Materials and methods

Study area

Hokkaido is the northernmost island of Japan and the vole is the most common small mammal there. The Forest Agency of the Japanese Government has been carrying out a monitoring census of vole populations in forests all over Hokkaido since 1954. A statistical report on hunting (Department of Health and Welfare, the Hokkaido Government 2000) showed that the population of red foxes increased until around 1980, and thereafter was fairly constant. Epidemiological surveys on *E. multilocularis* have been carried out by the Department of Health and Welfare of the Hokkaido Government, Council for Alveolar *Echinococcus*. We focused on two infected provinces, Nemuro and Abashiri, located in eastern Hokkaido, to allow us to adjust the model with the aid of ecological, epidemiological and meteorological parameters. Table 1 shows the prevalence of *E. multilocularis* in the fox population in these provinces.

Life cycle of *E. multilocularis*

The life cycle of *E. multilocularis* consists of a free-living egg phase and parasite stages in each of the two hosts. The vole has protoscoleces of *E. multilocularis* 1 or 2 months after orally ingesting eggs. Experimental infections in voles (*C. rufocanus*) showed that all voles were susceptible to *E. multilocularis* eggs, that the infectious protoscoleces in hydatid cysts increased with time, and that the protoscoleces were first detected in some voles at 41 days after ingestion, and in all voles by 44 days (Yagi and Ito 1998). Thus, we adopted 45 days as the period (τ_v) for the acquisition of infectiousness against the definitive host in voles.

The definitive host is infected with *E. multilocularis* by preying on voles which harbor multilocular *Echinococcus* with infectious protoscoleces. In experimental infections of foxes, parasite eggs were initially detected in the feces 29–33 days after infection (Nonaka et al. 1996), and at 29–32 days (Yagi and Ito 1998), but were not detected thereafter until 66–84 days after infection (Nonaka et al. 1996). Abundant eggs were excreted from the experimentally infected foxes during the initial week of egg output, and thereafter the number of eggs fell off rapidly (Yagi and Ito 1998). Thus, our model uses 30 days as the period (τ_f) for the maturity of *E. multilocularis* in foxes, 7 days as the duration (τ_h) of high parasite egg production and 60 days as that (τ_l) of low parasite egg production.

Longevity of *E. multilocularis* eggs

The longevity of *E. multilocularis* eggs is influenced by environmental factors. In particular, the duration of the egg's infectious ability is swayed by temperature and humidity (Yagi and Itoh 1999). The experimental formula for longevity (d days) at temperature (t°C) was obtained as $d=\exp[-0.135(t-43.4)]$ from experimental infections in mice (Ishige et al. 1993). However, this formula is inapplicable to low temperatures (Yagi and Ito 1999). As the seasonal climatic effect on the longevity of eggs governs the transmission dynamics of *E. multilocularis* from foxes to the intermediate hosts, we assume in the model that the seasonal transmission rate from foxes to voles is proportional to the relative seasonal longevity (e_r) of eggs as compared with the longevity of eggs at 13.8°C (temperature in autumn in Nemuro province), which is calculated by the experimental formula with the proviso that the upper bound of longevity is fixed at 100 days, taking into consideration a low temperature period and of the limitations of the formula. The relative longevity of eggs in Nemuro and Abashiri provinces is shown in Table 2. The relative longevity will be used in the model for the risk of the human population being infected with HAE.

Dynamics of host populations

It is important in terms of the dynamics of the transmission of *E. multilocularis* to consider the large-scale seasonal fluctuations in the host populations.

The breeding season of red foxes in Hokkaido is generally from the last third of March to the first third of April. Newborns emerge from their dens after weaning, which has been assessed at about 1 month after birth (Uraguchi and Takahashi 1998). We assumed that newborns would be exposed to *E. multilocularis* infection after weaning. The average litter size was estimated as 4.05 in Nemuro (Uraguchi and Takahashi 1998), and 4.0 in Koshimizu (Abe 1971) which was also in the eastern part of Hokkaido. The birth rate (b_f) in the population of foxes can be expressed by the product of litter

Table 1 Prevalence of *Echinococcus multilocularis* in the fox populations. Derived from the Department of Health and Welfare, the Hokkaido Government in Annual Report of the Council for alveolar echinococcus in Hokkaido, 2001–2002

Year	Nemuro			Abashiri		
	Number examined	Number positive	Prevalence (%)	Number examined	Number positive	Prevalence (%)
1995	82	62	75.6	28	12	42.9
1996	97	38	39.2	20	5	25.0
1997	89	28	31.5	22	10	45.5
1998	60	46	76.7	35	23	65.7
1999	23	7	30.4	22	10	45.5
2000	39	27	69.2	19	10	52.6
Total	390	208	53.3	146	70	47.9

Table 2 The relative longevity of *E. multilocularis* eggs. The mean air temperature was derived from the Japan Meteorological Agency, 1994–1999, for relative longevity the standard temperature was set as 13.89°C in autumn in Nemuro

Season	Nemuro		Abashiri	
	Mean air temperature (°C)	Relative longevity	Mean air temperature (°C)	Relative longevity
Spring	8.74	1.86	11.24	1.43
Summer	16.16	0.74	18.53	0.53
Autumn	13.89	1	13.71	1.02
Winter	−0.02	1.86	−1.14	1.86

Table 3 The assigned values for age- and season-dependent birth rates of voles

Age group (q)	Litter Size			Percentage of breeding females			Birth rate ($b_{v,q}$)		
	Spring	Summer	Autumn	Spring	Summer	Autumn	Spring	Summer	Autumn
2	0	4.5	5.2	0	50	50	0	1.13	1.3
3	0	4.5	5.2	0	60	85	0	1.35	2.21
4	6.2	4.8	5.3	100	70	100	3.1	1.68	2.65

size, sex ratio and percentage of breeding females; we adopted $b_f = 1.8$ where the values of litter size, sex ratio and percentage of breeding females were taken as 4.0, 0.5 and 90%, respectively. Generally, for any wild animal the death rate of juveniles is significantly higher than that of adults. We estimated the death rates of juveniles (under 1 year old) (δ_j) and adults (δ_a) as 0.82 and 0.33, respectively, on the assumption that the annual growth rate of the fox population would remain stable, based on the census data of the age structure (Uraguchi and Takahashi 1991, 1998). These figures are in accordance with the data obtained in Kushiro and Nemuro; $\delta_j = 0.86$ and 0.84 for 1971–1972 and 1977–1979. The corresponding values for the adults were $\delta_a = 0.33$ and 0.39 (Yoneda 1981). The density/km^2 (N_f) of the fox population has been used as a measure in the model. It was estimated that $N_f = 0.7 \sim 1.0$ before the breeding season at Nemuro in 1986–1996 (Uraguchi and Takahashi 1998).

The gray-sided vole (*C. rufocanus*) breeds in three seasons of the year (all except winter). Only females surviving the winter become pregnant in spring, and the spring-born females then mature during the summer to comprise a major part of the breeding population in the autumn. Thus, the pregnancy rate is higher in spring and autumn than in summer (Kaneko et al. 1998). The maturation period is about 30–60 days, and the gestation period is 18–19 days (Abe 1968). A typical litter size is 4–7, with an average of 5.3 (Fujimaki 1981; Yoccoz et al. 1998), and the order of decreasing litter size was reported to be spring, autumn and summer (Fujimaki 1975). The percentage of breeding females is higher in spring and autumn than in summer (Ota 1984). The birth rate (b_v) in the vole population, which can be defined as a product of litter size, sex ratio which is assumed to be 0.5, and percentage of breeding females, has two peaks a year, one in spring and one in autumn. 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 (Ohta 1984), while the survival rate in winter is higher than that in summer (Dewa 1975). Although the dynamics of vole populations in Hokkaido vary on a large scale annually and have certain geographical characteristics such as a periodical fluctuation with a 3.5–4.5 year cycle in the northern and eastern areas of Hokkaido, we assumed that the annual growth rate of the vole population would remain stable and assigned age- and season-dependent death and birth rates which would result from age- and season-dependent litter sizes, as well as the percentage of breeding females, as shown in Tables 3 and 4. As a measure of the abundance of the vole population, the model uses the density/km^2 (N_v). It was estimated that $N_v = 0.30 \times 10^3$ in spring (before the breeding season), giving 2.84×10^3 in autumn in Nemuro (1984–1993), and $N_v = 0.63 \times 10^3$ in spring, or 1.74×10^3 in autumn in Abashiri (1983–1992) (census data on vole populations from the Forest Agency of the Japanese Government).

Table 4 The assigned values for age- and season-dependent death rates of voles

Age group (q)	Death rate ($\delta_{v,q}$)			
	Spring	Summer	Autumn	Winter
0	0.5	0.5	0.5	0.25
1	0.25	0.25	0.25	0.25
2	0.2	0.2	0.2	0.25
3	0.2	0.2	0.2	0.25
4	0.25	0.25	0.25	0.25

Transmission model of *E. multilocularis*

We propose a deterministic model for the transmission of *E. multilocularis*. The model works well provided that there is no immigration or emigration of foxes in the target area. There is no need to take into account any immigration or emigration of voles owing to their narrow and exclusive home range (Saitoh 1991; Ishibashi et al. 1998). The fox population is divided into four epidemiological classes: negative, infected but not producing parasite eggs, high parasite egg production, and low parasite egg production, which are symbolized by x_1, x_2, x_3, x_4, respectively. The vole population is divided into three classes: negative, infected but not yet infectious, and infectious (holding protoscoleces in hydatid cysts), which are designated y_1, y_2, y_3, respectively. Each epidemiological class of the fox populations is classified into two age groups: juveniles (j) and adults (a), while each epidemiological class of the vole population is classified into five age groups: 0–1 month (0), 1–2 months (1), 2–3 months (2), 3–4 months (3), > 4 months (4). The symbols of age groups (a, j, 0~4) are used as suffixes of the above variables for epidemiological classes. All the variables used in the model are tabulated in Table 5.

