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Evidence for an increasing presence of *Echinococcus multilocularis* in foxes in The Netherlands

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Abstract

Echinococcus multilocularis, a tapeworm causing alveolar echinococcosis which is considered a serious zoonosis known to affect humans, appears to be expanding its geographical range in Europe. We studied the emergence of the parasite in the European western-most edge of its geographical distribution, based on two consecutive parasitological examinations of red foxes (*Vulpes vulpes*) sampled between 1996 and 2003 in The Netherlands. The average worm count increased from 2.6 worms per fox in the first surveillance to 16.6 worms per fox in the second. Using a mathematical model for a spatially spreading parasite, we found a strong indication that the parasite population is increasing in number and is spreading northward at the speed of 2.7 km per year. The reproduction number (R_0), reflecting the parasite's transmission process, is estimated from the surveillance data and it is likely to be more than 1 but not exceeding a value of 4. We analysed a parasite control strategy by estimating the critical fox density for parasite elimination. We conclude that *E. multilocularis* is an emerging parasite in The Netherlands and thus in the western part of Europe. Control will be very difficult given the current high fox population density.

Keywords: *Echinococcus multilocularis*; Red fox; Mathematical model; Reproduction number; Control

1. Introduction

Echinococcus multilocularis is a small tapeworm belonging to the family Taeniidae. In Europe, the life cycle of *E. multilocularis* is predominantly sylvatic, i.e. involving wild carnivores (mainly foxes of the genera *Vulpes* and *Alopex*) as definitive hosts and several species of small mammals (mainly rodents of the families *Arvicolidae* and *Cricetidae*) as intermediate hosts (Rausch, 1995; Eckert et al., 2001). Humans are considered accidental hosts for infection. The parasite is the causative agent of alveolar echinococcosis (AE), a very serious disease which may be life threatening in humans. The infection route to humans is through oral uptake of eggs from the environment, which have been shed by the definitive host. The incubation time of this infection can be 5–15 years and even then clinical signs are not typically related to the disease. At that time, infection of the liver and metastasis in the body can be so serious that treatment is difficult or even impossible. Mortality rates after diagnosis in untreated or inadequately treated AE patients has been reported to be as high as 100% after 15 years (Ammann and Eckert, 1996). Hence, prevention of this potentially life-threatening infection in humans is of major importance.

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Important factors enhancing the risk of exposure for humans include an increasing prevalence and increasing number of infective eggs of *E. multilocularis* shed in the environment by the definitive host. *E. multilocularis* is widely distributed in the northern hemisphere, where it is endemic in several regions in western and central Europe, most of northern and central Eurasia, and parts of North America (Eckert et al., 2001). At present, distribution of the parasite in western and central Europe includes regions in Austria, Switzerland, France, Germany, Liechtenstein, Luxemburg, Belgium, The Netherlands, Poland, Czech Republic, Slovak Republic, Denmark and the Norwegian Islands of Svalbard (Lucius and Bilger, 1995; Losson and Coignoul, 1997; Tackmann et al., 1998; van der Giessen et al., 1999; Romig et al., 1999; Vervaeke et al., 2003). Increases in red fox populations in many parts of Europe due to successful rabies control campaigns might have extended the parasite's distribution. An increase in parasite prevalence over time has only been demonstrated in southwestern Germany (Baden-Württemberg) (Romig et al., 1999). Hence, at present it cannot be determined whether *E. multilocularis* has recently extended its range or whether the parasite has simply remained undetected until now.

In The Netherlands, *E. multilocularis* was detected for the first time in a population of foxes sampled between October 1996 and March 1997 in the northern region of Groningen adjacent to Germany and the southern region of Limburg adjacent to Belgium (van der Giessen et al., 1999). These areas are considered the geographical westernmost border area of the parasite's distribution in Europe. Until now, no human cases have been reported in Groningen and Limburg, but due to the long incubation period, the first human case may only be recognised years after introduction of the parasite. Studies in the borderline areas might help to elucidate the dynamics of infection and provide more insight into possible risks for human health. Recently three new human patients were reported in Belgium, two patients from the provinces of Luxembourg and one from the province of Liege in 2004 (Detry et al., 2005) very close to Limburg, at the border area with The Netherlands. In Switzerland human alveolar echinococcosis cases doubled within 10 years, following a fourfold increase in the fox population (Schweiger et al., 2007).

In this study, we analysed *E. multilocularis* worm counts of individual foxes from one of the westernmost border areas in The Netherlands, derived in 1997 and 2003 with the aim of studying the spread of the parasite. We found that a mathematical model describing the parasite population increase and dispersal along a north-south axis is more consistent with the surveillance results than other models lacking either the growth or dispersal process. In addition, intervention strategies to control the parasite were analysed for this specific region using a transmission model described previously (Takumi and van der Giessen, 2005).

