cumulative mean temperature	−0.371**
3. Snowfall depth (total snow depth: cm; others: days)	
Cumulative snowfall depth	−0.371**
Number of days with snowfall deeper than 10 cm	−0.341**
Number of days with snowfall deeper than 20 cm	−0.390**
Number of days with snowfall deeper than 50 cm	−0.391**
4. Mean degree of slope (m/km)	−0.199
5. Mean altitude (m)	−0.227
6. Human population density (/km)	0.191
7. Number of milch cow per area (/km)	0.287*
8. Percentage of area of forest (transformed%)	−0.314**

*$P < 0.05$, **$P < 0.01$.

cumulative temperature and the number of days with snowfall deeper than 50 cm showed the highest correlation coefficients for the prevalence among the types of variables in respective categories. The correlation matrix among these eight types of variables on the respective categories indicated some significant correlations of pairs of the types of variables (Table 2). The smallest correlation coefficient was calculated between the variables on the population density of voles and the snowfall depth ($r = -0.005$, $P = 0.968$). Multiple regression analysis using these eight types of variables was performed to explain the prevalence of *E. multilocularis* in the foxes, and the following regression model was obtained:

$$y = 0.00979X_1 - 0.00037X_2 + 0.23832$$

$$(r = 0.32180, F = 16.84, P < 0.01)$$

In this model, Y, X_1 and X_2 mean the arcsin transformed prevalence, the captive number of voles in September and the number of days with snowfall deeper than 50 cm, respectively (see Appendix). The other variables were selected out from this model with the stepwise process. The standard error, F value (and P) and standardized partial regression coefficient of X_1 were 0.00232, 17.71 ($P = 0.0001$) and 0.41141, and those of X_2 were 0.00010, 15.50 ($P = 0.0002$) and −0.38485, respectively.

DISCUSSION

The present simple and multiple regression analyses showed that the population density of the red-backed voles is one of the important regional environmental factors on the prevalence of *E. multilocularis* in foxes in Hokkaido. Saitoh and Takahashi [6] performed similar epidemiological analyses on the prevalence of *E. multilocularis* in foxes in Hokkaido. However, their previous analyses treated only three categories of environmental factors i.e., density of voles, temperature and snowfall, and the regions adopted in the work were limited to three Districts in eastern Hokkaido.

Many works have addressed quantitative relationships between both abundance (or density) of parasites and their hosts from theoretical [7, 9] and empirical [6, 8, 10] aspects. These studies show that the higher abundance (or density) of hosts makes a higher prevalence and larger abundance of parasites possible. This result is demonsrated mainly with the more successful establishment of the life cycles of parasites in the higher abundance of hosts [7, 9], which is consistent with the present findings. As above mentioned, Saitoh and Takahashi [6] examined annual fluctuation of the prevalence of this cestode species in the fox in three districts in eastern Hokkaido by means of statistical comparison with density of the voles, and high correlations were shown between the prevalence of cestode and the density of voles of the respective and/or preceding years. Additionally, the functional response of predators (change of exploitation pattern related to the amount of their food resources) may play an important role in the relationships in the case of heteroxenous parasites depending on the prey-predator relationships of intermediate and final hosts [6, 11]. On the other hand, the population density of the foxes in Hokkaido has been measured in only a

Table 2. Correlation matrix among explanatory variables used in multiple regression analysis

	X_1	X_2	X_3	X_4	X_5	X_6	X_7
X_2	−0.492**						
X_3	−0.005	0.136					
X_4	−0.287*	0.287*	0.457**				
X_5	−0.263*	0.251*	0.344**	0.623**			
X_6	0.087	0.176	−0.134	−0.057	−0.102		
X_7	0.593**	−0.409**	−0.216	−0.255*	−0.183	−0.107	
X_8	−0.357**	0.248*	0.292*	0.352**	0.392**	−0.295*	−0.383**

*P < 0.05,**P < 0.01, X_1: number of voles captured in September; X_2: cumulative mean temperature; X_3: number of days with snowfall deeper than 50 cm; X_4: mean degree of slope; X_5: mean altitude; X_6: human population density; X_7: number of milch cow per area; X_8: percentage of area of forest.

few areas (for example, Morishima et al. [4]), so that this data is unfortunately unavailable in this study.

For the prevalence of some heteroxenous parasites dependng on prey-predator relationships between their intermediate and final hosts, predation pressure on the prey by the predator is assumed to be an important factor as well as the population densities of these hosts. The foxes are the important predators of voles in Hokkaido, so that many studies have been performed on their relationships [17-23]. In an area of eastern Hokkaido, it was shown that the hunting pressure on wild rodents, mainly composed of voles, by foxes changes seasonally, with the pressure becoming high in spring and autumn and low in summer and winter remarkably [20]. This phenomenon is attributable to a seasonal change in the amount of ground surface cover with grass and snowfall, which suppresses the hunting behavior of the foxes to the voles. This aspect can explain the function of the snowfall depth as another important environmental factor on the prevalence of *E. multilocularis* in foxes in Hokkaido, i.e, the snow is a barrier to infection of the foxes by this cestode species (also see Lindström [24]). Some reports show or discuss such relationships between the prevalence of *E. multilocularis* and the snowfall depth [4, 6]. Especially, Saito and Takahashi [6] obtained similar result from the present one based on the comparison among three districts with varied amount of snowfalls in Hokkaido. Some programs to control AHD in Hokkaido may be more effective if they are more intensively performed in years with a lower population density of voles and deeper snowfall.

Other than the two factors discussed above, three environmental factors showed significant positive or negative correlations in the single regression analysis, but they were not included in the present multiple regression model. The negative correlation between the prevalence and temperature may be attributable to the higher mortality of eggs of *E. multilocularis* in warmer environments [25]. On the other hand, the number of milch cows per area and the percentage of forest area correlated to the prevalence of *E. multilocularis* in foxes positively and negatively, respectively. It is known that foxes in Hokkaido frequently utilize livestock garbage such as milch cow placentae in winter as a compensative food resource in this season [17] and that the red foxes prefer complex habitats including various types of landscape and avoid uniform habitats simply composed of closed forests [26-27]. Additionally, Yamamoto [28] showed that red foxes in Japan tend to avoid severely sloped habitats, using radiotracking data. The mean degree of slope showed an inverse tendency to the prevalence of this cestode in the foxes, although it was statistically insignificant. As mentioned above, the population density data of the foxes in Hokkaido were unavailable; however, these results may suggest that these two (or three) factors influence the prevalence via the population density of the foxes. These factors showed positive or negative significant correlations with the vole density and/or snow depth (Table 2), so that it is impossible to exclude the possibility that their significant correlations in the simple regreesion analysis may be spurious correlations.