E. multilocularis can be transmitted to a fox when it ingests an infected vole containing protoscoleces in hydatid cysts. Therefore, the prevalence of *E. multilocularis* in the fox population is affected by their feeding habits; that is, the average number (NVF) of voles ingested by a fox each day. NVF depends on the density of the vole population and on the depth of snow (Abe 1975; Yoneda 1981). Thus, we introduce a feeding habit function $f(N_v, d_s)$ for vole density (N_v) and the depth of snow (d_s) as NVF. We assume that $f(N_v, d_s) = 0$ for $d_s > 50$ (cm), because a fox cannot prey on voles that live under deep snow. A fox feeds on voles by preference, but it does have other available food (Yoneda 1981). Thus, the model assumes that the NVF increases swiftly at a low fox density, while the degree of increasing is reduced at a high density, and that NVF becomes saturated at the maximum number (m_N) of voles ingested by a fox each day at a fairly high density, which was estimated as 8 (Abe 1975). Applying the logistic distribution (W) to the relationship between NVF and N_v, the feeding habit function is written as:

$$f(N_v, d_s) = g(d_s) m_N W(N_v),$$

$$W(N_v) = 1/\left(1 + \exp\left(-k(N_v - \overline{N}_v)\right)\right)$$

Table 5 The variables used in the *Echinococcus* transmission model. For symbol: $p = j,a$ representing the age group of juveniles and adults; $q = 0$–4 representing the age groups 0–1 month, 1–2 months old, 2–3 months old, 3–4 months old and > 4 months old, respectively

Host	Symbol	Description (density per 1 km^2)
Fox	$x_{1,p}$	Negative
	$x_{2,p}$	Infected, no parasite egg producing
	$x_{3,p}$	Infected, high parasite egg producing
	$x_{4,p}$	Infected, low parasite egg producing
	N_f	Total density
Vole	$y_{1,q}$	Negative
	$y_{2,q}$	Infected but not yet infectious
	$y_{3,q}$	Infectious (holding protoscoleces in hydatid cysts)
	N_v	Total density

where $g(d_s)$ stands for the function which obstructs foxes from preying on voles whose value decreases from 1 ($d_s < 10$) to 0 ($d_s > 50$). The shape parameters k and $\bar{N}_v$ were chosen as 0.003 and 1.2×10^3 from experimental data. The transfer rates among epidemiological classes in the fox population are composed of several epidemiological parameters. The symbol λ signifies the infection rate from the negative stage (x_1) to the infected, but no egg production, stage (x_2) and R, the recovery rate from the high production of eggs-stage (x_4) to the negative stage (x_1) in due consideration of multiple infection, while P_j, P_a, and Q_j, Q_a signify the transfer rates for the age groups of juveniles (j) and adults (a) from the infected but no egg production stage (x_2) to the high production of eggs-stage (x_3), and from the high production stage (x_3) to the low production stage (x_4), respectively. These transfer rates are expressed by the following formulae:

$$\lambda(t) = s_0 f(N_v(t)) y_3(t)/N_v(t)$$

$$P_p(t) = (1-\delta_{f,p})^{\tau_f}\lambda(t-\tau_f), \quad (p = j, a)$$

$$Q_p(t) = (1-\delta_{f,p})^{\tau_f+\tau_h}\lambda(t-(\tau_f+\tau_h)), \quad (p = j, a)$$

$$R(t) = \lambda(t)/(\exp(\tau_l\lambda(t)) - 1)$$

In the above formula, s_0 denotes the conditional probability that protoscoleces in a vole can mature in a fox; s_0 is assessed as 0.9 because of the high probability of infection with inoculated protoscoleces.

Voles can be infected with *E. multilocularis* by ingestion of free-living eggs that are excreted by the infected foxes. We introduce the transfer factors μ_0, μ and ρ. μ_0 is the basic infectious contact rate from the low egg producing class of foxes at the standard temperature, while μ is the infectious contact rate from the low egg producing class that is obtained by multiplying μ_0 by e_r (the relative seasonal longevity of eggs). ρ is the multiplicative factor for the basic infectious contact rate from the high egg producing class. Due to a lack of direct survey data, we will investigate the values of μ_0 and ρ on the basis of prevalence data for the populations of foxes and voles in the next section. In the model, we assume that a vole at the infected but not yet infectious stage moves to the infectious stage after τ_v days, and that no voles recover from *E. multilocularis*.

The basic scheme of the model is shown in Fig. 1a (for the population of foxes) and 1b (for the population of voles). The parameters used in the model are tabulated with the assumed and adjusted values in Table 6.

Results

Dynamics models of host populations

In order to act in concert with the transmission model for *E. multilocularis*, we constructed a seasonal population dynamics model of foxes based on a stable annual growth rate (the density/km^2 in spring being 1.0) on the basis of the ecological parameters in Table 6. We also constructed a seasonal population dynamics model of voles based on a stable annual growth rate (the density/km^2 in spring being 0.3×10^3) on the basis of the ecological parameters in Tables 3 and 4.

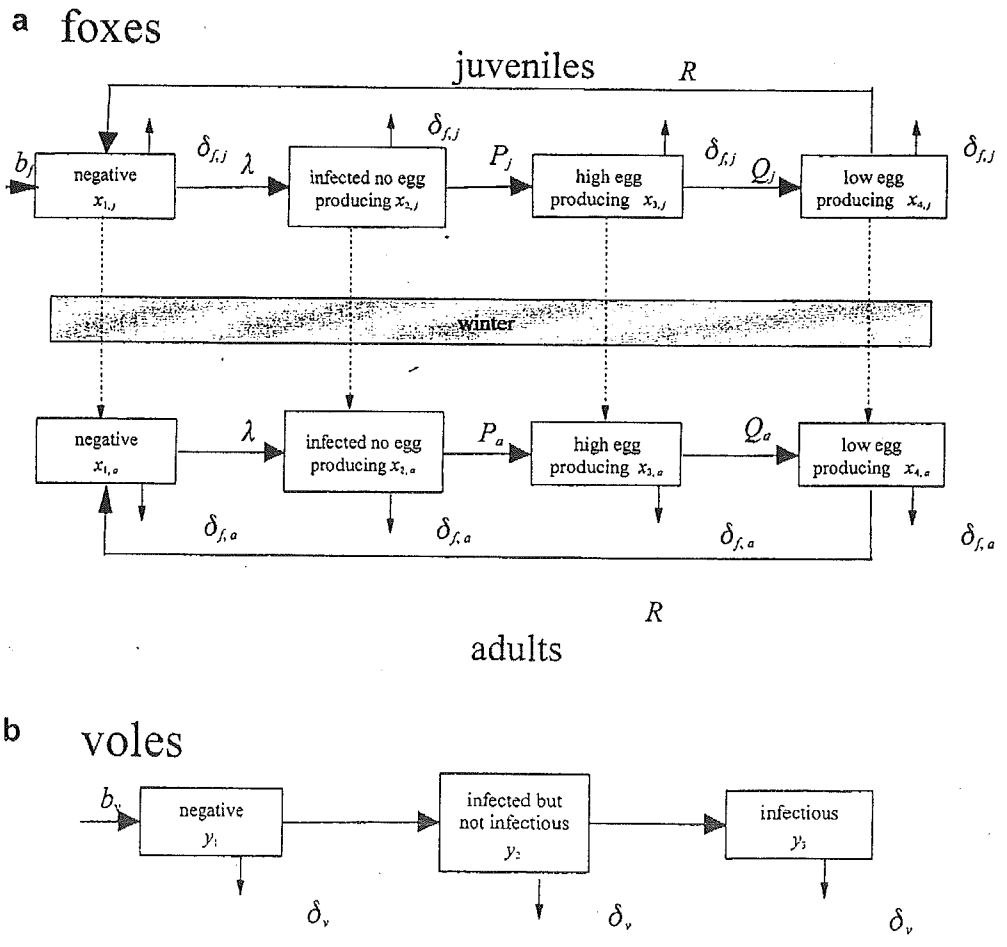


Fig. 1 The basic schemes for the transmission model for *Echinococcus multilocularis* in fox populations: **a** the transfers among four epidemiological classes, and vole populations, **b** the transfers among three epidemiological classes; the division into five age groups being omitted

Table 6 The model parameters together with their assumed and adjusted values. For basic infectious contact rate see text

Description	Symbol	Estimated value
Fox birth rate per year	b_f	1.8
Vole birth rate per month ($q=2,3,4$)	$b_{v,q}$	see Table 3
Fox death rate per year (juveniles)	$\delta_{f,j}$	0.82
Fox death rate per year (adults)	$\delta_{f,a}$	0.33
Vole death rate per month ($q=0,1,2,3,4$)	$\delta_{v,q}$	see Table 4
Period of no parasite egg production in foxes (days after infection)	τ_f	30
Duration of high parasite egg production (days)	τ_h	7
Duration of low parasite egg production (days)	τ_l	60
Period for acquiring infectious protoscoleces in voles (days after ingestion)	τ_v	45
The conditional probability of maturity in foxes	s_0	0.9
The basic infectious contact rate	μ_0	$(1.0\sim3.4)\times10^{-4}$
Multiplicative factor caused by high egg production	ρ	1~10

Fitting parameters

The basic reproductive rate (R_0) is the maximum number of secondary infections that are theoretically possible when one infected fox is introduced into a susceptible population: any secondary cases derive from the intermediate populations that are infected through eggs produced by one infected fox. The basic reproductive rate in the model, which can be calculated on the simplified assumption that any seasonal factors or any multiple infection are ignored, is expressed by the combination of several parameters in the model as:

$$R_0 = \left(\mu_0\rho\tau_h + \frac{\mu_0\tau_l}{1+\delta_f\tau_l}\right)\frac{1}{1+\delta_f\tau_f}\cdot\frac{1}{1+\delta_v\tau_v}\cdot\frac{1}{1+\delta_f\tau_h}\cdot\frac{s_0\bar{f}N_f}{\delta_v}$$

The symbols $\bar{f}$, δ_f and δ_v represent the average of NVF, the average of the daily death rates for the total population of foxes and that for the total population of voles, estimated as 6.6, 3.26×10^{-3} and 1.19×10^{-2}, respectively, while the other symbols in the formula are referred to in Table 6. In the previous sections, we assigned the estimated or adjusted values to all the above parameters, except for two transfer factors, the infectious contact rate from the low egg producing class (μ_0) and the multiplier of the basic infectious contact rate from the high egg producing class (ρ). Applying these values in the formula, R_0 can be written as a function of μ_0, ρ and N_f:

$$R_0 \approx 1.45\times10^4(1+1.39\times10^{-1}\rho)\mu_0 N_f$$

It is difficult to decide on the two parameters μ_0 and ρ from the field data because of the presence of free-living eggs in the environment, although it is more important to decide on an applicable value of μ_0 than of ρ. Since the ratio of parasite egg discharge in the high production period compared to that in the low production period was found to be more than five times greater in the experimental infection of foxes (Yagi and Ito 1998), ρ is limited to within the scope of (1, 10) and a value of 5 is adopted in the following simulations. When the permissible range of R_0 is arranged from 1 to 20, the value of μ_0 is allowed to vary within the range (4.0×10^{-5}–8.1×10^{-4}), (2.7×10^{-5}–5.4×10^{-4}) or (1.9×10^{-5}–3.8×10^{-4}) at $\rho=1$, 5 or 10, respectively, on choosing 1.5/km^2 as the average density of foxes N_f. We examined the highest, lowest and average yearly prevalence rates of *E. multilocularis* in the fox population for the range (μ_0) from 0.1×10^{-4} to 5.0×10^{-4} in steps of 1.0×10^{-5} with the model simulations under the Nemuro situation. The curves for the prevalence rates are shown in Fig. 3.

