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A field study to control *Echinococcus multilocularis*-infections of the red fox (*Vulpes vulpes*) in an endemic focus

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SUMMARY

Foxes harbouring *E. multilocularis* represent an important source for human infection with this parasite which causes alveolar echinococcosis. To minimize the risk of human infection, a control study was conducted to reduce the prevalence of *E. multilocularis*-infection in foxes in an focal endemic area of 5000 km². Foxes were given access to baits containing 50 mg praziquantel. Twenty baits per km² were distributed by airplane during 14 campaigns. The effects of control measures were monitored by parasitological examination of 9387 foxes shot before and during the control trial. A distinct reduction of the prevalence of *E. multilocularis* was observed for both, the initially endemic area and the low-endemic periphery. The effect was more pronounced in adult than in juvenile foxes. Under control conditions, the risk area decreased in size. However, an eradication of the parasite was not reached with the chosen strategy.

INTRODUCTION

Echinococcus multilocularis (Leuckart, 1863) is a cestode parasite of the northern hemisphere. The parasitic cycle probably exists in all regions in central Europe [1], but also for instance in large areas of China [2]. In North America the parasite has been detected in the central northern part as well as in Alaska and is considered to be of increasing concern [3]. The translocation of infected foxes to non-endemic regions may be a special epidemiological risk in the USA [4].

Foxes represent the main definitive host of *E. multilocularis* in Europe. Recent studies investigating the risk potential in urban areas in central Europe indicate that infected foxes seem to represent an important reservoir for the parasite even under urban conditions [5]. While dogs, cats and other carnivores can also harbour the tapeworm, their epidemiological importance in Europe is unclear [6, 7]. Different, mostly arvicolid rodent species (especially *Microtus arvalis*, *Arvicola terrestris*, *Ondathra zibethicus*) serve as intermediate hosts and contract the infection by oral uptake of oncospheres (tape worm 'eggs') which are shed in faeces of infected definitive hosts after 4 weeks of prepatency.

The larval stage of *E. multilocularis* can cause human alveolar echinococcosis (AE), a serious hepatic disease which is usually lethal if left untreated (reviewed in [8]). AE is thus considered as the most dangerous autochthonous parasitic zoonosis in central Europe [9]. Although it is generally assumed that humans contract the infection via the oral route, the precise risk factors for human infection have not yet been identified. AE has been a major problem in the

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Alaskan Eskimo population [10, 11]. In the contiguous United States it seems to be a very rare human disease since only two cases have been reported so far [3, 12]. For the endemic regions of central Europe, annual incidence rates of 0·02–1·4 cases per 100000 inhabitants have been estimated [1].

Knowledge about the distribution of *E. multilocularis* among red foxes in Germany has changed completely in recent years. Until about 10 years ago the parasite was assumed to be restricted to the south-western part of Germany, an area long known for AE in humans. The epidemiological situation in Germany is now characterized by endemic areas with a higher prevalence in many western, especially in south-western parts of the country, whereas a low-endemic situation with some spotted foci prevails in the East [13–15]. Despite this geographical distribution of *E. multilocularis* in the fox population, cases of human AE are reported almost exclusively from south-western regions of Germany. Since the occurrence of this parasite in foxes has only recently been discovered in some regions and because of an apparent increase of prevalence in certain areas, an increasing risk for human infection cannot be excluded. This warrants the investigation of the chances and limitations of intervention strategies against *E. multilocularis*. This paper presents results of a field study in which praziquantel-containing baits were used to control the parasite within a circumscribed focus endemic for *E. multilocularis* in foxes [15].

MATERIALS AND METHOD

Baits

Praziquantel (Droncit®, Bayer AG, Leverkusen, Germany) was homogeneously incorporated into the coat of baits (Rabifox®, Impfstoffwerk Dessau-Tornau GmbH, Dessau, Germany) which are commonly used for the oral vaccination of foxes against rabies. Each bait contained 50 mg praziquantel, i.e. at least 5 mg/kg body mass. In some treatment campaigns, rabies vaccine blisters were also included in the baits.