The distribution of *E. multilocularis* and AHD is changing in various areas in the world, including Japan [1-2]. Especially in this country, there are three recent problems on the temporal change on the worm and disease: distribution expanding in western Hokkaido, remarkable increase of the prevalence in the foxes in most of Hokkaido, and possibility to colonize in Honshu [2, 29] (also see the Homepage of Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University; URL: http://vpcserv.vetmed.hokudai.ac.jp/). The present analyses can explain at least partly the contemporary spatial difference of the prevalence of *E. multilocularis* in the foxes in many regions in the Hokkaido with a few environmental factors. In the near future, more available models must be developed to explain both spatial and temporal difference of the distribution and prevalence of the worm in not only Hokkaido, but also other areas in Japan and other counties.

ACKNOWLEDGEMENTS

We thank Dr. K. Nakata, of the Hokkaido Forestry Research

Institute, Dr. T. Saitoh, of the Field Science Center for Northern Biosphere, Hokkaido University, and Drs. M. Ishige and K. Takahashi, of the Hokkaido Institute of Public Health for their useful advice and information. We are also grateful to Dr. S. Takai, of the Information Technology Center, Toyama University for support on the statistical treatments. This work was supported by Grant-in-Aid from the Ministry of Education, Science and Culture, Japan (No. 02954107).

要　約

北海道の各地で 1985 年から 1990 年にかけて捕獲されたアカギツネ *Vulpes vulpes* における多包条虫(タホウジョウチュウ) *Echinococcus multilocularis* の感染率と，地域ごとの環境因子の関係を単回帰および重回帰分析法を用いて分析した。8 つのカテゴリーに属する 15 種類の説明変数のうちから，単回帰分析によってカテゴリーごとに 1 つ，計 8 つの変数を選択した。それらを用いたステップワイズ重回帰分析において，以下の重回帰モデルが得られた：$Y = 0.00979X_1 - 0.00037X_2 + 0.23833$ (Y：平方根ー逆正弦変換を行ったキツネにおける多包条虫の感染率，X_1：9 月におけるヤチネズミ類 *Clethrionomys* spp. の捕獲数，X_2：50 cm 以上の積雪のあった日数，$r = 0.32180$，$P = 0.0001$)。ヤチネズミ類の密度が高いほど多包条虫の生活環が成立しやすくなり，積雪による本条虫の感染率への負の効果は，積雪がキツネの捕獲行動を妨げるためであると考えられた。

キーワード：ヤチネズミ類，多包条虫，環境因子，重回帰分析，アカギツネ

REFERENCES

1. Rausch RL. 1986. Life-cycle patterns and geographic distribution of *Echinococcus* species. In: *The Biology of Echinococcosis and Hydatid Disease* (Thompson RCA, ed.), pp. 44-80. George Allen & Unwin, London.
2. Ohbayashi M. 1986. Echinococcosis-Especially multilocular echinococcosis in Hokkaido. In: *Progress in Veterinary Science 1986* (Izawa H, Shimizu Y, eds.), pp. 59-72. Kindai Shuppan, Tokyo (in Japanese).
3. Iwaki T, Hatakeyama, S, Nonaka N, Miyaji S, Yokohata Y, Okamoto M, Ooi H-K, Oku Y, Kamiya M. 1993. Survey on larval *Echinococcus multilocularis* and other hepatic helminths in rodents and insectivores in Hokkaido, Japan, from 1985 to 1992. *Jpn J Parasitol* 42: 502-506.
4. Morishima Y, Tsukada H, Noknaka N, Oku Y, Kamiya M. 1999. Coproantigen survey for *Echinococcus multilocularis* prevalence of red foxes in Hokkaido, Japan. *Parasitol Int* 48: 121-134.
5. Takahashi K, Mori C. 2001. (translated title) Host animals and prevalenceof *Echinococcus multilocularis* in Hokkaido. *Pub Health Hokkaido* 27: 73-80 (in Japanese).
6. Saitoh T, Takahashi K. 1998. The role of vole populations in prevalence of the parasites (*Echinococcus multilocularis*) in foxes. *Res Popul Ecol* 40: 97-105.
7. Anderson RM, May RM. 1978. Regulation and stability of host-parasite population interactions. I. Regulatory processes. *J Anim Ecol* 47: 219-247.
8. Haukisalmi V, Henttonen H, Tenora F. 1987. Parasitism by helminths in the grey-sided vole (*Clethrionomys rufocanus*) in northern Finland: influence of density, habitat and sex of the host. *J Wildl Dis* 23: 233-241.
9. Dobson AP. 1990. Models for multi-species parasite-host communities. In *Parasite Communities: Patterns and Process* (Esch GW, Bush AO, Aho JM, eds.), pp. 261-288. Chapman and Hall, London.
10. Haukisalmi V, Henttonen H. 1990. The impact of climatic factors and host density on the long-term population dynamics of vole helminths. *Oecologia* 83: 309-315.
11. Haukisalmi V, Henttonen H. 1993. Population dynamics of *Taenia polyacantha* metacestodes in the bank vole *Clethrionomys glareolus*. *Ann Zool Fennici* 30: 81-84.
12. Hinz E. 1988. Geomedical aspects of parasitology. In *Parasitology in Focus* (Melhorn H, ed.), pp. 607-618. Springer-Verlag, Berlin.
13. Ugbomoiko US, Obiamiwe BA. 1991. Helminths of small mammals in a forest zone of Southern Nigeria. *Helminthologia* 28: 19-22.
14. Saitoh T. 1987. A time series and geographical analysis of population dynamics of the red-backed vole in Hokkaido, Japan. *Oecologia* 73: 382-388.
15. Saitoh T, Stenseth NH, Bjornstad ON. 1998. The population dynamics of the vole *Clethrionomys rufocanus* in Hokkaido, Japan. *Res Popul Ecol* 40: 61-76.
16. SAS Institute Inc. 1985. *SAS User's guide: statistics, version 5 edition.* SAS Institute Inc., North Carolina.
17. Abe H. 1975. Winter food of the red fox, *Vulpes vulpes schrencki* Kishida (Carnivora: Canidae), in Hokkaido, with special reference to vole populations. *Appl Ent Zool* 10: 40-51.
18. Misawa E. 1979. Change in the food habits of the red fox *Vulpes vulpes schrencki* Kishida according to habitat conditions. *J Mamm Soc Jpn* 7: 311-320 (in Japanese with English summary).
19. Yoneda M. 1982. Influence of red fox predation upon a local population of small rodents. II. Food habit of the redfox. *Appl Ent Zool* 17: 308-318.
20. Yoneda M. 1983. Influence of red fox predation upon a local population of small rodents. III. Seasonal changes in predation pressure, prey preference and predation effect. *Appl Ent Zool* 18: 1-10.
21. Kondo N, Takahashi K, Yagi K. 1986. Winter food of the red fox, *Vulpes vulpes schrencki* Kishida, in the endemic area of multilocular echinococcosis. *Mem Preparative Off Nemuro Mun Mus* 1: 23-31.
22. Tsukada H, Nonaka N. 1996. Foraging behavior of red foxes *Vulpes vulpes schrencki* utilizing human food in the Shiretoko National Park, Hokkaido. *Mamm Study* 21: 137-151.
23. Tsukada H. 1997. External measrements, breeding season, litter size, survival rate, and food habits of red foxes (*Vulpes vulpes schrencki*) in the Shiretoko National Park. *Bull Shiretoko Mus* 18: 35-44 (in Japanese with English summary).
24. Lindström ER. 1994. Vole cycles, snow depth and fox population. *OIKOS* 70: 156-160.
25. Ishige M, Ito T, Yagi K. 1993. Temperature effects on life span of *Echinococcus multilocularis* eggs. *Rep Hokkaido Inst Public Health* 43: 49-51.
26. Nakazono T. 1989. The habitat utilization pattern of Japanese red fox, *Vulpes vulpes japonica*, in Kyushu. *Mamm Sci* 29: 51-62 (in Japanese with English summary).