When the permissible ranges of the highest and average prevalence rates (%) have their limits set to (55, 90) and (40, 75) making allowance for Table 1, the value of μ_0 is allowed the bounds (1.0×10^{-4}–3.4×10^{-4}). We carry out simulations within these bounds in the next section.

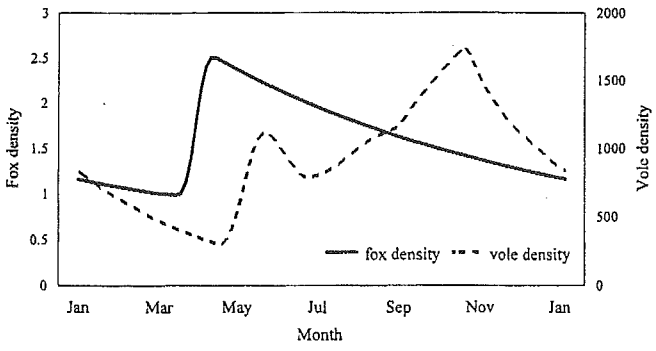


Fig. 2 The seasonal population dynamics models of foxes and voles. The *solid line* and the *dotted line* show the variations in fox or vole density/km^2, respectively, on the basis of our population dynamics models

Simulations for the prevalence of *E. multilocularis*

The transmission model for *E. multilocularis* was programmed by Fortran 90 to work on any computer under the Microsoft Windows TM platform.

Firstly, to investigate the effect of seasonal transitions on the prevalence of *E. multilocularis* in both host populations, foxes and voles, under the situations in

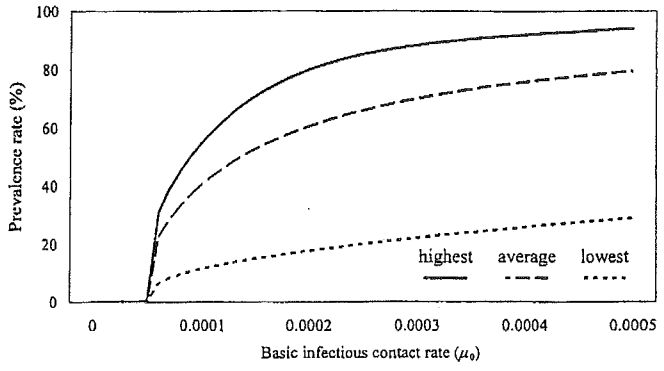


Fig. 3 The prevalence in the fox population corresponding to the basic infectious contact rate (μ_0) in the model. The *solid line*, the *broken line* and the *dotted line* show the highest, the average and the lowest yearly prevalences, respectively

Nemuro and Abashiri, we planned the simulations with μ_0 chosen as 1.5×10^{-4}, the value that realized the average prevalence rates during 1995–2000 in Nemuro (53%) and Abashiri (48%) (Table 1). There is a great difference between the two provinces in terms of snowfall. The Japanese Meteorological Agency (1986–1997) reported that the average depth of snow in the winter 3 months (January–March) was 36.1 cm and 66.2 cm, and that the number of days above 50 cm was 0 days and 72 days in Nemuro and Abashiri, respectively. The simulations indicate that the density of infected foxes goes down to its lowest during the second third of March (Nemuro), and the first third of April (Abashiri) before the breeding season, thereafter reaching its peak during the second third of June due to an increase in infected juveniles. Although the prevalence rate in fox populations is highest during the first third of October due to a decrease in the total population, the timing of the lowest prevalence is synchronized with that of the lowest infectious density. This result also shows that there are some differences in the winter prevalence of the fox populations between the Nemuro and Abashiri areas, that is, the winter density of infected foxes maintains a certain level in Numero, while it falls to a low level in Abashiri.

Secondly, we investigated the risk to the human population of being infected with HAE using model simulations. In this article, such a risk is expressed as the proportion of parasite eggs in the environment relative to the maximum abundance in the Nemuro area, which is referred to as the hazard index. The simulations were also carried out for the environs of Sapporo, the capital of Hokkaido, where the fox population density was assumed to be half as much as that in Nemuro. The simulation for Nemuro indicates that the hazard index is highest during the first third of June when infected juveniles begin to discharge parasite eggs, and that it goes down for a while during a high temperature period and then rises again to reach a second peak in October. A comparative study indicates that the hazard index fluctuates widely and has a sharp peak under the Nemuro conditions (high density of fox population), while it varies little and remains at a low level throughout the whole year in the environs of Sapporo (low fox density).

Discussion

It is important to consider the relationship between the model and reality. It is unavoidable for the transmission model for *E. multilocularis* to have a somewhat complicated structure, and consequently to involve many ecological, as well as epidemiological, parameters because *E. multilocularis* has a complicated life cycle involving two kinds of hosts. Through experimental and field data, we obtained estimated values for all parameters with two exceptions (μ_0, ρ) and the number of exceptional parameters is low considering the complicity of the model. We set bounds for the above two parameters on the basis of field data for the prevalence rate of *E. multilocularis*, so that the model fits regional situations where *E. multilocularis* prevails.

A distinctive feature of the model is the division of the production stage in foxes into two classes according to egg output; this is adequate for investigating the risk of infection in the human population. In the model, we considered neither the acquisition of immunity nor intensity of infection which is related to the protoscolice burden in the voles that are preyed on by the fox. Further precise research on transmission models based on the intensity of infection, or models incorporating immunity, is desirable for tracking the progress of experimental infections in foxes.

The process of transmission of *E. multilocularis* from foxes to voles is controlled by the state of free living eggs in the environment the longevity of which depends on environmental factors. We considered the effect of air temperature on the longevity of eggs, but not the effect of humidity, which also has an influence (Yagi and Itoh, 1999). This leaves some room for the consideration that there would be some lag time between the discharge of eggs from foxes and the oral ingestion of eggs by voles, and that the free living eggs would accumulate in the environment. However, to avoid a more complicated structure in the model, we assumed that the discharge of eggs had an immediate contribution to the transmission of *E. multilocularis* in voles.

On the other hand, the process transmitting *E. multilocularis* from voles to foxes seems more clear, as it is governed by predation on the voles. We think that the introduction of a feeding habit function for foxes, which depends on the vole density and depth of snow, is adequate to describe the above transmission stage.

For the allowable bounds (1.0×10^{-4}–3.4×10^{-4}) of the basic infectious contact rate (μ_0), the bounds of the highest, the average and the lowest yearly prevalence rates (%) in the population of voles were estimated as (2.8, 12.2), (1.1, 4.7) and (0.4, 1.6), respectively, while the Annual Report of the Council for Alveolar *Echinococcus* in Hokkaido, 2001–2002 (Department of Health and Welfare, the Hokkaido Government 2002) reported that

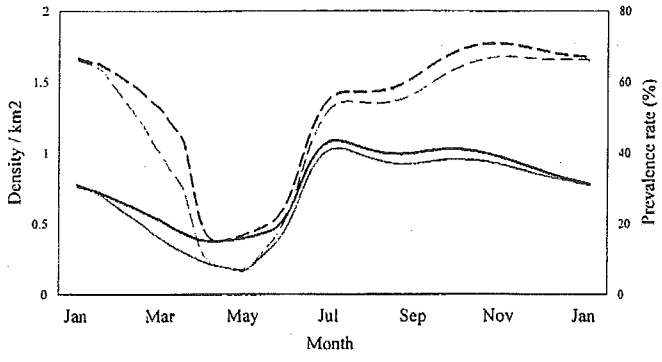


Fig. 4 Seasonal variation in the density/km^2 of infected foxes for *E. multilocularis* (*solid lines*) and seasonal variation in the prevalence of *E. multilocularis* in the fox population (*broken lines*). The *black* and *gray lines* show the Nemuro and Abashiri situations, respectively

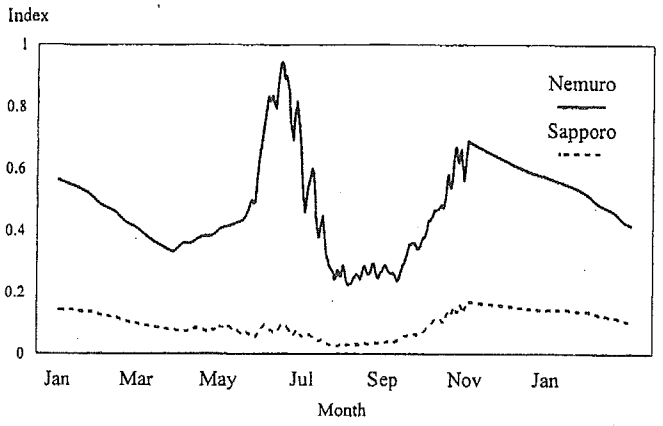


Fig. 5 Seasonal variation in the hazard index. The *solid line* shows the Nemuro situation, and the *dotted line* the environs of Sapporo

the prevalence rate in the vole population of Nemuro, northern Hokkaido varied widely from 0.0% to 17.3%, and that its average for 20 years (1981–2000) was 4.6%. The above bounds of μ_0 seem adequate to use in simulations from the point of view of prevalence in voles; it takes into account the difficulty in finding the exact prevalence in vole populations in the field, as well as the fact that there are some hot spots of voles infected with *E. multilocularis*.