2. Materials and methods

2.1. Study area

Surveillance was conducted in 1996–1997 to determine whether *E. multilocularis* was crossing over the 500 km long border with Germany and Belgium (van der Giessen et al., 1999). In the province of Limburg where *E. multilocularis* was detected for the first time, follow-up surveillance was conducted in 2003 to investigate the prevalence of the parasite and the spread of the infection near the detection point. Therefore, a larger number of foxes per unit area were sampled in the second surveillance (Fig. 1). In both surveillances, foxes were sampled in the months between November and February. Foxes were collected by hunters and were sent to the National Institute for Public Health and Environment (RIVM) and carcasses were frozen at −80 °C for 1 week prior to necropsy. At necropsy, small intestines and contents of colons were removed. In both surveys, age of the foxes was classified as either juvenile (less than 1 year of age) or adult, based on the extent of tooth wear (Eckert et al., 2001).

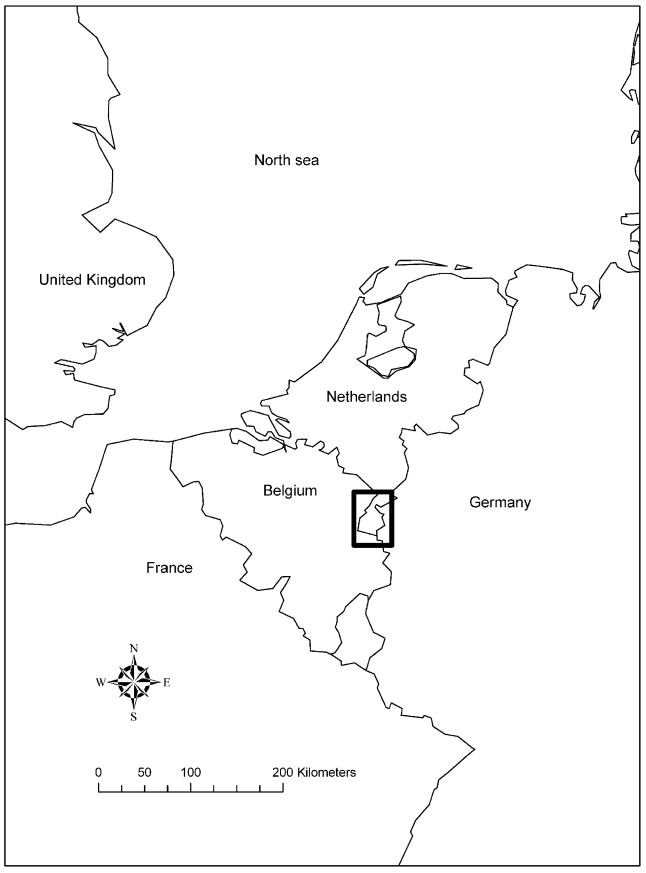

Fig. 1. Surveillance area in the Province of Limburg, The Netherlands, where surveillance took place between 1996 and 2003, is indicated by a rectangle. The enclosed area is approximately 800 km^2.

2.2. Parasitological examination

Parasitological examination of the small intestines was carried out by microscopical examination of mucosal smears as recommended by the WHO Collaborating Center for Parasitic Zoonosis in Zurich (Deplazes and Eckert, 1996). PCR was carried out on colon contents as described previously (van der Giessen et al., 1999). Similar methods were used in the two consecutive surveillance studies between 1996 and 2003.

2.3. Spatial and temporal parasite distribution

The number of parasites in individual foxes can be described by the negative binomial distribution (Anderson and May, 1991). The probability that j worms are present in the intestine of a fox is equal to,

$$\frac{\Gamma(j+k)}{\Gamma(k)\Gamma(j+1)}\left[\frac{M}{k}\right]^{j}\left[1+\frac{M}{k}\right]^{-k-j}$$

The symbol M is the mean worm burden per fox and k is inversely related to the degree of parasite aggregation, small k indicates high aggregation. The negative binomial distribution arises in a situation, where the numbers of larvae in individual infected rodents are random and foxes eat a random number of infected rodents. For a large value of k (i.e. much larger than 1), the negative binomial distribution becomes Poisson distribution.