27. Jedrezejewski W, Jedrezejewska B. 1995. Foraging and diet of the red fox *Vulpes vulpes* in reaction to variable food resources in Bialowieza National Park, Poland. *Ecography* 15: 212-20.
28. Yamamoto Y. 1996. Home range and habitat utilization of the red fox *Vulpes vulpes japonica* in the sub-alpine belt in central Japan. *Nat Envir Sci Res* 9: 47-61 (in Japanese with English summary).
29. Kamiya K, Iwaki T, Yokohata Y. 2002. *Echinococcus* spp. In: *Handbook of Alien Species in Japan* (The Ecological Society of Japan, ed.), pp. 224-225. Chijin Shokan, Tokyo (in Japanese).

Appendix. Two significant variables of environmental factors in the present analysis (number of voles captured in September (X_1) and number of days with snowfall deeper than 50 cm (X_2)) in 74 regions in Hokkaido, Japan.

Regions	X_1	X_2	Regions	X_1	X_2	Regions	X_1	X_2
Chitose	9.0	252	Asahi	18.8	596	Obihiro	12.6	28
Mori	6.9	105	Teshio	36.7	325	Sarabetsu	15.8	172
Minamikayabe	6.4	46	Haboro	10.6	391	Memuro	13.2	74
Shikabe	9.5	77	Engaru	13.1	149	Makubetsu	9.9	97
Bibai	9.0	420	Wakkanai	21.4	290	Otofuke	12.3	52
Esashi	6.1	434	Sarufutsu	19.5	465	Shihoro	19.5	21
Kaminokuni	2.7	118	Utanobori	16.2	612	Kamishihoro	8.2	246
Assabu	6.4	250	Oumu	18.5	217	Teshikaga	12.3	141
Kumaishi	3.9	279	Kunneppu	8.1	139	Taiki	16.4	253
Otobe	5.4	179	Bihoro	18.8	186	Shintoku	18.6	61
Imagane	9.0	349	Memanbetsu	10.4	54	Shimizu	22.1	66
Kitahiyama	4.0	268	Abashiri	13.0	54	Shikaoi	13.9	59
Kuttyan	11.1	603	Shari	15.6	241	Ikeda	8.9	4
Kimobetsu	8.3	501	Kiyosato	14.4	271	Toyokoro	11.6	40
Kuromatsunai	16.8	344	Koshimizu	16.2	271	Kushiro (City)	20.9	0
Yoichi	10.4	435	Soubetsu	16.0	251	Hamanaka	18.3	0
Yubari	6.8	186	Otaki	7.7	526	Shiranuka	8.7	5
Kuriyama	12.7	272	Noboribetsu	23.9	152	Onbetsu	12.2	64
Iwamizawa	15.1	483	Atsuma	3.2	66	Akkeshi	14.7	41
Mikasa	12.7	374	Hayakita	9.1	134	Nemuro	39.8	27
Takanosu	13.5	482	Mukawa	14.3	0	Nakashibetsu	24.3	162
Aibetsu	18.3	553	Shiraoi	7.2	176	Bekkai	39.2	52
Nayoro	17.4	487	Oiwake	4.5	134	Sapporo	9.5	294
Bifuka	23.2	585	Shizunai	13.2	116	Hakodate	14.0	54
Shibetsu	11.2	538	Biratori	6.8	131			

these monitoring data for critical factors that determine the regional degree of endemicity of AHD from many candidate regional emvironmental factors, and to clarify relationships between the incidence and these factors, based on single and multiple regression analyses.

DATABASE AND METHODS

The regional incidence of AHD in Hokkaido has been monitored yearly in the prevalence of *E. multilocularis* in red foxes, racoon dogs (*Nyctereutes procyonoides* ; Gray 1834), rodents, feral cats, feral dogs, and domestic animals including horses and pigs [5]. However, datasets on these animals other than the red foxes were available only in small parts of Hokkaido and their sample sizes were often very small or unknown. Hence, in this study, the prevalence of this cestode in the foxes examined from 1985 to 1990 was used as the criterion variable in 74 regions (Cities, Towns and Villages; see Appendix) where more than 40 foxes were examined in this period.