The effect of multiple infection introduced into the model has a great influence on the prevalence of *E. multilocularis* in fox populations. A comparative study shows that the highest and average yearly prevalence rates in the fox population with multiple infection are about 1.4–1.6 and 1.4–1.8-fold higher than those without multiple infection for $\mu_0 > 1.4\times10^{-4}$, and that the highest prevalence rate without multiple infection asymptomatically tends towards the equilibrium prevalence rate, which is calculated on the simplified assumption (referred to in the fitting parameter subsection) and is given as the formula for the basic reproductive rate (R_0): 0.65 ($1-1/R_0$), as μ_0 tends to be larger. The field data on the prevalence in Nemuro (Table 1) support the effect of multiple infection in the model.

Although little is known about the seasonal transition in the prevalence of *E. multilocularis* due to the lack of seasonal prevalence data in the populations of foxes and voles, the simulation clarifies the mechanism of seasonal transmission for *E. multilocularis* quantitatively, and this can be helpful in designing strategies for its control. Bearing in mind that the peak density of infected foxes does not necessarily coincide with that of the prevalence (Fig. 4), it is essential to pay more attention to transition, rather than density, when attempting to control the source of infection. A difference in the prevalence between Nemuro and Abashiri was also indicated by the field data (Table 1; Saitoh and Takahashi 1998); the simulation showed that this was caused by a difference in snowfall between the two provinces.

The hazard index depends not only on the air temperature, but also on other environmental factors, including humidity. Although we only took air temperature into account for the hazard index, it is reasonable to assume that there is some danger an individual would be infected with HAE during the high hazard index period as shown in Fig. 5. Eckert et al. (2002) proposed preventive measures and safety precautions to prevent human echinococcosis. These included education, careful handling of wild berries and vegetables etc., but they did not mention the seasonal fluctuations in egg dispersal in the environment which are shown in the present report. Consideration of seasonal fluctuations in parasite egg dispersal is necessary when planning more suitable preventive measures against *E. multilocularis*, although there may be different types of seasonal fluctuations in egg dispersal according to the countries or provinces, as shown between Nemuro and Sapporo in the present article. Therefore, further careful research is necessary before planning measures relating to different countries and provinces because there may be various types of seasonal fluctuations of temperature, snow fall and ecological factors such as the population density of vole predators.

Acknowledgments We would like to thank Profs. M. Kamiya, Y. Oku and T. Saitoh of Hokkaido University, Dr. K. Takahashi of Hokkaido Institute for Public Health, and Prof. A. Ishii of the Jichi Medical School for their helpful comments. We are indebted to the Forestry Agency of the Japanese Government for providing the census data on vole populations, and also to the Department of Health and Welfare, the Hokkaido Government for providing the hunting data on foxes. This work was supported in part by Grant-in Aid for Scientific Research from Japan Society for the Promotion of Science (grant no. 13640116) and by grants from the Ministry of Health, Labour and Welfare, Japan for "The control of emerging and reemerging diseases in Japan".

References

Abe H (1968) Growth and development in two forms of *Clethrionomys*, 1: external characters, body weight, sexual maturity and behavior (in Japanese with English summary). Bull Hokkaido Forest Exp Stn (Bibai) 6:69–89

Abe H (1971) Studies on the ecology of a fox in the eastern part of Hokkaido (in Japanese). Department of Health and Welfare, Hokkaido Government, Sapporo

Abe H (1975) Winter food of red fox, *Vulpes vulpes schrencki* Kishida, in Hokkaido, with special reference to vole population. Appl Entomol Zool 10:40–51

Anonymous (1995) Annual Reports of the Council for Alveolar *Echinococcus* in Hokkaido 1994. Department of Health and Welfare, Hokkaido Government

Dewa H (1975) Seasonal variation of the daily activity rhythms in snow season (in Japanese with English summery). Res Bull College Exp For Hokkaido Univ 22:105–120

Doi R (1995) A critical situation of the prevalence of echinococcosis (alveolar hydatid disease)—necessity immediate action for the prevention (in Japanese). Jpn J Public Health 42:63–68

Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS (2002) WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern. WHO, Paris

Fujimaki Y (1975) Reproductive activity in *Clethrionomys rufocanus bedfordiae*. 3. Regional differences in autumn reproductive activity (in Japanese with English summary). Bull Hokkaido Forest Exp Stn 13:38–45

Fujimaki Y (1981) Reproductive activity in *Clethrionomys rufocanus bedfordiae*. 4. Number of embryos and prenatal mortality. Jpn J Ecol 31:247–256

Ishibashi Y, Saitoh T, Kawata M (1998) Social organization of the vole *Clethrionomys rufocanus* and its demographic and genetic consequences: a review. Res Popul Ecol 40:39–50

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

Kaneko Y, Nakata K, Saito T, Stenseth NC, Bjornstad ON (1998) The biology of the vole *Clethrionomys rufocanus*: a review. Res Popul Ecol 40:21–37

Nonaka N, Iida M, Yagi K, Ooi HK. Oku Y, Kamiya M (1996) Time course of coproantigen excretion in *Echinococcus multilocularis* infections in foxes and an alternative definitive host, golden hamsters. Int J Parasitol 26:1271–1278

Ohbayashi M (1996) Host of *Echinococcus multilocularis* in Hokkaido. In: Uchino J, Sato N (eds) Alveolar echinococcosis: strategy for eradication of alveolar echinococcosis of the liver. Fujishoin, Sapporo, pp 59–64

Ota K (1984) Study on wild murid rodents in Hokkaido (in Japanese). Hokkaido University Press, Sapporo

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

Saitoh T (1991) The effects and limits of territoriality on population regulation in gray red-backed voles, *Clethrionomys rufocanus bedfordiae*. Res Popul Ecol 32:391–406.

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

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

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, p 228

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 Inst 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

Yoccoz NG, Nakata K, Stenseth NC, Saitoh T (1998) The demography of *Clethrionomys rufocanus*: from mathematical and statistical models to further field studies. Res Popul Ecol 40:107–121

Yoneda M (1981) Red fox, *Vulpes vulpes schrencki* Kishida: population and their food habits, 2 (in Japanese). Hoppou Ringyo/Northern Forestry 33:285–288

Exp. Anim. 52(1), 25–30, 2003

Establishment of an Enzyme-Linked Immunosorbent Assay for Detection of Hantavirus Antibody of Rats Using a Recombinant of Nucleocapsid Protein Expressed in Escherichia coli

Akira TAKAKURA[1)], Kazuo GOTO[1)], Toshio ITOH[1)], Kumiko YOSHIMATSU[2)], Ikuo TAKASHIMA[3)], and Jiro ARIKAWA[2)]

[1)]Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki, 216-0001, [2)]Institute for Animal Experimentation, School of Medicine, Hokkaido University, Kita-ku, kita-15, Nishi-7, Sapporo, 060-0817, and [3)]Laboratory of Public Health, Department of Environmental Veterinary Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Kita-ku, Kita-18, Nishi-9, Sapporo, 060-0818, Japan

Abstract: *A recombinant nucleocapsid protein of Hantaan virus (HTN) 76–118 strain expressed in* E. coli *was applied as a serodiagnostic antigen in an enzyme-linked immunosorbent assay (rHTN-ELISA) for detection of hantavirus antibody in rat sera. The sensitivity and specificity of the rHTN-ELISA were compared with those of the indirect immunofluoresent assay (IFA) using virus-infected cells. The sensitivity of rHTN-ELISA was similar to that of the IFA both in experimentally SR-11 infected rat and naturally infected rat sera. Sera showing a low antibody titer in IFA and suspected to be negative by other methods were also found to be negative in rHTN-ELISA. These results indicate that rHTN-ELISA is effective as a screening method for serodiagnosis of hantaviruses, because of its high sensitivity, specificity, safety and suitability for processing large number of samples.*
Key words: *ELISA, hantavirus, rats, recombinant antigen*

Introduction

Hemorrhagic fever with renal syndrome (HFRS) is a rodent-borne viral zoonosis characterized by fever, hemorrhagic manifestations and renal disorder [8–10, 14]. The causative agents hantaviruses, are classified into the genus *Hantavirus*, family *Bunyaviridae*. To date, eight virus types have been grouped in the genus according to antigenetic and genetic characteristics [1, 2, 4, 6, 7, 10]. These are Hantaan, Seoul, Puumala, Prospect Hill, Thailand, Dobrava, Thottapalayam and Sin Nombre viruses. Among these, five are pathogenic to humans. Hantaan, Seoul, Puumala and Dobrava viruses have been identified as etiologic agents of HFRS and recently, Sin Nombre virus was identified as the causative agent of adult respiratory distress syndrome (ARDS) or hantavirus pulmonary syndrome (HPS) [3]. Air-borne transmission of hantavirus between rodents

(Received 12 January 2002 / Accepted 22 July 2002)
Address corresponding: A. Takakura, Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki, 216-0001, Japan

or from rodents to human beings has been achieved via excreta of persistently infected rodents [8, 10]. Therefore, extensive serologic monitoring of rodents and eradication of seropositive animals is most important for the prevention of human infection in laboratory animal facilities.

The indirect immunofluoresent antibody (IFA) test with infected Vero cell antigen has been widely used as the standard method for serologic diagnosis of hantavirus infection [2, 9, 10]. However, the IFA has practical problems: requirement of a biosafety level 3 containment laboratory to prepare antigens and avoid biologic hazard, and specificity and sensitivity problems related to exclusion of false-positive and false-negative test results due to strong background staining which may overwhelm the specific reaction, or production of dots closely resembling the specific profiles. Confusion at the time of IFA evaluation due to the appearance of false-positive and negative reactions has caused problems in microbiological control of laboratory animal facilities. In addition, IFA is not suitable for screening large numbers of samples.

Enzyme-linked immunosorbent assay (ELISA) has been used for the detection of many virus-specific antibodies [5, 13, 15, 19]. ELISA measuring virus-specific serum IgG antibodies have been shown to be more sensitive than the hemagglutination inhibition test or the complement fixation test [13], and are suitable for screening large numbers of samples. In the preparation of viral antigens used in ELISA, propagation, concentration, purification, inactivation and quality control are usually required.

A serological test using recombinant antigens has been reported for many pathogens [5, 11, 12, 15–18]. If a recombinant viral protein is used as an antigen for ELISA, the risk in the processing of antigen preparations can be eliminated and many high quality antigens can be produced. In the present study, we attempted to develop an ELISA using biotinylated recombinant hantavirus nucleocapsid (NP) protein expressed in *E. coli* coated avidin-coated microplates as an antigen for hantavirus antibody screening in rats.