The mean worm burden per fox is potentially specific to the year and the location of surveillance. We model spatial and temporal change of the worm population using the model proposed by Skellam (1951).

$$\frac{\partial M}{\partial t} = sM + D\frac{\partial M}{\partial y^2}$$

$M(y,t)$ is the mean worm burden at a given time t and a location y. The symbol s is the rate of exponential growth per year. The symbol D is the diffusion coefficient in unit km^2 per year. Since we are investigating the initial phase of parasite emergence, we ignore any density-dependent process in the parasite's life cycle and assume the simplest model for population growth (i.e. exponential growth). The expression $2\sqrt{sD}$ is the rate of spread in km per year (Skellam, 1951). We assume an initially localised infection focus (in the province of Limburg) 1 year before the onset of surveillance, i.e. a Dirac function with the mass being equal to e^{α} (Edelstein-Keshet, 1987).

The mathematical model produces a series of nested models in increasing complexity for the observed spatial and temporal distribution of the worm burdens. The simplest model when $s = 0$ and $D = 0$ corresponds to the mean worm burden per fox being constant over space and time. The model with only $s = 0$ corresponds to the situation in which the parasites are transported to nearby locations but the total number of parasites in the survey area remains constant over time. Lastly, when both parameters s and D are positive, the parasites are spreading geographically and their numbers are increasing over time.

We fitted the mathematical model to the worm burden data and calculated maximum likelihood estimates (MLE), and profile likelihood based 95% confidence intervals (CIs) of the parameters (Venzon and Moolgavkar, 1988). No surveillance data are available to determine the initial condition. Thus we hypothesised that the initial mean worm burden was not greater than the maximum number of adult worms detected in the first surveillance, i.e. $\alpha \leqslant \ln(100)$. Two-dimensional surveillance location data were reduced to one dimension by using y coordinates along a north-south direction only. The benefit of adding the parameters of the model is assessed by the likelihood ratio test. P-value were calculated based on the deviance of two nested models and the chi-squared distribution with 1 degree of freedom.

2.4. R_0 estimation

Echinococcus multilocularis has a complex life cycle involving eggs, larvae and adult worms parasitising two species of mammalian hosts (mainly foxes and rodents). The reproduction number (R_0) of the parasite can be expressed in terms of the parasite's transmission parameters as previously described (Takumi and van der Giessen, 2005). The system of equations describing the (non-spatial) dynamics of the egg (G), larvae (L), and adult worm (M) stages of *E. multilocularis* is (Takumi and van der Giessen, 2005),

$$\frac{\mathrm{d}}{\mathrm{d}t}G(t) = \lambda\varphi n M(t) - \mu_3 G(t) - \beta_2 a G(t)$$
$$\frac{\mathrm{d}}{\mathrm{d}t}L(t) = \beta_2\pi_2\sigma G(t-\tau_2) - \mu_2 L(t) - \beta_1 n L(t)$$
$$\frac{\mathrm{d}}{\mathrm{d}t}M(t) = \beta_1\pi_1 a L(t-\tau_1) - \mu_0 M(t) - \mu_1 M(t)$$

The parameters are the inverse of the average life expectancies of foxes (μ_0), adult parasite worms (μ_1), rodents (μ_2) and parasite eggs (μ_3), the time for the parasite to mature in the fox (τ_1) and in rodents (τ_2), the average number of protoscolices per infected rodent (σ), probabilities of infection per parasite in foxes (π_1) and in rodents (π_2), the release rate of proglottids (λ), the average number of eggs per proglottid (ϕ), the rate of predation by a fox (β_1), the rate of egg uptake by a rodent (β_2), the mean fox population density (n), and the mean rodent population density (a).

The parasite persists in the population of rodents and foxes when $R_0 > 1$, where,

$$R_0 = \frac{\lambda\varphi\sigma\pi_1\beta_1 n\pi_2\beta_2 a}{(\mu_0+\mu_1)(\beta_1 n+\mu_2)(\beta_2 a+\mu_3)} \quad (1)$$

Some of the transmission parameters in the expression for the reproduction number are specific for certain areas, in our case for Limburg. However, it is costly in terms of labour, time and money to collect data that support estima-

tion of individual transmission parameter values for this specific region. We circumvent this by expressing the reproduction number as a function of the growth rate s, which can be estimated using the surveillance data in the Limburg area.

Substituting the exponential function e^{st} into the system of differential equations we find that the rate s must satisfy the equation

$$s = (\mu_0 + \mu_1) \times \left(\frac{\lambda\varphi\sigma\pi_1\beta_1 n\pi_2\beta_2 a}{(\mu_0+\mu_1)(s+\beta_1 n+\mu_2)(s+\beta_2 a+\mu_3)} e^{-s(\tau_1+\tau_2)} - 1 \right)$$

We solve for small values of the rate s by expanding the right-hand side in s near zero. Keeping in mind that R_0 is expressed by Eq. (1), the zero order term is,

$$(\mu_0 + \mu_1)(R_0 - 1)$$

and the first order term has the coefficient,

$$-(\mu_0+\mu_1)R_0\left(\tau_1+\tau_2+\frac{1}{n\beta_1+\mu_2}+\frac{1}{a\beta_2+\mu_3}\right)$$