In general, the degree of endemicity of parasitosis in humans and animals and the prevalence of their causal parasites are often dependent on various environmental factors, such as host density [7-11], climate [6, 10, 12-13] and topography [12]. In this study, 15 types of candidate explanatory variables belonging to eight categories of environmental factors, i.e., population density of the voles, temperature, depth of snowfall, mean degree of slope, mean altitude, human population density, number of milch cows per area and percentage of forest area, were collected from various sources. The datasets of the population density of voles were accumulated every July, August and September at hundreds of census points in national, prefectural and private forests in Hokkaido by the Forestry Agency of the Japanese National Government and the Hokkaido Prefectural Government, based on the number of voles captured with each 50 snap trap set in an area of 0.5 ha for serial three nights [14-15]. The density data of the voles used in the present study was collected from 1980 to 1989 in private forests, which are more frequently located near human activity and probably more important on the epidemiology of AHD than the national and prefectural forests. Most of these data are on gray red-backed voles (*Clethrionomys rufocanus* ; Sundevall 1846), and very small numbers of *C. rutilus* (Pallas 1779) and *C. rex* Imaizumi 1971 are included in them [15]. The data on climatic factor were obtained from datasets by Sapporo District Meteorological Observatory from 1985 to 1989, from which six types of variables were used for the analyses, including total mean and cumulative mean temperatures, cumulative snowfall depth and number of days with snowfall deeper than 10, 20 and 50 cm (The detasets are shown in the homepage of Japan Meteorological Agency; http://www.data.kishou.go.jp/index.htm). The total mean and cumulative mean temperatures were from the monthly mean of respective values for the five years. The cumulative snowfall depth was the sum of snowfall depth for each day of the snowfall in each month from January to April and in December. The mean degree of slope in an inland region was obtained from the altitudinal difference divided by the horizontal distance between the highest and lowest points in the region. In seaside regions, the alternative mean degrees of slope were calculated according to the following formula:

$$M.D.S.=2A\,/\,(N+F)\ ,$$

where M.D.S. and A are the mean degree of slope and the altitude of the highest point, respectively. N and F are the respective horizontal distance from the highest point to the nearest and farthest points on the coastline of the region. The mean altitude is the mean of the altitudes of the highest and lowest points in each region. These altitudes and distances were measured on 1/200,000 topographical maps of Hokkaido by Geographical Survey Institute of Japan. Data of the human population density and the number of milch cows were based on the National Population Census of the 1985 (shown in the homepage of Hokkaido Prefectural Government; http://www.pref.hokkaido.jp/skikaku/sk-kctki/index.html) and 1980 World Census of Agriculture, respectively. The area of forest was based on the datasets of public information by the Hokkaido Prefectural Government in 1990. Some regions lacked data on the density of the voles, temperature or snowfall depth, where the means of variables of surrounding regions were used instead.

The prevalence of *E. multilocularis* in the foxes and the percentage of forest area were used after arcsin-root transformation. Simple correlation coefficients were calculated between this prevalence and all 15 types of candidate explanatory variables of the eight categories of regional environmental factors (Table 1) to determine any apparent relationships between the prevalence of the cestode in foxes and these environmental factors (Table 1). In three categories with multiple types of variables, i.e., population density of voles, temperature and depth of snow, each type of variable showing the highest simple correlation coefficient in this analysis was sellected for adoption in later analyses. A correlation matrix among the eight variables of respective categories was constructed to examine the relationships between each pair of the variables. Multiple regression analysis using the prevalence of *E. multilocularis* in the foxes and these eight variables as criterion and explanatory variables, respectively, with a selection process of these explanatory variables by stepwise method was performed with the REG and STEPWISE Procedures in SAS Program Version 5 [16].

RESULTS

Simple correlation coefficients between the prevalence of *E. multilocularis* in foxes and each of the 15 types of variables were shown in "r" of the Table 1. The population density of voles, temperature, snowfall depth, number of milch cows per area and percentage of forest area significantly correlated with the prevalence. The captive number of voles in September, the

エキノコックス症感染源対策の経済評価

Economic evaluation of counter-measures against echinococcosis using baits with anthelminthics

大石卓史*・嘉田良平*・有路昌彦*・山根史博**

Takafumi OISHI*, Ryohei KADA*, Masahiko ARIJI* and Fumihiro YAMANE**

要旨：本稿では，人獣共通感染症であるエキノコックス症対策として，近年有効性が確認されつつある感染源対策（キタキツネや犬の体内からのエキノコックスの駆虫）の実施によりもたらされるリスク削減便益を，CVM（Contingent Valuation Method：仮想評価法）を用いて検証した。北海道内の4市町を対象に二段階二肢方式のCV調査を実施し，パラメトリック推定法により感染源対策に対する支払意志額（WTP）を計測した結果，1世帯あたりの年間WTPは，中央値でおよそ2,000ー3,000円，平均値でおよそ2,500ー4,500円の範囲にあることがわかった。

キーワード：エキノコックス症対策，CVM

Abstract：In this paper, we try to evaluate risk reduction benefits by counter-measures against echinococcosis using baits with anthelminthics. We applied contingent valuation method based on double-bounded dichotomous choice approach at 4 areas in Hokkaido. As a result, The median WTP estimated are between about 2,000 - 3,000 yen per year. The mean WTP are between about 2,500 - 4,500 yen as well.