Materials and Methods

Anti sera, experimentally infected rat sera and naturally infected urban rat sera

Anti-Seoul virus SR-11 [1, 6] strain rat serum and anti-Hantaan virus B-1 strain [2, 8, 9] rat serum were used for the determination of the optimal antigen concentration and as positive controls. A total of 10 female 5-week-old Slc:Wistar rats were inoculated intraperitoneally with 10^4 FFU of SR-11 strain, and sera were collected at 28 days after infection. The seroconversion for each serum sample was confirmed by IFA, and the sera were then pooled. This serum was used as anti SR-11 serum in this study. The experimental protocol and design were approved by the Central Institute for Experimental Animals (CIEA) Animal Experimentation Committee and performed according to the CIEA Guidelines for Animal Experimentation. Anti-B-1 strain rat sera were provided by Dr. Nishimune, Research Institute for Microbial Disease, Osaka University. Experimentally SR-11 infected Wistar rat sera collected on the 3rd, 5th, 7th, 10th, 14th, 21st and 28th day after infection from a infected rat, and 28 naturally infected urban rat (*Rattus norvegicus*) sera from a Seoul virus contaminated region were provided by Dr. Kariwa, Hokkaido University [1, 6].

Rat sera showing low antibody titer in IFA

Twenty-five sera (including BB, SHR and GK strains) showing low antibody titer in IFA were obtained from rat breeding colonies at an animal laboratory. These sera had already been evaluated as negative by the neutralization test and Western blotting.

Non- infected rat sera

One-hundred ten (30 from 32-week old female and 15 from 10 week-old male Jcl: Wistar , 30 from 32-week-old female and 15 from10-week-old male Jcl: SD, and 10 from 32-week-old female and 10 from 10-week-old male Jcl: F344) rat sera were used as hantavirus non-infected rat sera in this study.

Preparation of rHTN-ELISA plates

The biotinylated recombinant HTN 76-118 strain NP protein-expressing antigen in *E. coli* (rHTN Ag) was prepared by the PinPoint Xa system (Promega Inc. USA) as described previously [17]. This antigen is

expressed on the portion of the gene coding for amino acids 1–103 of SR-11 nucleocapsid protein (NP). The portion showed high cross-reactivity to Seoul, Puumala and Prospect Hill viruses [17]. The HTN NP biotinylated fusion protein was extracted from *E. coli* transfected with the plasmid. Cultured *E. coli* was collected by centrifugation and suspended in 1% Triton X-100 and 0.03% SDS-phosphate buffer saline (PBS). The suspension was ultrasonicated, and then centrifuged to remove the insoluble fraction. Solubilization of recombinant antigen was confirmed by Western blot staining with peroxidase-conjugated avidin. It was then diluted with carbonated bicarbonated buffer (CBB, pH 9.8) and used to coat each well of a streptavidin-coated microtiter plate (avidin plate; Boehringer-Mannheim, USA). After coating, each well was washed three times with PBS and masked with 0.1% skim milk. After removal of masking solutions, each well was dried at 37°C for 2 h. The solublized fraction of *E. coli* coated avidin plates was also prepared by the same method as the control antigen plate (E-ELISA).

ELISA procedure

The ELISA was performed as described previously [13]. Optical density (OD) was measured at 492 nm by a microplate reader (BIO-RAD, USA)

IFA procedure

IFA was performed as described in a previous paper [1, 2, 16, 18]. Vero E6 cells infected with SR-11 [16, 18] were used as antigen for IFA. An antibody titer of 1:16 or higher was evaluated as positive.

Results

Determination of optimal antigen concentration for rHTN-ELISA

The optimal concentration of rHTN antigen for rHTN-ELISA was determined by checkerboard titration. Each well of a streptavidin-coated plate was coated with 5, 10 or 20 μg/ml protein concentrations of rHTN antigen diluted with CBB and reacted with anti SR-11, anti B-1 rat serum and negative rat serum serially diluted from 1: 40 to 1: 5, 120. The reaction of 5 μg/ml protein concentration of rHTN antigen was lower than that of 10 and 20 μg/ml, while 20 μg/ml showed a high reaction with both antisera; however 20 μg/ml showed a higher OD value in negative rat serum than that of 10 μg/ml. The results for 10 μg/ml are shown in Fig. 1. This protein concentration showed a similar reaction to that of 20 μg/ml and a lower OD value in negative rat serum. Therefore, the rHTN antigen concentration of 10 μg/ml was considered to be the optimum and was used in the following assays.

Response to rat sera from non-infected facilities

rHTN-ELISA and E-ELISA were performed for examination of non-specific reactions in 40-fold diluted non-infected rat sera. The results are shown in Table 1. The OD values of both types of ELISA in these rat

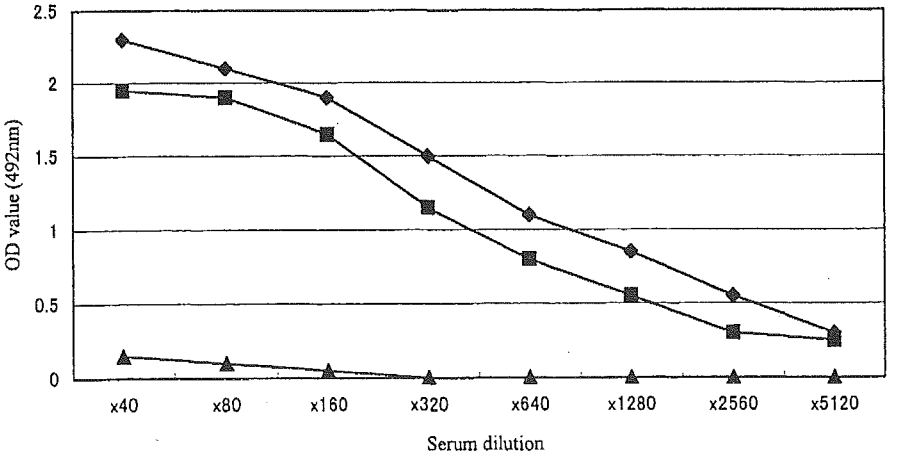


Fig. 1. Responses of 10 μg/ml protein concentration of rHTN antigen to anti B-1 (◆), anti SR-11 (■) and negative (▲) rat serum in rHTN-ELISA.

Table 1. Responses of rHTN-ELISA to rat sera from non-infected facilities

Strain	Sex	Age	No.	rHTN-ELISA	E-ELISA
Wistar	♀	32w	n=30	0.067 ± 0.033[1]	0.076 ± 0.029
	♂	10w	n=15	0.024 ± 0.024	0.019 ± 0.017
SD	♀	32w	n=30	0.099 ± 0.072	0.080 ± 0.049
	♂	10w	n=15	0.034 ± 0.012	0.031 ± 0.013
F344	♀	32w	n=10	0.056 ± 0.034	0.043 ± 0.028
	♂	10w	n=10	0.079 ± 0.040	0.087 ± 0.065

1: OD value mean ± SD in 40-fold diluted non-infected rat sera

Table 2. Comparison of sensitivities of rHTN-ELISA and IFA in urban rat sera collected from a contaminated area

	rHTN-ELISA (OD value)	
	pos.	neg.
IFA pos. (n=12)	11 (1.872 ± 0.243)[1]	1 (0.215)[2]
IFA neg. (n=16)	1 (0.501)[3]	15 (0.159 ± 0.071)

1: IFA antibody titer was from 1:128 to 1:4096. 2: IFA antibody titer was 1:32. 3: IFA antibody titer was < 1:16.

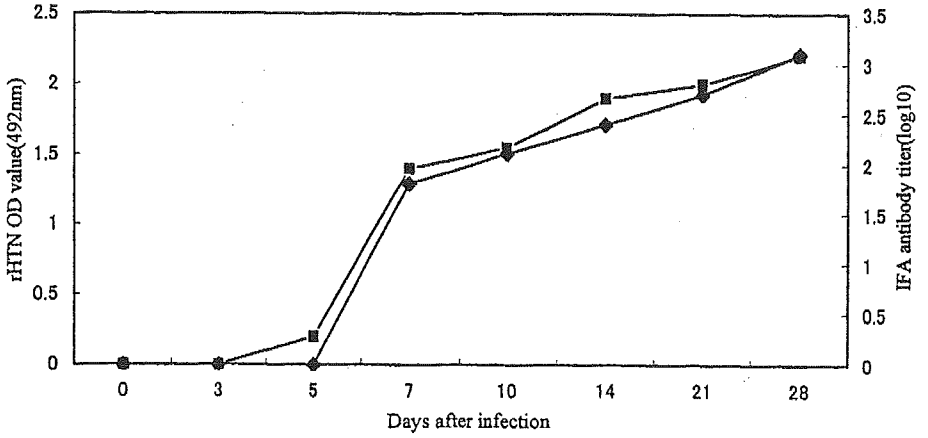


Fig. 2. Comparision of sensitivities of rHTN-ELISA (■) and IFA (◆) to rat serum experimentally infected with SR-11.

sera were very low (mean OD 0.059 ± 0.084 in rHTN-ELISA, mean OD 0.056 ± 0.034 in E-ELISA) with no relation to animal strain, age or sex. Cross-reactions between *E. coli* and rat sera were not found in E-ELISA. From this result, an OD value of 0.4 or greater was evaluated as positive at a 40-fold serum dilution in this study based on the mean ± 3 SD OD values of non-infected rat sera.

Comparison of sensitivities of rHTN-ELISA and IFA in rat serum experimentally infected with SR-11 strain

Rat sera experimentally infected with the SR-11 strain were examined by rHTN-ELISA to compare with the antibody detection sensitivity of IFA. The results are shown in Fig. 2. In rHTN-ELISA, the antibody was detected on the 7th day after infection and the OD value increased to 2.187 on the 28th day. In IFA, the antibody was also detected from the 7th day after infection and the IFA titer increased to 1:1280 on the 28th day. The sensitivity of rHTN-ELISA was the same as that of IFA.

Comparison of sensitivities of rHTN-ELISA and IFA in naturally infected urban rat serum

Twenty-eight urban rat (*Rattus norvegicus*) sera from a Seoul virus contaminated area were examined by rHTN-ELISA and IFA to compare the sensitivities for detection of hantavirus antibody (Table 2). Fifteen sera showed negative reactions by both methods, and among 13 antibody positive sera, one was positive in rHTN-ELISA but negative in IFA, one was positive in IFA but negative in rHTN-ELISA and 11 were positive by both methods. From the results of these comparative investigations, rHTN-ELISA and IFA showed a high rate of agreement rate, 91.7% for positive results and 93.8% for negative results.