We simplify further by noting that predation by foxes is a minor cause for rodent death ($n\beta_1 \ll \mu_2$) and that the average time for parasite eggs to be ingested by a rodent is much longer than the life expectancy of the eggs ($a\beta_2 \ll \mu_3$). Discarding second and higher order terms, the rate s can be approximated as

$$s = \frac{(\mu_0+\mu_1)(R_0-1)}{1+(\mu_0+\mu_1)R_0(\tau_1+\tau_2+\mu_2^{-1}+\mu_3^{-1})}$$

Solving for R_0, the new expression for R_0 reads.

$$R_0 = \frac{\mu_0\mu_2\mu_3+\mu_1\mu_2\mu_3+\mu_2\mu_3 s}{(\mu_0+\mu_1)(\mu_2\mu_3 - s(\mu_2+\mu_3) - s(\tau_1+\tau_2)\mu_2\mu_3)} \quad (2)$$

The expression for R_0 now involves only the parameters related to life expectancies and maturation times. Life expectancies are assumed to be 450 days for foxes (Mulder et al., 2004), 182 days for rodents (Roberts and Aubert, 1995) and 57 days for parasite eggs (Veit et al., 1995). Life expectancy of adult worms are assumed to be 12 days (Nonaka et al., 1996; Takumi and van der Giessen, 2005), 17 days, or 44 days (Kapel et al., 2006). The maturation periods are 30 days for ingested larvae to mature in foxes (Nonaka et al., 1996) and 112 days for ingested eggs to mature into larvae in rodents (Matsumoto et al., 1998). The lower and upper estimates for the growth rate s (Table 4) were substituted into Eq. (2) to calculate the lower and upper estimates for R_0.

2.5. Assumptions of the model

The following assumptions were made about host populations in deriving the expression for R_0. First, the mean fox population density and the mean rodent population density are constant. Second, infected foxes and rodents neither enter nor leave the surveillance region. In addition, infected rodents were assumed to be infected for their lifetime.

2.6. Intervention strategies and evaluating intervention

Intervention alters the parasite transmission process, aiming to reduce the reproduction number of the parasite to less than 1 so that it eventually disappears from the contaminated area. We evaluate one particular intervention strategy, control of fox population density. Because predation by foxes is not the major cause of rodent death ($n\beta_1 \ll \mu_2$) the reproduction number expressed by Eq. (1) is proportional to the fox population density n,

$$R_0 \sim n \quad (3)$$

Therefore, when the fox population density is reduced by some numerical fraction, the reproduction number R_0 reduces by the same fraction.

The reproduction number is also proportional to the probability that an ingested larva matures into an adult worm in the intestine of the fox (π_1) so that,

$$R_0 \sim \pi_1 n \quad (4)$$

As a result of successful control of the parasite, the proportion of foxes lacking acquired immunity to the parasite will increase, resulting in increasing π_1. Thus the fox population density must be further reduced by the same factor to compensate for the increase in R_0 due to the loss of immunity.

3. Results

3.1. Prevalence and spatial distribution

The first *E. multilocularis*-infected foxes in the southern province of Limburg in The Netherlands (Fig. 1) were detected during the surveillance conducted between the months of November 1996 and February 1997 (van der Giessen et al., 1999). A total of 39 foxes were examined in the Limburg region, of which 14 foxes were adults (older than 1 year) and 25 foxes were juveniles (Table 1). One fox was infected with a moderate number of worms in the intestine and two foxes were positive by PCR but no worms could be detected (Table 2). All three positive foxes were found close to the border with Belgium (Fig. 2). All other foxes sampled in a northward direction were negative

Table 1
Number of foxes positive and negative to infection using PCR by age group in the two periods of surveillance (1996–1997 and 2002–2003) in The Netherlands

Year	1996	2003
PCR + juvenile[a]	2	10
PCR − juvenile	23	60
PCR + adult	1	13
PCR − adult	13	112
Total	39	195

[a] Less than 1 year of age.

Table 2
Survey results 1996–1997

PCR	Worm count	No. of foxes
–	0	36
+	0	2
+	100	1

Foxes were tested for the presence and the quantity of *Echinococcus multilocularis* by PCR and by microscopy using intestinal scrapings. The number of foxes having the specified combination of the test outcomes are shown in the third column.