Key Words：Counter-measures against echinococcosis, Contingent Valuation Method

はじめに

エキノコックス症は，エキノコックスと呼ばれる寄生虫の虫卵がヒトの口から体内に入り，幼虫となって肝臓などに寄生することで，10ー15年の無症状期を経た後，重い肝機能障害などを引き起こす人獣共通感染症であり，切除以外に有効な治療法がなく致死率も高い。ヒトへの感染経路としては，エキノコックスが寄生したキタキツネや犬などの終宿主やその糞に触れる，それらの糞で汚染された農産物を食べる，沢水・わき水を飲むなどのケースがあると考えられている。また，1980年代以降，キタキツネのエキノコックス感染域が北海道東部域から全道域に拡大したことに伴い，近年，札幌市周辺などの北海道東部域以外の地域においても，エキノコックス症認定患者数の増加が見られており[1]，感染者数の増加などの人的リスクに加え，北海道の基幹産業である農業や観光業などへの経済的損失の発生といった，エキノコックス症関連リスクを最小限に抑えるための体制をいかに構築するかが喫緊の課題となっていると言える[2]。

エキノコックス症への対策[3]としては，患者の早期発見や治療の実施，衛生教育・予防啓発活動，飲み水対策としての上水道整備，感染源であるキタキツネの捕殺などがこれまでに行われてきた。

これらの対策のうち，患者の早期発見や治療の実施は，エキノコックス症に感染した患者の生存率を高めることを目的としたものであり，また，衛生教育・予防啓発活動や上水道整備は，キタキツネや犬などの終宿主のエキノコックス感染率が高まりつつある状況下において，ヒトのエキノコックス症への感染を防止することを目的としたものである。これらは，現在存在するエキノコックス症感染リスクの対策として非常に重要であると

* (株) UFJ総合研究所，** 京都大学大学院地球環境学舎

言えるものの，感染源対策，すなわち，キタキツネや犬などの終宿主のエキノコックス感染率を低減させるものではないために，エキノコックス症の撲滅という根本的な問題解決には直結しないものである。

また，キタキツネの捕殺は，感染源対策として位置付けられ，年間1万頭前後の規模での捕殺が行われた時期もあったものの，対策に要する費用的・時間的制約が大きいために，捕殺が間引き程度の規模に留まってしまったこと，また，動物愛護の観点から，キタキツネを捕殺することに対する合意形成が困難であったことなどから，十分な対策効果を上げることができなかった[4]。

このような中，キタキツネの捕殺に代わる感染源対策として，ベイトと呼ばれる駆虫薬入りの餌を屋外に散布することで，キタキツネや犬の体内からエキノコックスを駆虫する（虫下しを行う）方法の有効性が近年の研究で確認されてきている（神谷（2003），野中（2003））。この方法は，キタキツネや犬などの終宿主を捕殺する必要がないため，それらの動物との共生関係を保ちつつ，エキノコックス感染率を低減できるという利点を有しており，その本格実施に向けた検討を行うことが急務であると考えられる[5]。

そこで本稿では，駆虫薬散布による感染源対策を我が国におけるエキノコックス症撲滅に向けた手段と位置付け，対策の実施によりもたらされるリスク削減便益を，表明選好アプローチの1つである CVM（Contingent Valuation Method：仮想評価法）を用いて検証する。

本稿の構成は以下の通りである。1．では，エキノコックス症感染源対策の概要について述べる。2．では，本稿で使用するデータについて述べる。3．では，感染源対策の実施によりもたらされるリスク削減便益の計測方法及びその計測結果について述べる。最後に，本稿の帰結及び今後の課題についてまとめる。

1．エキノコックス症感染源対策の概要

エキノコックス症感染源対策として，キタキツネや犬などの終宿主の体内から，エキノコックスの駆虫（虫下し）を行う研究が北海道大学や北海道立衛生研究所により行われている。北海道大学が1997年から5年間にわたり小清水町で行った試験では，ベイトと呼ばれる駆虫薬入りの餌を屋外に散布することで，散布域内のキタキツネのエキノコックス感染率が概ね 70%から 10%へと低減し，効果的にキタキツネを駆虫できる可能性が確認された。また，現在，この感染源対策の実用化に向け，同様の研究が小樽市や根室市において行われている（神谷（2003），野中（2003））。

本稿では，このようにエキノコックス症対策として有効性が確認されつつある感染源対策の実施によりもたらされるリスク削減便益を，CVM を用いて検証する。

2．分析データ

本稿では，平成16年1月に郵送方式にて実施された，エキノコックス対策に関するアンケート調査において得られた CV データを対象に分析を行う。このアンケート調査は，図1に示す北海道内の4市町（札幌市（中央区・北区），小樽市，富良野市，小清水町）において実施されたものである[6]。アンケート調査票の回収状況及び WTP 推定に用いた標本サイズは表1に示すとおりである。

ここで，被験者に支払意志額（Willingness to pay；以下，WTP）を尋ねる質問方式としては，図2に示す二段階二肢方式を採用し，一段階目及び二段階目の提示額は表2に示すA～Dの4タイプを設定した[7]。なお，被験者のシナリオへの理解を高めるため，図2の質問に先駆け，小清水町における感染源対策の効果（散布域内のキタキツネのエキノコックス感染率が 70%から 10%へと低減した点）やキタキツネのエキノコックス感染率とヒトへの感染との関係についての説明を行っている。

また，標本サイズの決定は，WTP 推定に関わるアンケート項目にすべて回答している被験者を標本として取り出した後，抵抗回答を表明している被験者を除外することで行っている。

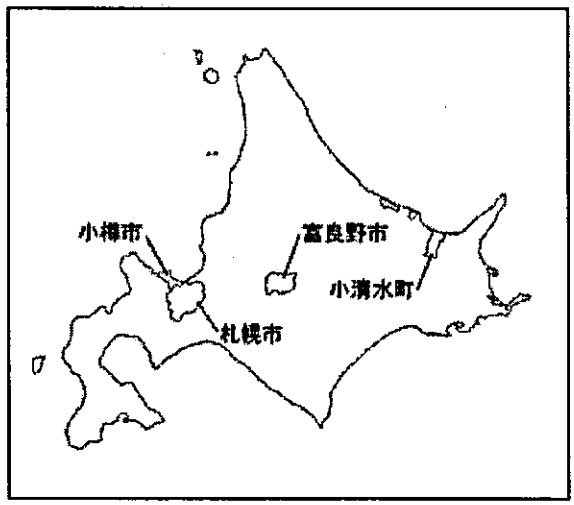

図1　アンケート調査実施地区

表1　アンケート調査票の回収状況・標本サイズ

	配布数	回収数	標本サイズ
札幌市	600	153	84
小樽市	600	143	86
富良野市	600	184	91
小清水町	600	248	177
計	2,400	728	438