Response to rHTN-ELISA in rat sera showing low antibody titer in IFA

Twenty-five rat sera showing low antibody titers (1:32 to 1:64) in IFA were examined by rHTN-ELISA in a 40-fold dilution. The response of these rat sera to the rHTN-ELISA showed low OD values. The mean OD values were 0.138 ± 0.070 in the 1:32 IFA titer group and 0.168 ± 0.084 in the 1:64 IFA titer group. These samples were also evaluated as antibody negative in rHTN-ELISA. Similar results were obtained in the neutralization test and Western blotting.

Discussion

In this study, rHNT-ELISA was found to have a sensitivity similar to that of IFA in experimentally and naturally infected rat sera, and a higher specificity than that of IFA in false positive rat sera. rHTN-ELISA could also detect both Seoul virus and Hantaan virus antibodies in reactions to the antisera using the recombinant Hantaan virus 76–118 strain [17]. The reduced specificity of ELISA is caused by the presence of nonspecific reactions because of the low purity of antigen [13, 15]. However, the OD values of rHTN-ELISA in sera of non-infected animals and in sera with low IFA titers were very low. These results showed that r-HTN-ELISA has high specificity. We think that the reason for this is the use of the avidin plate coated with biotinylated recombinant antigen. Because of the avidin-biotin reaction on the wells of the microplate, it was possible to coat the microplate with almost pure antigen [15, 17]. This has already been demonstrated in a previous report by Shenai *et al.* [15]. Therefore, the background of the ELISA reaction was eliminated in this rHTN-ELISA.

The preparation of antigens for the serological test of many zoonotic pathogens requires a biosafety level 3 containment laboratory to avoid biologic hazards and a high quality mass production system for the testing of a large number of samples. The rHTN-ELISA of this study combines recombinant antigen and ELISA and avoids these problems. Therefore, we think that rHTN-ELISA will be useful for screening hantavirus antibody in laboratory rats.

Diagnosis of infectious diseases by serological tests makes it possible to test many items using small amounts of sample, and this method is also useful in exposing inapparent infections such as hantavirus in rats. However, no matter what type of method is used, there are differences in degree, and non-specific reactions such as false negative and false positive reactions occur. False negative reactions can be eliminated by using animals with normal immune responses that have been reared in the same animal room for at least 4 weeks, i. e. by using appropriate animals and a test method with a high antibody detection sensitivity. However, it is difficult to eliminate false positive reactions even using methods with high specificity. This also applies to rHTN-ELISA and the appearance of false positive reactions cannot be ruled out because of such factors as higher OD values in older animals than in younger animals. Therefore, to obtain a more reliable, definite diagnosis, it will be necessary to confirm the results concurrently by other methods such as Western blotting of rHTN-ELISA positive sera [16, 18].

Acknowledgments

The authors thank Wakamoto Pharmaceutical Co., Ltd. for kindly providing us with the reagents and excellent technical advice. This study was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 06558112).

References

1. Arikawa, J., Ito, M., Yao, J.S., Kariwa, H., Takashima, I., and Hashimoto, N. 1994. Epizootiological studies of hantavirus infection urban rats in Hokkaido, Japan: evidences of the persistent infection from the sero-epizootiological surveys and antigenic characterizations of Hantvirus isolates. *J. Vet. Med. Sci.* 56: 27–32.
2. Dohmae, K., Koshimizu, U., and Nishimune, Y. 1993. In utero and mammary transfer of hantavirus antibody from dams to infant rats. *Lab. Anim Sci.* 43: 557–561.
3. Gavrilovskaya, I., Lamonica, R., Fay, M-E., Hjelle, B., Schmaljohn, C., Shaw, R., and Mackow, E. R. 1999. New York 1 and Sin Nombre viruses are serotypically distinct viruses associated with Hantavirus pulmonary syndrom. *J. Clin. Microbiol.* 37: 122–126.
4. Homberger, F.R., Romano, T.P., Seiler, P., Hansen, G.M., and Smith, A.L. 1995. Enzayme-linked immunosorbent assay for detection of antibody to lymphocytic choriomeningitis virus in mouse sera, with recombinant nucleoprotein as antigen. *Lab. Anim. Sci.* 46: 493–496.
5. Kariwa, H., Kimura, M., Yoshimatsu, S., Arikawa, J., Yoshimatsu, K., Takashima, I., and Hashimoto, N. 1996.

Mode of Seoul virus infection persistency in newborn rats and transiency in adult rats. *Arch. Virok.* 141: 2327–2338.

6. Kitamura, T., Morita, C., Komatsu, T., Sugiyama, K., Arikawa, J., Shiga, S., Takeda, H., Imaizumi, K., and Oya, A. 1983. Isolation of virus causing hemorrhagic fever with renal syndorome (HFRS) through a cell culture system. *Jpn. J. Med. Sci. Biol.* 36: 17–25.
7. Lee, H.W. 1982. Hemorrhagic fever with renal syndrome (HFRS). *Scand. J. Infect. Dis. Suppl.* 36: 82–85.
8. Lee, H.W., Lee, P.W., and Johnson, K.M. 1978. Isolation of the etiologic agent of Korean hemorrhagic fever. *J. Infect. Dis.* 137: 298–302.
9. Lee, P.W., Amyx, H.L., Gibbs, C.J., and Lee, H.W. 1981. Propagation of Korean hemorrhagic fever microbiological monitoring of laboratory animals. *Infect. Immun.* 31: 334–338.
10. Riley, L.K., Knowles, R., Purdy, G., Salome, N., Pintel, D., Hook, R.R., Franklin, C.L., and Besch-williford, C.L. 1996. Expression of recombinant parvovirus NS1 protein by a baculovirus and application to serologic testing of rodents. *J. Clin. Mcrobiol.* 34: 440–444.
11. Shenai, B.R., Komalam, B.L., Arvind, A.S., Krishnaswamy, P.R., and Rao, P.V.S. 1996. Recombinant antigen-based avidin-biotin microtiter enzyme-linked immunosorbent assay for serodiagnosis of invasive amebiasis. *J. Clin. Microbiol.* 34: 828–833.
12. Takakura, A., Kagiyama, N., and Terada, E. 1991. Studies on the development of an ELISA kit for microbiological monitoring of laboratory animals. *Exp. Anim.* 40: 7–15.
13. Tsai, T.F. 1987. Hemorrhagic fever with renal syndrome: mode of transmission to humans. *Lab. Anim. Sci.* 37: 428–430.
14. Voeten, J.T.M., Alphen, D., Claas, E.C.J., Groot, R., Osterhaus, A.D.M.E., and Rimmelzwaan, G.F. 1998. Use of recombinant nucleoproteins in enzyme-linked immunosorbent assay for detection of virus specific immunoglobulin A (IgA) and IgG antibodies in influenza virus A- or B-infected patients. *J. Clin. Microbiol.* 36: 3527–3531.
15. Yoshimatsu, K., Arikawa, J., Li, H., Kariwa, H., and Hashimoto, N. 1996.Western blotting using recombinant Hantaan virus nucleocapsid protein expressed in silkworm as aserological confirmation of hantavirus infection human sera. *J. Vet. Med. Sci.* 58: 71–74.
16. Yoshimatsu, K., Arikawa, J., Tamura, R., Yoshida, R., Lundkvist, A., Niklasson, B., Kariwa, H., and Azuma, I. 1996. Characterization of the nucleocapsid protein of Hantaan virus strain 76–118 using monoclonal antibodies. *J. Gen. Virol.* 77: 695–704.
17. Yoshimatsu, K., Arikawa, J., Yoshida, R., Li, H., Yoo, Y-C., Kariwa, H., Hashimoto, N., and Azuma, I.1995. Production of recombinant hantavirus nucleocapsid protein expressed in silkworm larvae and its use as a diagnostic in detecting antibodies in serum from infected rats. *Lab. Anim. Sci.* 45: 641–646.
18. Zöller, L.G., Yang, SI., Gött, p., Bautz, E.K.F., and Darai, G. 1993. A novel μ-capture enzyme-linked immunosorbent assay based on recombinant proteins for sensitive and specific diagnosis of hemorrhagic fever with renal syndrome. *J. Clin. Microbiol.* 31: 1194–1199.

幼虫移行症の原因としてのアライグマ回虫

杉山　広・森嶋康之・坂本京子・亀岡洋祐・川中正憲

Racoon roundworm, *Baylisascaris procyonis*, as a cause of larva migrans

Hiromu Sugiyama, Yasuyuki Morishima, Kyoko Sakamoto, Yosuke Kameoka, and Masanori Kawanaka

獣医寄生虫学会誌　第２巻第１号（2003）別冊
日本獣医寄生虫学会

最近注目される人獣共通寄生虫症（下）

顎口虫症，動物由来蛔虫症

神谷 晴夫（かみや はるお） / 弘前大学医学部寄生虫学講座 教授

表1　国内で認められる顎口虫

	終宿主	終宿主での寄生部位	第2中間宿主／待機宿主	ヒトへの推定感染源	主たる国内分布
有棘顎口虫 *Gnathostoma spinigerum*	ネコ科，イヌ科動物	胃壁	淡水魚類，両生類，鳥類，爬虫類，哺乳類	雷魚，ボラ，コイ，フナなど魚類，ヘビ	中部，南日本
ドロレス顎口虫 *G.doloresi*	イノシシ，ブタ	胃壁	イモリ，サンショウウオ，カエル，マムシ，ブルーギルなど	ヤマメ	南日本，特に九州
日本顎口虫 *G.nipponicum*	イタチ	食道壁	ドジョウ，ウグイ，ナマズ，ヤマメ，ヤマカガシ，シマヘビなど	ドジョウ，コイ，ヒメマス，シラウオなど	中部・北日本，特に青森，秋田
剛棘顎口虫 *G.hispidum*	ブタ，イノシシ	胃壁	魚類，両生類，鳥類	ドジョウ	輸入寄生虫症例は中部日本以南，特に関西方面に多い