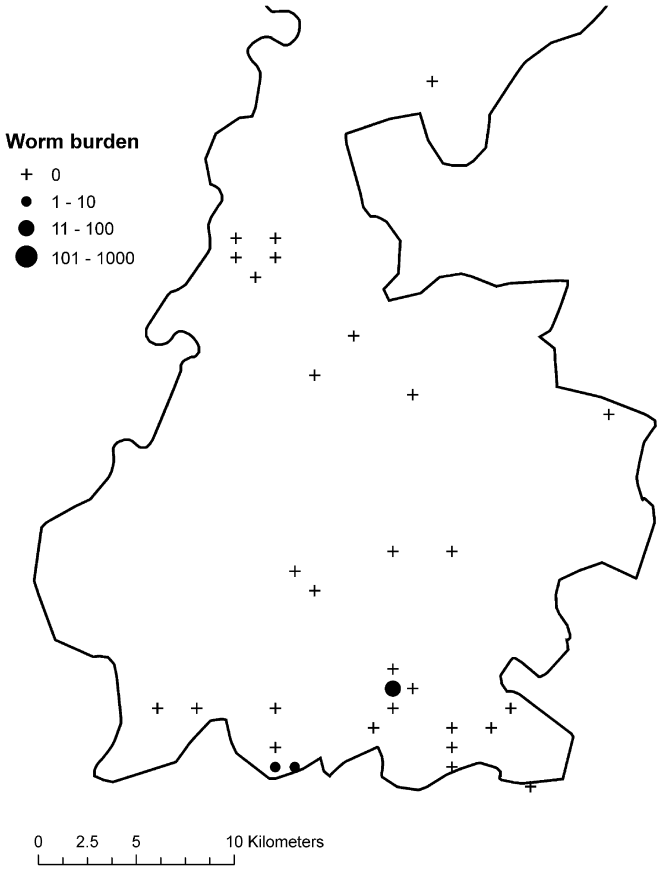

Fig. 2. Spatial distribution of *Echinococcus multilocularis*-positive and -negative foxes as analysed in 1996–1997. Foxes that tested negative for *E. multilocularis* both by PCR and by intestinal scraping are indicated by crosses. Foxes that tested positive by either method are indicated by black circles. The numbers of worms determined by intestinal scraping are illustrated by the size of the black circles. Foxes that tested positive by PCR only were assigned a worm burden equal to 1.

both by PCR and by intestinal scraping. Subsequently a survey was conducted in the months between November 2002 and February 2003 to determine a baseline prevalence with the aim to follow trends over time. In total 196 foxes were examined, of which 125 were adults, 70 were juveniles, and one was unclassified (Table 1). The number of infected foxes was 23. Worm counts in individual foxes were highly dispersed. A few of most heavily infected foxes harboured the majority of the total number of the parasitic worms

Table 3
Survey results 2002–2003

PCR	Worm count	No. of foxes
–	0	173
+	0	8
+	1	2
+	5	2
+	6	1
+	10	1
+	25	1
+	50	2
+	100	1
+	200	1
+	300	1
+	500	1
+	1000	2

Foxes were tested for *Echinococcus multilocularis* by PCR and by microscopy as shown in Table 2.

(Table 3). Fitting the negative binomial distribution to each of the surveillance data sets separately resulted in small values for the parameter k (0.015 for the first and 0.019 for the second surveillance) and the fit was significantly better than Poisson distribution in both cases ($P > 0.99$). This observation is in accordance with a study conducted in Switzerland (Hofer et al., 2000). When sampling locations of the foxes were ignored, the means of the negative binomial distributions were not found to be significantly different based on the likelihood ratio test. In the follow-up survey in Limburg most severely infected foxes were found in the southern region where the first infected foxes were found in the period 1996–1997 (Fig. 3). Infected foxes with worm burdens comparable to those of the first survey are now detected across a wider region of the Limburg province (Fig. 3).

We used the likelihood ratio to test whether negative binomial variation alone can explain the observed spatial and temporal change in parasite distribution or, alternatively, whether growth of the parasite population due to its recent introduction into this region and/or the spreading of the parasite were more likely. Compared with the negative binomial variation alone, spreading of the parasite in a Northern direction significantly improved the model fit ($P = 0.95$). An additional assumption that the parasite population is increasing in number also improved the fit ($P = 0.83$). Resulting parameter estimates are listed in Table 4. Estimate of R_0 for *E. multilocularis* based on Eq. (2) was 1.6 (1.1–3.5 using profile-likelihood based 95% CI on the growth rate s). The effects of varying the life expectancies of foxes and parasites on R_0 were limited. Increasing the life expectancy of foxes to 2 years changed the estimated R_0 by less than one decimal place. Increasing the life expectancy of adult worms to 44 days changed the estimated R_0 to 1.7. Another inference from the model fit is that the parasite appears to expand its niche in the province of Limburg at a speed of 2.7 km per year ($= 2\sqrt{sD}$) in a northward direction.