表2　提示額の設定

	初期提示額	二段階目の提示額	
		高提示額	低提示額
A	500円	1,000円	250円
B	1,000円	3,000円	500円
C	3,000円	5,000円	1,000円
D	5,000円	10,000円	3,000円

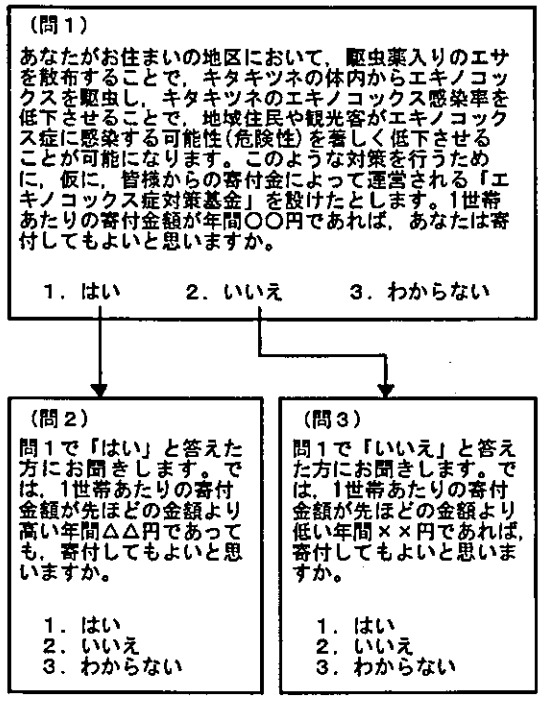

図2　WTP質問方式

3．リスク削減便益の計測

3．1　計測モデル

WTPを推定するための計測モデルとしては，寺脇(2002)を参考に，間接効用アプローチによるパラメトリック推定法を採用した。

また，WTP分布として対数ロジスティック分布を仮定した上で[8]，以下のStepにより，支払行動関数の特定化を行った。

まず，Step1：表3に示すすべての説明変数を含むモデルを推定し，t値の絶対値が1以下となる説明変数を除外した。Step2：次に，1度目のモデル推定において除外された説明変数を除いた残りの説明変数を含むモデルを再び推定し，t値の絶対値が1以下の説明変数を除外した。Step3：Step2の作業をt値の絶対値が1以下の説明変数が存在しなくなるまで続けることで，説明変数の組み合わせを決定した。

3．2　支払行動関数の推定結果

支払行動関数の推定結果は表4に示すとおりである[9]。

各市町の支払行動関数に含まれている説明変数の係数の符号についてみると，LBD（提示額の対数）は，4市町すべてのモデルに含まれており，係数の符号は負となっている。これは提示額が高くなるほど支払行動をとりにくくなることを示しており，整合的な結果であると言える。

INCOME（年収）及びPET（ペット（犬，ネコ）の飼い方）は，小樽市，富良野市のモデルに含まれており，その係数の符号はともに正となっている。これは年収が高くなるにつれて，また，ペットを屋外で飼っているほど，支払行動をとりやすくなることを示しており，整合的な結果であると言える。

RECOG2（駆虫薬散布の知識）は，富良野市のモデルに含まれており，その係数の符号は正となっている。これは駆虫薬散布の知識がある場合に支払行動をとりやすくなることを示しており，整合的な結果であると言える。

CIRCUM（周辺環境）及びANXIOUS（エキノコックス症感染への不安）は，小清水町のモデルに含まれており，その係数の符号はともに正となっている。これは家の周囲にキタキツネが生息しやすい環境がある場合，また，エキノコックス症感染への不安がある場合に支払行動をとりやすくなることを示しており，整合的な結果であると言える。

一方，SEX（性別）やYHABIT（居住年数）も複数の市町のモデルに含まれているが，地域により係数の符号が異なる結果となった。

表3　支払行動関数の推定時に用いた説明変数の候補

名称	定義	変数内容
SEX	性別	「男性」=1，「女性」=0
AGE	年齢	「20歳代」=1，「30歳代」=2，「40歳代」=3，「50歳代」=4，「60歳代」=5，「70歳以上」=6
INCOME	1世帯あたりの所得	「200万以下」=100，「201万ー400万円」=300，「401万ー600万円」=500，「601万ー800万円」=700，「801万ー1,000万円」=900，「1,001万ー1,500万円」=1,250，「1,501万ー2,000万円」=1,750，「2,001万円以上」=2,250
NHOUSE	世帯員数	数値データ（人）
NCHILD	小学生以下の子供の有無	「小学生以下の子供有」=1，「小学生以下の子供なし」=0
OCCUP	職業（農家）	「農家」=1，「非農家」=0
YHABIT	居住年数	「1年未満」=1，「1ー5年」=2，「6ー10年」=3，「11ー20年」=4，「21年以上」=5
CIRCUM	周辺環境	「家から500m以内に河川敷，野山，自然公園，田・畑，防風林のいずれかあり」=1，「なし」=0
PET	ペット（犬，ネコ）の飼い方	「飼っていない」=1，「いつも屋内で飼っている」=2，「だいたい屋内で飼っている」=3，「だいたい屋外で飼っている」=4，「いつも屋外で飼っている」=5
OUTDOOR	登山・キャンプ・自然公園への訪問回数	「行かない」=1，「年間1ー2回」=2，「年間3ー4回」=3，「年間5ー9回」=4，「年間10回以上」=5
RECOG1	認識1（エキノコックス）	「エキノコックスの流行を知っている」=1，「エキノコックスの流行を知らない」=0
RECOG2	認識2（駆虫薬散布の知識）	「駆虫薬散布による感染源対策を知っている」=1，「知らない」=0
ANXIOUS	エキノコックス症感染への不安	「日頃不安である」=1，「そうでない」=0
LBD	提示額の自然対数	数値データ（円）

表4　支払行動関数の推定結果

札幌市

変数名	係数	t値	p値
CONST	11.27	6.86	0.00
SEX	0.37	1.81	0.07
LBD	-1.62	-6.83	0.00
対数尤度			-86.80
AIC			167.59