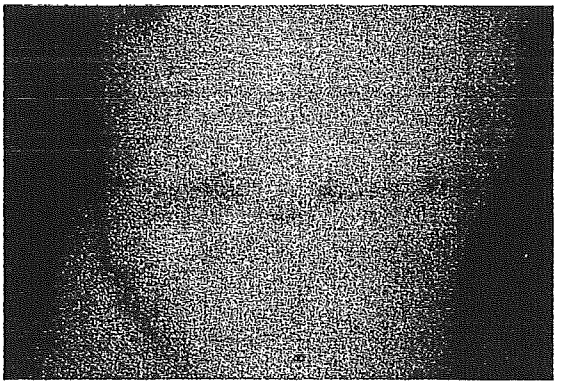


図1　日本顎口虫症患者皮膚爬行疹
左側胸部．腹側より背側に向かって直線的に移動．

❖顎口虫症

国内では4種の顎口虫が報告されている（表1）．重要な人獣共通寄生虫である．最近，東北地方，特に青森県，秋田県において日本顎口虫症が多発している．いずれの場合でも，その幼虫移行による皮膚爬行疹（図1・2）の出現で医療機関を受診している．受診時，爬行の移動方向を考慮して皮膚を切除し，そこから虫体（図3・4）が確認されている．感染部位切除後の再発はわれわれの自験例では認められていない．感染源は，ドジョウ，シラウオなど淡水魚であるが，ドジョウを除いて，患者が食した魚から感染期幼虫は検出されていない．

また，アメリカで感染したと考えられる有棘顎口虫症例も経験している．さらに，ホタルイカなどを感染源とする近縁種の旋尾線虫感染による類似の皮膚爬行疹が発現するので，鑑別が必要である．青森県産のスケソウダラにその旋尾線虫Type-Xが高率に感染していたとの報告もあり，今後いっそうの注意が必要であろう．

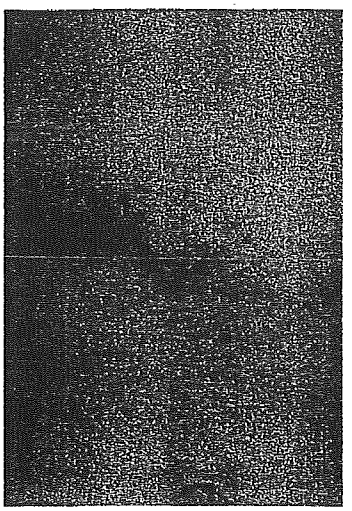


図2　日本顎口虫症患者皮膚爬行疹
上腹部の限局した部位を移動.

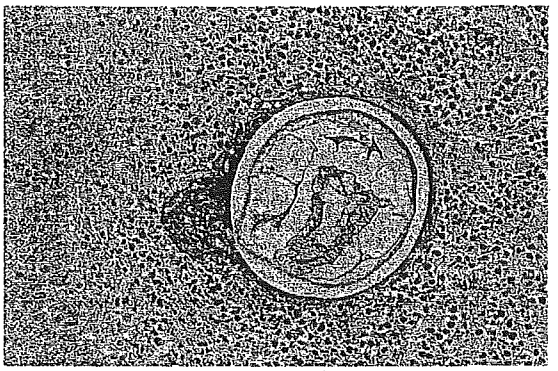


図3　日本顎口虫症患者の切除皮膚の虫体横切像
顕著な好酸球の集族が認められる.

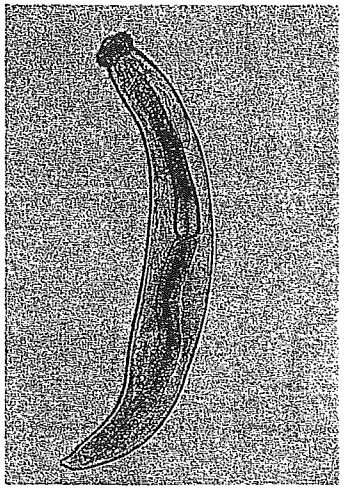


図4　患者の切除皮膚から検出した日本顎口虫幼虫
体長約2 mm. 頭部に鉤を備えた頭球がみられる.

❖動物由来蛔虫症

1）アライグマ蛔虫症

海外からの移入動物の問題が深刻になっている. アライグマは「あらいぐまラスカル」のアニメ人気以降, ペットとして海外からの輸入が急増した. しかし性格が粗暴なこともあり, 飼主が手に負えず野外に放逐し, それが野生化・繁殖して北海道など日本各地で重大な問題となっている. それら動物自体による生態系の破壊はもちろんであるが, 付随して持ち込まれる感染症も看過できなくなっている.

ところが最近, 東北地方の私設動物牧場で, 本来日本国内には分布しなかったアライグマ蛔虫(図5)に感染したカイウサギに, 中枢神経症状を呈する幼虫移行症が集団発生した(図6). 実験的に感染させたラットでも, 顕著な神経症状を呈した(図7). アライグマ蛔虫の幼虫は, 脳, 特に小脳に高頻度に侵入し, 幼虫が大きいこともあり, 重篤な傷害を及ぼす典型的な内臓幼虫移行症である(図8). アメリカでは子どもの感染死亡例が報告されており(図9), 今後, わが国でも重大な関心を払う必要があろう.

2）ブタ蛔虫症

九州地方で, ブタ蛔虫による内臓幼虫移行症が報告され注目されている. ヒトの蛔虫とブタ蛔虫は近縁であるが, 本種は通常はヒトでは成虫に発育せず, 幼虫のまま肝臓などに寄生する. ヒトへの感染源は, 本幼虫に感染したニワトリやウシの肝臓の生食やブタ蛔虫の虫卵で汚染された野菜などが考えられている. 感染数によるが, 肺炎様症状や肝機能障害を引き起こす. 免疫学的に診断されている.

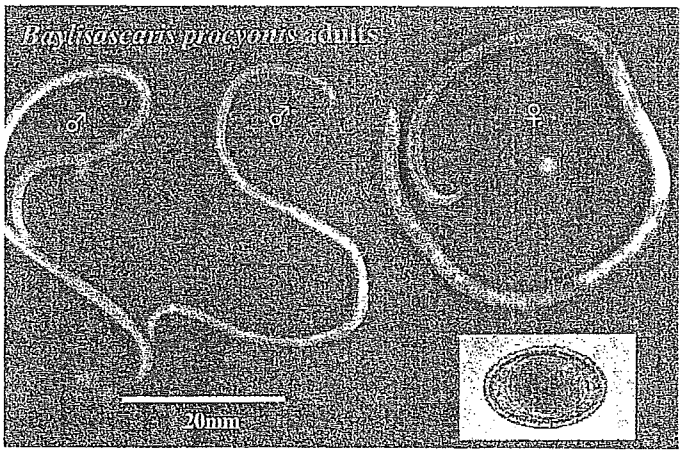


図5　アライグマ蛔虫
右下の枠内は産出された未分割虫卵．大きさ約70×60μm.

図6　中枢神経障害を示すウサギのアライグマ蛔虫幼虫移行症
臨床的に小脳障害である斜頸から起立不能を呈する．

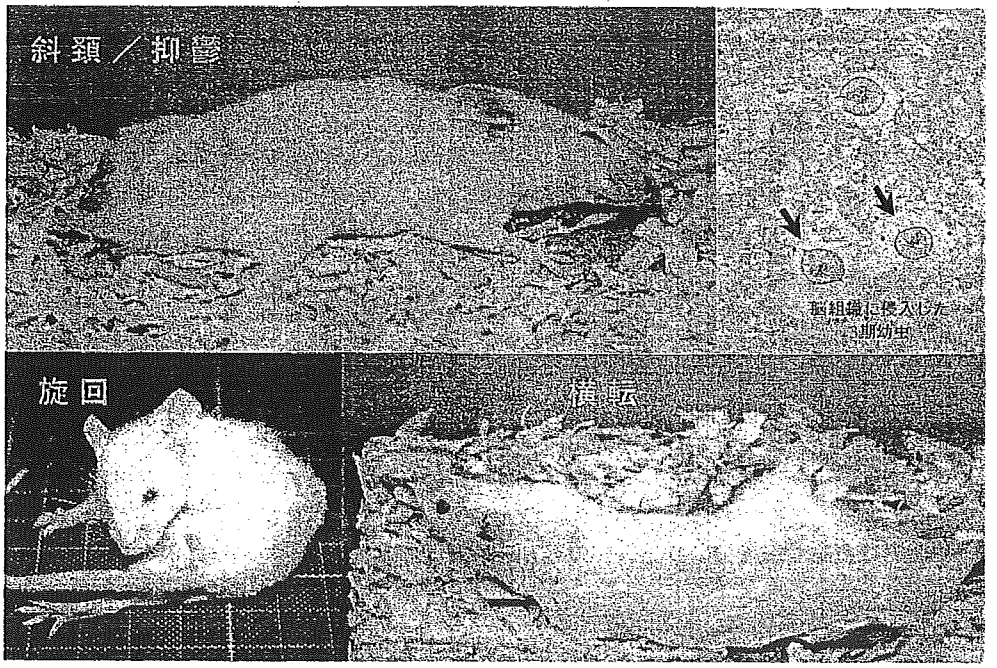


図7　マウスでの実験的アライグマ蛔虫幼虫移行症
斜頸，旋回，横転等の神経症状を示す．右上枠内は，その脳病理組織中の幼虫横切像．

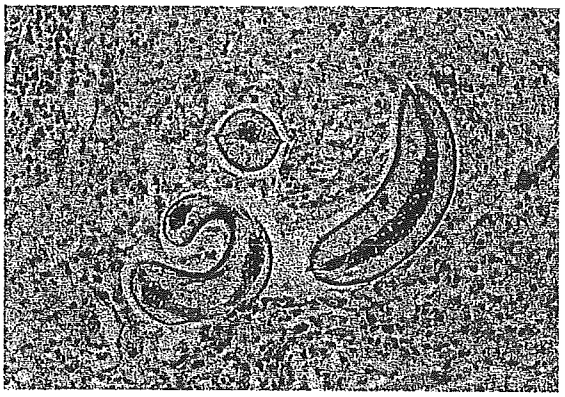


図8　アライグマ蛔虫感染ウサギ小脳組織中の幼虫．HE染色．

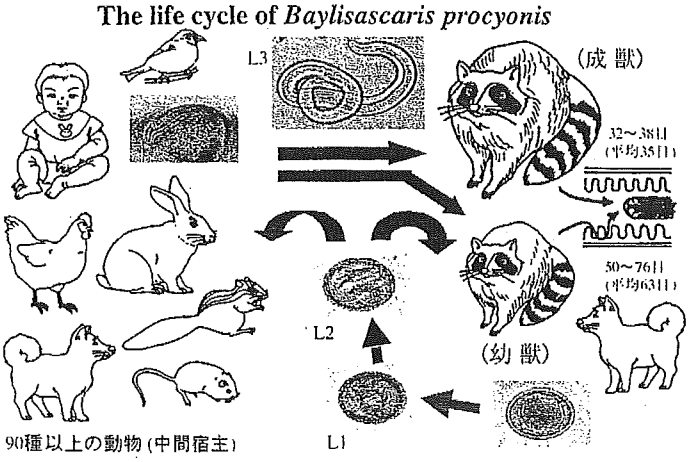


図9　アライグマ蛔虫の生活環

アライグマから排出された虫卵は，外界で第2期幼虫（L2）に発育し，ヒトを含む中間宿主に感染し，第3期幼虫（L3）となり，成獣のアライグマに摂取され成虫となる．一方，アライグマが幼獣の際には第2期幼虫含仔虫卵からも感染し，成虫となり得る．