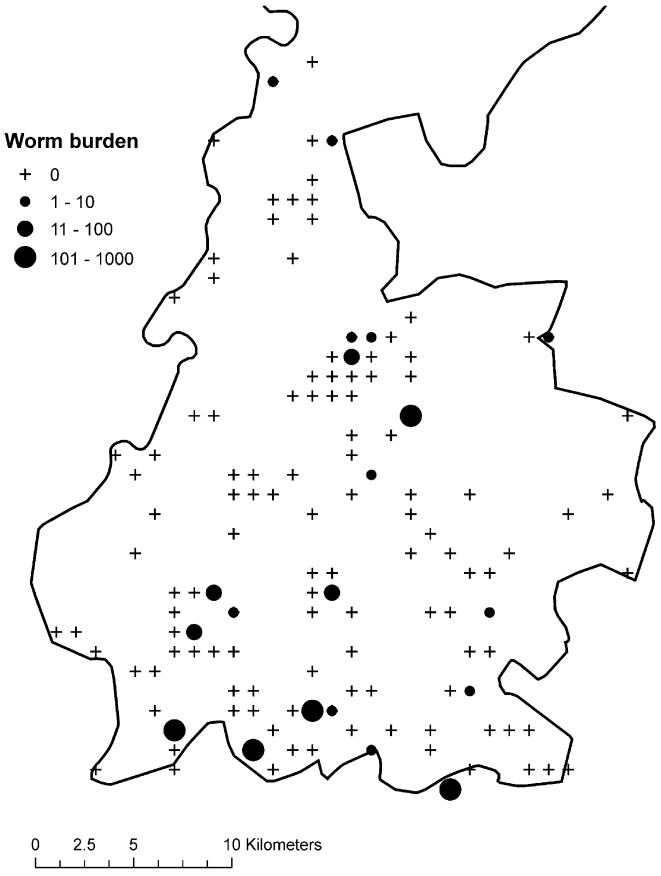

Fig. 3. Spatial distribution of *Echinococcus multilocularis*-positive and -negative foxes as analysed in 2002–2003. Foxes that tested negative for *E. multilocularis* both by PCR and by intestinal scraping are indicated by crosses. Foxes that tested positive by either method are indicated by black circles. The numbers of worms determined by intestinal scraping are illustrated by the size of the black circles. Foxes that tested positive by PCR only were assigned a worm burden equal to 1.

Table 4
Estimates of the parameters of the mathematical model describing the population increase and the geographical spread of *Echinococcus multilocularis*

Description	Symbol	Estimate	95% CI[a]	Unit
Log-worm count of the first infected fox	α	3.2	(0.93, 4.6)	None
Rate of increase in the mean worm count	s	0.36	(0.12, 0.68)	year^{-1}
Rate of dispersal in space	D	4.9	(3.1, 30)	km^2 year^{-1}
Parasite aggregation index	k	0.02	(0.01, 0.03)	None

[a] Confidence interval, based on profile-likelihood.

4. Discussion

About 100 km southward into Belgium (from the current surveillance area in Limburg), there is a region where a large number of foxes infected with *E. multilocularis* were detected (Vervaeke et al., 2003, 2006) and in recent years the parasite has been expanding its range in a northern direction towards Limburg along the river Maas. In addition, an area as far as 200 km north of Limburg was parasite-free at the time of the 1996–1997 surveillance (0/187 foxes) (van der Giessen et al., 1999). Therefore it is very likely that the two surveillances reported here document the movement of the parasite's infection front in the years between 1996 and 2003.

Based on our surveillance data we estimated that the reproduction number R_0 is greater than 1, indicating that *E. multilocularis* is an emerging parasite in the fox population in the border area of Limburg. Parasite emergence in this study is defined neither by the prevalence nor by the mean worm count ignoring the sampling locations. When parasites emerge, we would expect to observe the parasite numbers in a specific pattern in space and time. The spatial–temporal pattern of observed parasite numbers is best explained by assuming the spatial spreading and parasite population growth, rather than the two alternative explanations, namely the parasite population is constant in space and time, i.e. only the negative binomial variation explains the observed pattern, or the parasite population is not growing but spreading into a wider region.