小樽市

変数名	係数	t値	p値
CONST	19.89	5.10	0.00
SEX	-0.87	-1.31	0.19
INCOME	9.26E-04	1.13	0.26
NCHILD	-1.25	-1.55	0.12
YHABIT	-0.31	-1.35	0.18
PET	0.50	1.07	0.29
LBD	-2.55	-5.23	0.00
対数尤度			-55.92
AIC			97.85

富良野市

変数名	係数	t値	p値
CONST	12.65	6.68	0.00
SEX	0.94	1.74	0.08
INCOME	1.50E-03	2.75	0.01
PET	0.42	1.54	0.12
RECOG2	1.24	1.76	0.08
LBD	-1.89	-7.13	0.00
対数尤度			-67.84
AIC			123.67

小清水町

変数名	係数	t値	p値
CONST	11.46	6.34	0.00
SEX	-1.07	-1.97	0.05
NHOUSE	0.23	1.32	0.19
NCHILD	-0.68	-1.04	0.30
YHABIT	0.46	2.82	0.00
CIRCUM	0.98	2.06	0.04
ANXIOUS	0.86	1.93	0.05
LBD	-1.77	-7.95	0.00
対数尤度			-121.567
AIC			227.134

3．3　リスク削減便益の推定結果

各地域で推定された支払行動関数から算出した，1 世帯あたりの年間 WTP の中央値及び平均値を図3に示した[9]。

これによると，WTP 中央値はおよそ 2,000ー3,000 円，WTP 平均値はおよそ2,500ー4,500円の範囲にあることがわかった[10]。

WTP 評価額が最も高くなったのは小清水町であり，WTP 中央値は3,120 円，WTP 平均値は4,580 円となった。これは，小清水町では，1997 年から感染源対策の研究が行われており，被験者の感染源対策に関する認知度やその効果に対する理解度が高かったことや，世帯主をアンケート調査の対象としたことが一因であると思われる[11]。

また，小清水町以外の市町村では，被験者の感染源対策に対する認知度が低かったにもかかわらず，一定程度の評価額が得られており，特に，富良野市の評価額は，WTP 中央値が 2,828 円，WTP 平均値が 4,054 円と小清水町に近い値となっている。このことは，エキノコックス症関連リスクが高まりつつある地域において，リスクの内容や感染源対策の目的・効果についての理解を図ることで，感染源対策の強化・推進が可能であることを示唆するものと言えるだろう。

おわりに

本稿では，北海道内の 4 市町を対象に，エキノコックス症対策として，近年，その有効性が確認されつつある感染源対策の実施によりもたらされるリスク削減便益を，CVM を用いて検証した。その結果，1 世帯あたりの年間 WTP は，中央値でおよそ 2,000ー3,000 円，平均値でおよそ 2,500ー4,500 円の範囲にあることがわかった。

今後は，本稿で明らかとなった WTP 評価額を参考に，駆虫薬の生産・散布方法やそれに要する費用，行政や地域住民などのステイクホルダー間での費用の負担割合など，感染源対策の本格実施に向けた検討を行う必要があるであろう。また，同時に，感染源対策と患者の早期発見・治療や衛生教育・予防啓発活動などの対策との効果的な組み合わせ方法，ステイクホルダー間でのリスクコミュニケーションをより適切に行うための体制などについて検討を行うことも必要と考えられる。これらは残された課題である。

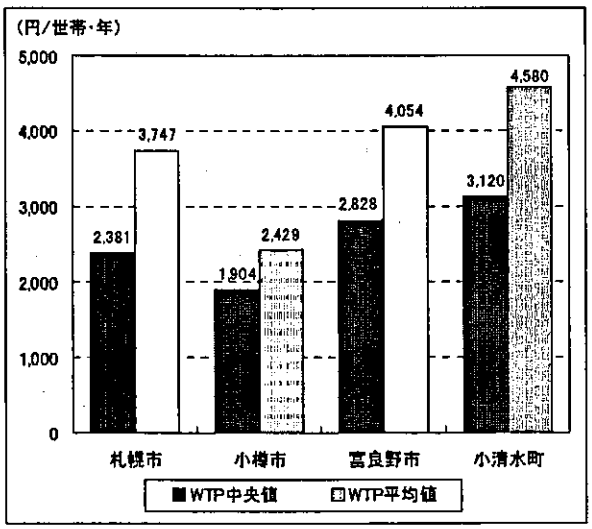

図3　WTP 推定結果

謝　辞

本研究を進めるにあたり，北海道大学大学院獣医学研究科寄生虫学教室の神谷正男教授，奥祐三郎助教授，野中成晃助手，巌城隆客員研究員からは，多くのご支援・ご指摘を得ました。また，アンケート調査の実施に当たっては，札幌市，小樽市，富良野市，小清水町の各自治体担当者の皆様に多大なご協力を賜りました。記して感謝の意を表します。

補　注

1) 現在，北海道内のキタキツネの感染率は 60%前後であると言われている（厚生労働省ホームページ<http://www.forth.go.jp/mhlw/animal/page_j/i04-2.html>）。また，通常，野生動物での感染状況は，数年から 10 数年経てヒトの感染者数に反映すると考えられており，1995 年時点において，今後 15ー20 年の間に約 1,000 名の新規患者が発生するとの試算も行われている（土井，1995）。なお，2004 年（1 月 1 日ー9 月 5 日）に報告されたエキノコックス症患者数は計 22 名であり，そのうち 12 名が札幌市保健所管内からのものとなっている（北海道感染症情報センターホームページ<http://www.iph.pref.hokkaido.jp/kansen/402/data.html>）を参照されたい。

2) このような状況を受け，平成 15 年 11 月に施行された「感染症の予防及び感染症の患者に対する医療に関する法律及び検疫法の一部を改正する法律」では，エキノコックス症は，消毒，動物の輸入禁止等の対策措置が必要な新 4 類感染症に分類されている。

3) エキノコックス症対策の詳細については，北海道大学大学院獣医学研究科寄生虫学教室のホームページ<http://133.87.224.209/index.html>，北海道立衛生研究所のホームページ<http://www.iph.pref.hokkaido.jp/default.htm>を参照されたい。