3）アニサキス性アレルギー

臨床家にとってアニサキス症は，比較的遭遇する機会の多い寄生虫症であろう．海産魚類等を生食後，短時間のうちに発症する急性腹症の場合，内視鏡で胃壁に刺さった虫体が摘出される．しかし最近，アニサキス抗原によるアレルギーの可能性が強く示唆される症例報告が急増している．アニサキスに感作された人が，その後アニサキスに感染した加熱調理済みの魚を食して，アレルギーを生ずるというものである．

アニサキス抗原は，耐熱性で加熱処理しても抗原性は残る．このような新しい概念の症例は，1990年にわが国で最初に報告されているが，最近，スペインからも多く報告され注目されている．アニサキス抗原に曝露されれば，アレルギーが必ず発現するものでもないが，寄生虫アレルゲンとしての把握が，今後必要であろう．

❖おわりに

「最近注目される人獣共通寄生虫症」の中で，現在激しく流行し深刻な状況を呈しているものや，これからその可能性のあるものについて，自験例をもとにそれらのカラー像を示し概説した．人・物の大移動時代での感染症の広がりは，さまざまな過去・現在・未来の人の営みとの係わりから把握することが必要であろう．医療のフロンティアで，感染者とまず向き合う臨床医家の方々の大きな関心をお願いしたい．

付図の一部は本学部外科学第二講座，皮膚科学講座，小児外科講座より提供していただいた．記して感謝申し上げます．

研究報告

青森県のエキノコックス症の現状と対策

—北海道から本州への伝播を考慮して—

弘前大学寄生虫学
教授 神谷晴夫

緒言

国内には二種類のエキノコックス（*Echinococcus* spp.）が分布する。多包条虫（*Echinococcus multilocularis*）と単包条虫（*E. granulosus*）である。両種とも世界的に重要な人獣共通寄生虫病の原因虫である。それらの中間宿主はそれぞれ、野鼠など齧歯類とヒツジやウシなどの有蹄類で、終宿主は両種ともイヌ、キツネなどの肉食獣である（図1・2）。

国内においては、前者は北海道の野生動物間で激しく流行し、患者も年々増加している。一方、後者の単包条虫では、ヒツジなど中間宿主での感染や患者の発生が報告されているが、幸いなことに国内の終宿主からの感染はみつかっていない。

現在、エキノコックス症（多包虫症）は、国内で流行している寄生虫症として最も深刻な様相を呈している[1-5]。しかも北海道と本州との間は、青函トンネルの開通やフェリーの運航等による人的・物的交流の増大が、本州への流行拡大要因（例えば虫卵に汚染した牧草の移入、感染した終宿主動物の移動など）として指摘されている。一方、北海道外での患者の発生は、時空的に関係の深い青森県で最も多い[5,6]。

このような背景をもとに、われわれは今まで継続的に野生動物や家畜での疫学的調査を青森県で実施してきた。加えて、一九九七年より六年間にわたって厚生労働科学研究補助金の交付を受け新興・再興感染症研究事業が行われた。それらの結果をもとに、青森県でのエキノコックス症の現状と対策に関して、本州への流行拡大の最前線としての視点から言及し、併せて北海道外の臨床医家の方々の本症に対する関心を期待したい。

一、北海道でのこれまでの流行経緯の概略

多包条虫は主に北緯四〇度以北に分布するが、近年の世界的自然環境破壊やヒト、物の地球的規模での交流に起因し、アジアや北米においては南下傾向にある。国内では北海道のほぼ全域が高度の汚染地域となり、さらに青森県のブタから感染が発見され、本州への伝播が濃厚となった。

北海道では礼文島において、一九三七年に最初の患者が報告され、それ以降一三一名の患者が発生したが、上下水道の整備等種々の対策が講じられた結果、最終的に同島での流行は終息した[3]。世界で唯一の本症駆除成功例である。しかし一九六五年、根室市で七歳の少女の感染が特定され、しかもその近辺地域では、すでにキタキツネとエゾヤチネズミとの間に自然界で生活環が維持されていることが明らかになった[3,7]。

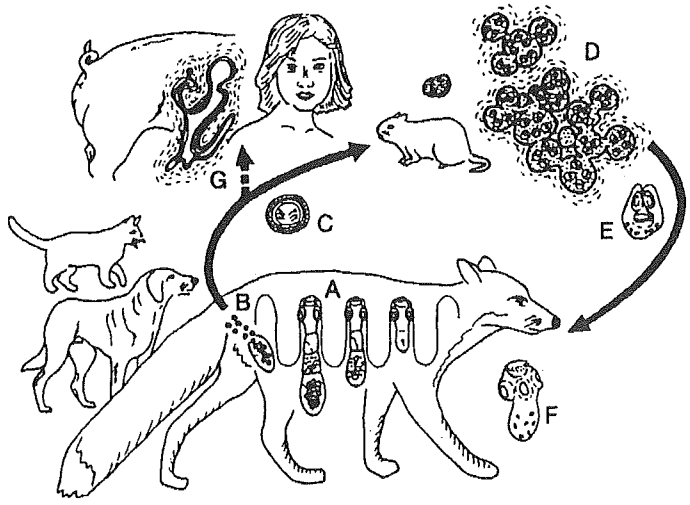
その後、長らく流行は主に根室、釧路、網走の三支庁に限定されているものと捉えられていた。ところが、一九八三年、網走支庁東藻琴食肉衛生検査所で、食肉検査を受けたブタに感染が発見された[8]。それ以降、食肉検査に供されたブタでの感染を指標にして調査したところ、すでに北海道内に多包虫症は広く拡散・流行している深刻な状況が明らかになっていった[3,9]。

一方、二〇〇一年度までに本症と認定された累計患者数は四二四名で、新規認定患者は毎年五～二〇名である[10]。さらに北海道では、検診を受けて抗体陽性者等、要観察者が四〇〇～五〇〇名に達している。

また、北海道のキツネでの寄生

率は平均一五%、地域によっては四〇～六〇%と高く、その流行状況は深刻である。飼育犬での感染率は約一%との報告[2]もある。さらに最近、札幌の室内飼育犬での感染が報告され、その重大性が改めて強く認識された[3]。また、流行地のネコ、タヌキでの感染もしばしば報告されており、終宿主動物として今後の慎重な対応が求められている。

図1　多包条虫の生活環



A：キツネ等イヌ科動物の腸管に寄生する多包条虫
B：虫卵・虫体片節の糞便中への排出
C：感染源となる六鉤幼虫を容れた虫卵
D：エゾヤチネズミなど好適中間宿主での多包虫
E, F：原頭節
G：ヒト，ブタなど非好適中間宿主での発育が抑えられた多包虫

二、青森県での現状と疫学的流行監視状況

(1)エキノコックス症患者発生状況

青森県では、現在までに二四例の多包虫症患者の報告があり、そのうち九例が県内原発例と考えられている[5][6]。その他、一九八六年には、青森県で感染したと考えられている一例の単包虫症患者が検出されている[12]。

(2)野生動物の棲息状況と感染調査

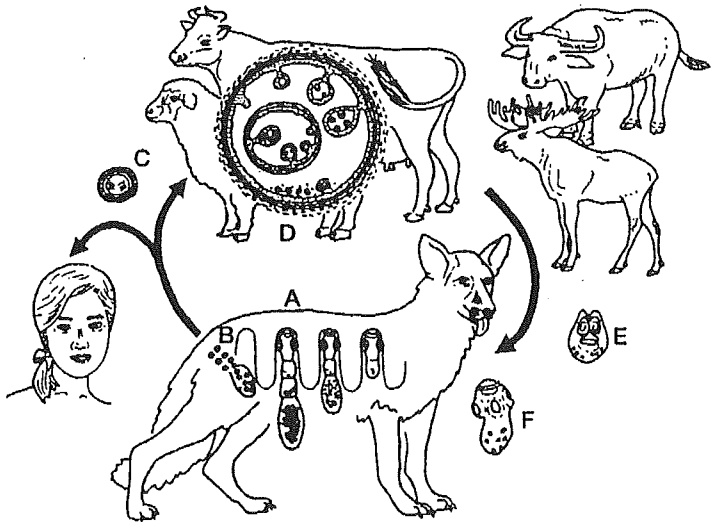
(1)終宿主動物：一九九七～二〇〇二年度内に、ホンドキツネ、ホンドタヌキ、テン等、多数を検査したが、多包条虫は検出されていない[13]～[15]。また最近、青森県北部で、キタキツネに類似しヒトによく慣れたキツネが頻繁に目撃されており、キタキツネの青函トンネルを介した本州への移動も慎重に監視する必要がある。今までのところ、終宿主動物から多包条虫の感染はみつかっていない。

ところが最近、ハンターが北海道で捕獲し、青森県弘前市の剝製業者に持ち込んだキタキツネに重度の感染が検出された(神谷ら、未発表)。このことは北海道からのイヌの移動を含め人為的な宿主動物の移動に関して、早急に厳しい対応が必要なことを示唆している[18]。

(2)中間宿主動物：一九九〇年より青森県内の野生中間宿主動物の感染調査を継続して行っている。それ以前の一九七〇年代に行われた県内の動物寄生虫相の膨大な調査では、中間宿主並びに終宿主動物から多包(条)虫の感染はみつかっていない[19]。

さらに一九九五年からは、定点監視区

図2　単包条虫の生活環



A：オオカミ等イヌ科動物の腸管に寄生する単包条虫
B：虫卵・虫体片節の糞便中への排出
C：感染源の六鉤幼虫を容れた虫卵
D：好適中間宿主とその中での単包虫の発育
E, F：原頭節