The estimated R_0 is not likely to exceed 4. The magnitude of R_0 determines the amount of effort needed to control the parasite. A possible strategy for parasite control was demonstrated in Germany and Switzerland in experimental settings where baits containing an anthelmintic drug were dispersed using an aeroplane (Tackmann et al., 2001; Hegglin et al., 2003). It was shown that this method is effective as long as it is enforced but it never eliminated the parasite completely. We have suggested that the parasites might have been completely removed from the fox population but the larvae still remained in the population of rodents, causing re-emergence of the parasite following the apparent disappearance of the parasite from the fox population (Takumi and van der Giessen, 2005). While the baiting strategy is partially successful, the same cannot be said about controlling fox population density. Because R_0 is proportional to fox population density (Eq. (3)) a condition for successful control might be to reduce the fox population density by a factor of four or more to reduce R_0 below one. Although it is very difficult to determine fox density in a particular area, the best professional judgment after the second surveillance in Limburg, based on age distribution of the fox population investigated, reproduction ratio of the females and hunting index, was an estimated fox density of 2–4 per km^2 in spring and 4–9 per km^2 in autumn (Mulder et al., 2004). To control *E. multilocularis*, the fox density should be reduced to 0.5–1 per km^2 in the spring and 1–2 per km^2 in autumn. One possible complication is that some of the foxes in the Limburg area may have acquired immunity against the parasite. When the parasite is eliminated, there will be more foxes in the area without acquired immunity to the parasite. In naive foxes, almost 100% of the ingested larvae become adult worms (Kapel et al., 2006). R_0 is also proportional to the probability of infection in foxes (π_1 in Eq. (4)) so that if the foxes became twice as susceptible due to loss of

immunity, the fox population density must be reduced further by a factor of two to compensate for the susceptibility increase. This would mean that unless the fox population density is kept below 0.25–0.5 per km^2 in spring and 0.5–1 in autumn, intervention is expected to fail to locally eliminate the parasite. Achieving this low fox population density and keeping it permanently low by human intervention (e.g. culling) is practically impossible. The role of host immunity was also discussed elsewhere (Torgerson, 2006).

Estimation of R_0 rests on the assumption that all foxes acquired infection locally within the surveillance area. However, we cannot rule out the possibility that the foxes were infected somewhere distant and later moved into the surveillance area at the time when our first surveillance ended in 1997. If infected foxes came from distant places we would have overestimated R_0. However, we hypothesise that this effect is limited because if foxes did indeed acquire their infections remotely and then travelled a long distance to enter the surveillance area, we would have seen a mix of lightly and heavily infected foxes over the whole study region. Therefore the observed north-south gradient in worm burden is consistent with locally, rather than distantly acquired infections. In animal disease surveillance it is virtually impossible to infer the locations, where the animals acquired their infections, but the origin of infection may be assessed indirectly. If the sampled foxes were shown to be related, for example, by examining their mitochondrial DNA, they are more likely to have acquired their infections locally, an indication that local environmental conditions support the persistence of the parasite life cycle.

Using a one-dimensional growth diffusion model, we modelled anisotropy in worm burden distribution (north-south gradient), but heterogeneity in the environment was not modelled. However, spatial heterogeneity in the environment may only be important on a small spatial scale, not on our coarse scale analysis in which local variations are averaged over the surveillance area.

Recently three human patients were reported in Belgium, two patients from the province of Luxembourg and one from the province of Liege in 2004 (Detry et al., 2005). The latter province is less than 10 km from Limburg. The prevalences of infection in the local foxes populations in these provinces in the period between 1997 and 2002 were 30% (97/321 foxes) in Luxembourg and 15% (26/171) in Liege. Because overall parasite prevalence in Limburg is 11% (26/235), comparable to the Liege area, it seems reasonable to anticipate the first case of alveolar echinococcosis in Limburg in coming years.

In conclusion, using this approach we showed that *E. multilocularis* is spreading and thus is an emerging zoonotic parasite in the southern province of Limburg in The Netherlands, the westernmost border in Europe. Furthermore, parasite control cannot be achieved by reducing fox density. Increasing presence of *E. multilocularis* in foxes might indicate that human cases could appear in the future.

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References

Ammann, R.W., Eckert, J., 1996. Cestodes. *Echinococcus*. Gastroenterol. Clin. North Am. 25, 655–689.

Anderson, R.M., May, R.M., 1991. Infectious Diseases of Humans. Oxford University Press, New York.

Deplazes, P., Eckert, J., 1996. Diagnosis of the *Echinococcus multilocularis* infection in final hosts. Appl. Parasitol. 37, 245–252.

Detry, O., Honore, C., Delwaide, J., Demonty, J., De Roover, A., Vivario, M., Thiry, A., Hayette, M.P., Belaiche, J., Meurisse, M., Honore, P., 2005. Endemic alveolar echinococcosis in Southern Belgium? Acta Gastroenterol. Belg. 68, 1–4.

Eckert, J., Gemmell, M.A., Meslin, F.-X., Pawlowski, Z.S., 2001. WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern. World Health Organization, Paris.

Edelstein-Keshet, L., 1987. Mathematical Models in Biology. The Random House, New York.

