A stochastic model of *Echinococcus multilocularis*
focusing on protoscoleces

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

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

1. INTRODUCTION

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

Humans and voles become infected with *E.*

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

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

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

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

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

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

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

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

2. MATERIALS AND METHODS

2-1 Population dynamics of hosts

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

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

The breeding season of voles is recognized as occurring in three seasons every year. The main breeding seasons are spring and autumn, since the breeding rate in summer is much less than that in the

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

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

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

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

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

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

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

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

2-3 Growth of protoscoleces in voles

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

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

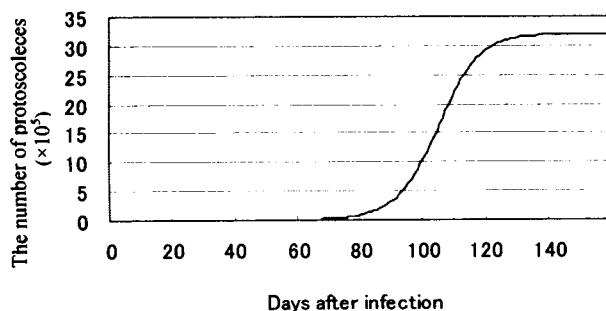with P_{max}, r being the maximum number of protoscoleces and the growth rate, respectively.

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

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

A fox becomes infected with E. multilocularis after

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

1. The number of voles preyed on by a fox per day follows the food habit function (Ohga et al., 2002)

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

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

Then, the sum of $f(k)$ over $k \geq 1$ is the probability that a fox will become infected per day.

3. For an infected fox, we decide the age of infected voles preyed on by the fox following from the age distribution (the number of protoscoleces in the vole following the protoscoleces-day model).

4. The worm burden in the fox is determined by both the protoscoleces-day model and the immune response, which will be discussed in the next section. The infected fox discharges parasite eggs constantly, depending on its worm burden after the latent period (30 days) during a 50 days period (from the end of latent period).

2-5 Immune responses against E. multilocularis in foxes

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

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

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

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

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

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

with n being the number of infection experiences.

3. RESULTS

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

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

Prevalence of infection of host population

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

Table 2 Structure of characteristics in fox in the model

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

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

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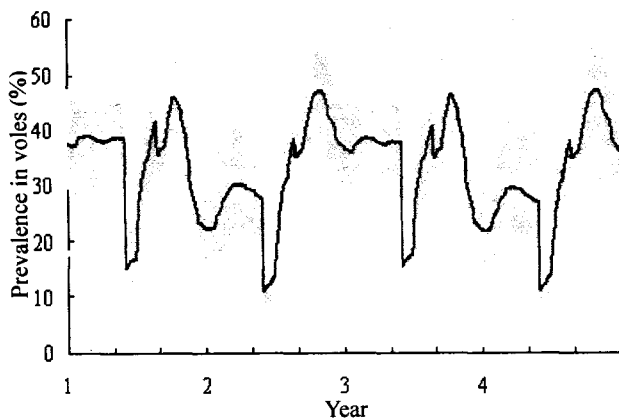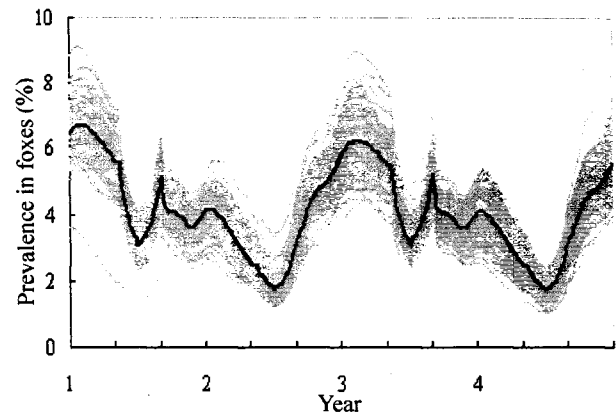small. In contrast, the prevalence in voles increases

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

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

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

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

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

Worm burden in foxes

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

4. DISCUSSION

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

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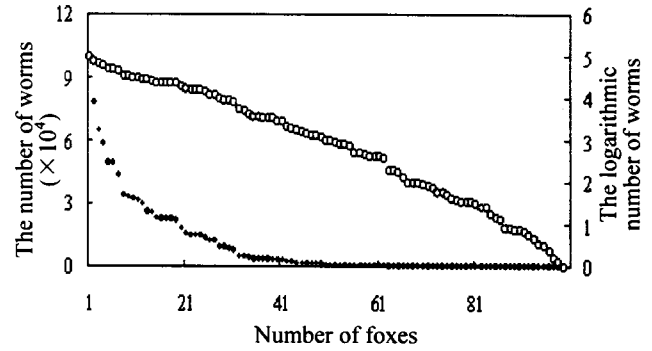

Fig. 4 Distribution of the worm burden in the infected foxes obtained by simulation. The number of worms (worm burden) and the log number of worm burden are shown by dots and circles, respectively.

further studies.

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

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

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

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

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

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