4) キタキツネの捕殺については，捕殺を行った地域に新たなキタキツネが流入し，キタキツネの移動が促進されることで，エキノコックスの感染域が拡大する可能性についても指摘されている。

5) 厚生労働省においても，従来の対策だけでは不十分であり，患者の早期発見や治療の実施に加え，早急に感染源対策が必要である，との認識がなされている。（厚生労働省ホームページ<http://www.forth.go.jp/mhlw/animal/page_j/i04-2.html>）

6) 本アンケート調査は，「動物由来寄生虫症の流行地拡大防止対策に関する研究（厚生労働省科学研究費補助金（新興・再興感染症研究事業））」の一環として実施したものである。アンケート実施地域については，地域属性を勘案しつつ，札幌市（都市的地域），小樽市（地方中核都市），富良野市（農村地域（駆虫薬未散布地域）），小清水町（農村地域（駆虫薬散布地域））の 4 市町を選定した。なお，札幌市，小樽市，富良野市は選挙人名簿登録者全数を，小清水町は選挙人名

簿登録者のうち世帯主のみを抽出対象とし，無作為抽出により配布先の選定を行った。また，アンケート調査票の冒頭において，世帯主もしくは主な収入を得ている方に回答を依頼する旨の文章を添えた。

[7] 図2に示した各質問において「わからない」と回答した被験者は，分析対象外とした。

[8] WTP 分布としては，対数ロジスティック分布もしくは対数正規分布のいずれかを仮定することが一般的となっているが，どちらの分布を仮定しても，WTP 推定結果に大きな差異が生じないことが多いことから，本稿では，対数ロジスティック分布のみを採用し，分析を行った。

[9] WTP 平均値は，最高提示額（10,000 円）で生存関数の切断を行い算出した。

[10] 各市町で得られた WTP 推定結果に，住民基本台帳に登録されている世帯数（平成 16 年 1 月 31 日時点）及び標本選択率（=標本サイズ/調査票回収数）を乗じることで年間総便益の試算したところ，札幌市の年間総便益は 1,144 百万円（WTP 中央値），1,799 百万円（WTP 平均値）に，小樽市の年間総便益は 77.5 百万円（WTP 中央値），98.9 百万円（WTP 平均値）に，富良野市の年間総便益は 14.8 百万円（WTP 中央値），21.2 百万円（WTP 平均値）に，小清水町の年間総便益は 4.9 百万円（WTP 中央値），7.1 百万円（WTP 平均値）になった。

[11] エキノコックス対策に関するアンケート調査では，感染源対策の認知度に関する質問も行っている。有効回答の中で，感染源対策を「よく知っている」もしくは「聞いたことはある」と答えた割合は，札幌市26.4%，小樽市26.6%，富良野市32.6%に対し，小清水町では59.3%と高くなった。

引用文献

土井陸雄(1995) 憂慮されるエキノコックス症(多包虫症)流行の拡大 ―今こそ第一次予防対策推進を―．日本公衆衛生雑誌，42(2)，63-68.

神谷正男(2003)エキノコックス症感染源対策は急務！．第 50 回日本寄生虫学会・日本衛生動物学会 北日本支部合同大会 国際シンポジウム．

寺脇 拓(2002)農業の環境評価分析．勁草書房，東京，247pp.

野中成晃(2003)キツネとペットにおけるエキノコックス症対策― 北大・獣医・寄生虫学教室の取り組み―．第 50 回日本寄生虫学会・日本衛生動物学会 北日本支部合同大会 国際シンポジウム．

弘 前 医 学 56:37 44, 2005

総 説

条虫の石灰小体の性状とその機能

長 内 理 大　神 谷 晴 夫

抄録 すべての条虫類は，石灰小体と呼ばれるミネラルを多量に含む構造物を持っている．これが条虫の生存・寄生適応に深くかかわっている可能性が強く示唆されている．しかしながら，その機能については分子生物学的な側面からの知見も含め未知の部分が多い．この総説では，これまでの知見を基に，今後，石灰小体に係わるタンパク質や遺伝子発現などの研究がどのように進んでいくか考察した．

弘前医学 56:37—44, 2005

キーワード: 条虫; エキノコックス; 石灰小体; 機能.

REVIEW

PROPERTIES AND FUNCTIONS OF CESTODE CALCAREOUS CORPUSCLES

Arihiro Osanai and Haruo Kamiya

Abstract All cestodes contain mineral concretions termed calcareous corpuscles and these concretions are likely to be involved in the parasite survival in host environment. Nevertheless, the precise function of calcareous corpuscles is still unclear and has not been studied from the view of biochemistry and molecular biology. This review intends to summarize the published litertures about the properties, formation and function of calcareous corpuscles and to discuss how the biochemical and molecular biological approaches on calcareous corpuscles will be concerned in the host-parasite relationship.

Hirosaki Med. J. 56:37—44, 2005

Key words: cestode; *Echinococcus* spp.; calcareous corpuscles; function.

1. 緒　言

石灰小体 (Calcareous corpuscles) は，条虫を特徴付ける構造物である．古くは，18世紀にすでに石灰小体に関する記述があるが[1]，実際に生物学的な研究が始まったのは1950年以降で，構成成分，形態，形成過程，化学的性状などについての報告がなされてきた．しかし，石灰小体が，条虫の寄生・生存にいかなる役割を果たしているのか，つまりその機能についてはほとんど明らかにされていない．今後，エキノコックス症など難治性幼条虫症の駆虫薬開発のために，条虫の巧みな寄生戦略を解析し，その機能を明らかにする過程は避けて通れない．この総説においては，これまでの石灰小体に関わる情報を整理し，また最近少しずつ報告されるようになってきた石灰小体とタンパク質との係わりについてまとめ，さらに石灰小体という条虫特有な構造を標的とした駆虫薬の開発の可能性を探りたい．なお，石灰小体の生理学的研究に関しては，簡潔にかつ網羅的にまとめ

Department of Parasitology, Hirosaki University School of Medicine
Correspondence: H. Kamiya
Received for publication, January 20, 2005
Accepted for publication, Janurary 31, 2005

弘前大学医学部医学科寄生虫学講座
別刷請求先：神谷晴夫
平成17年1月20日受付
平成17年1月31日受理