Hegglin, D., Ward, P.I., Deplazes, P., 2003. Anthelmintic baiting of foxes against urban contamination with *Echinococcus multilocularis*. Emerg. Infect. Dis. 9, 1266–1272.

Hofer, S., Gloor, S., Muller, U., Mathis, A., Hegglin, D., Deplazes, P., 2000. High prevalence of *Echinococcus multilocularis* in urban red foxes (*Vulpes vulpes*) and voles (*Arvicola terrestris*) in the city of Zurich, Switzerland. Parasitology 120 (Pt. 2), 135–142.

Kapel, C.M., Torgerson, P.R., Thompson, R.C., Deplazes, P., 2006. Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats. Int. J. Parasitol. 36, 79–86.

Losson, B.J., Coignoul, F., 1997. Larval *Echinococcus multilocularis* infection in a dog. Vet. Rec. 141, 49–50.

Lucius, R., Bilger, B., 1995. *Echinococcus multilocularis* in Germany: increased awareness or spreading of a parasite? Parasitol Today 11, 430–434.

Matsumoto, J., Yagi, K., Nonaka, N., Oku, Y., Kamiya, M., 1998. Time-course of antibody response in mice against oral infection with eggs of *Echinococcus multilocularis*. Parasitology 116 (Pt. 5), 463–469.

Mulder, J.L., Jansman, H.A.H., van der Giessen, J., 2004. Ecologisch onderzoek aan geschoten vossen in Zuid-Limburg, 2002–2003. Alterra, Wageningen.

Nonaka, N., Iida, M., Yagi, K., Ito, T., Ooi, H.K., 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.

Rausch, R., 1995. Life cycle patterns and geographic distribution of *Echinococcus* species. In: Thompson, R.C.A., Lymbery, A.J. (Eds.), *Echinococcus* and Hydatid Disease. CAB International, Wallingford, pp. 89–134.

Roberts, M.G., Aubert, M.F., 1995. A model for the control of *Echinococcus multilocularis* in France. Vet. Parasitol. 56, 67–74.

Romig, T., Bilger, B., Dinkel, A., Merli, M., Mackenstedt, U., 1999. *Echinococcus multilocularis* in animal hosts: new data from western Europe. Helminthologia 36, 185–191.

Schweiger, A., Ammann, R.W., Candinas, D., Clavien, P.-A., Eckert, J., Gottstein, B., 2007. Human alveolar echinococcosis after fox population increase, Switzerland. Emerg. Infect. Dis. 13, 878–882.

Skellam, J.G., 1951. Random dispersal in theoretical populations. Biometrika 38, 196–218.

Tackmann, K., Loschner, U., Mix, H., Staubach, C., Thulke, H.H., Conraths, F.J., 1998. Spatial distribution patterns of *Echinococcus*

multilocularis (Leuckart 1863) (Cestoda: Cyclophyllidea: Taeniidae) among red foxes in an endemic focus in Brandenburg, Germany. Epidemiol. Infect 120, 101–109.

Tackmann, K., Loschner, U., Mix, H., Staubach, C., Thulke, H.H., Ziller, M., Conraths, F.J., 2001. A field study to control *Echinococcus multilocularis*—infections of the red fox (*Vulpes vulpes*) in an endemic focus. Epidemiol. Infect. 127, 577–587.

Takumi, K., van der Giessen, J., 2005. Transmission dynamics of *Echinococcus multilocularis*; its reproduction number, persistence in an area of low rodent prevalence, and effectiveness of control. Parasitology 131, 133–140.

Torgerson, P.R., 2006. Canid immunity to *Echinococcus* spp.: impact on transmission. Parasite Immunol. 28, 295–303.

van der Giessen, J.W., Rombout, Y.B., Franchimont, J.H., Limper, L.P., Homan, W.L., 1999. Detection of *Echinococcus multilocularis* in foxes in The Netherlands. Vet. Parasitol. 82, 49–57.

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

Venzon, D.J., Moolgavkar, S.H., 1988. A method for computing profile likelihood based confidence intervals. Appl. Stat. 37, 87–94.

Vervaeke, M., Dorny, P., Vercammen, F., Geerts, S., Brandt, J., Van Den Berge, K., Verhagen, R., 2003. *Echinococcus multilocularis* (Cestoda, Taeniidae) in red foxes (*Vulpes vulpes*) in northern Belgium. Vet. Parasitol. 115, 257–263.

Vervaeke, M., van der Giessen, J., Brochier, B., Losson, B., Jordaens, K., Verhagen, R., Coulander Cde, L., Teunis, P., 2006. Spatial spreading of *Echinococcus multilocularis* in red foxes (*Vulpes vulpes*) across nation borders in western Europe. Prev. Vet. Med. 76, 137–150.