Area

The baits were distributed in an area of 5000 km² situated in the Northwest of Brandenburg, Germany. Due to logistic reasons, foxes were sampled for parasitological examination in two counties of 4450 km² which are comprised in the control area. The region is characterized by two neighbouring endemic foci of *E. multilocularis* in foxes, approximately 432 km² in size, and a low-endemic periphery [15].

Treatment campaigns

Based on previous experiences with praziquantel-containing baits [16] and rabies vaccination campaigns, the following design was chosen: 14 treatment campaigns were conducted between April 1995 and June 1997 at a density of 20 baits/km². The baits were dispersed from airplanes. Each campaign was carried out over 2 consecutive days. During the first year of treatment intervals of 6 weeks were chosen between individual campaigns. In the second and third year of the study, treatments were performed every 12 weeks. In the treatment campaigns 13 and 14, the initial treatment area of 5000 km² was reduced to a core region of 1200 km² which included the endemic foci and their periphery.

Examination of foxes

To analyse changes in the prevalence of the parasite in foxes during the control period, data of foxes shot in the study area between January 1992 and August 1997 were taken into consideration. For the control period a sampling plan was designed to detect at least one positive fox per year in a regional raster of 100 km² with 99% statistical safety if a prevalence of 1% was given. The population density of foxes was assumed to exceed 10000 animals per raster unit. Thus, a total number of 4732 foxes had to be examined [17]. Six collection points for shot foxes were installed in different parts of the study area and equipped with freezers (−20 °C). For each fox, name and address of the hunter, the precise location where the fox had been shot (marked on a map) and the date of shooting were registered. The foxes were then handled and examined as described [15]. For detection of *E. multilocularis*, the intestinal scraping technique (IST) was used [18]. To increase sensitivity, at least 12 scrapings for each juvenile fox, and at least 21 for each adult fox were examined.

In addition, 224 dogs and 387 cats from the same area, euthanized and submitted by veterinary practitioners, were investigated in the same way as the foxes.

Statistics

Data were either obtained before control [15] or recently sampled under control conditions. Only foxes

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which could unambiguously be attributed to the control area and the respective time interval were included in the analysis. The total sample was stratified according to (i) control status (before and under control), (ii) temporal interval (month; quarter of the year (I, II, III, IV); year), (iii) age class (juvenile, J; adult foxes, A) and (iv) epidemiological status of the region (endemic focus, E; low-endemic periphery, L). Strata were designated with letters or combinations of letters used as abbreviations for age class (J, A) and region (E, L).

For the strata, period prevalence (percentage of positives out of investigated foxes) and the respective 95% two-sided confidence intervals (CI) were estimated [19]. Relevant combinations were tested for prevalence differences with the χ^2 or Fisher's exact test. The temporal fluctuation in prevalence was analysed for systematic effects on short (months) and longer time-scale (years) for both the 'before control' stratum and the total data-set. For this purpose, the monthly stratified prevalence values were polished from deviation of the overall mean prevalence (mean polishing, [20]) by assigning deviations from the mean to different factors [21]. Even if the method originally was developed for space-time data the different temporal windows of control impact (i.e. long-term) and fox biology (i.e. short-term) motivate the consideration of two temporal main effects in the application. First, the observed proportion of cases (π_{ij}) in month i of year j of the study period was compared to the proportion of positives (p_{ij}) expected from the respective population at risk under the assumption of a homogeneous distribution. The resulting discrepancies ($\pi_{ij} - \mathrm{p}_{ij}$) were then modelled as a linear combination of the main effects [21] according to the equation:

$$g(\pi_{ij}, p_{ij}) = \mu + \alpha_i + \beta_j + \gamma_{ij},$$

where μ is the mean discrepancy, α_i the main effect associated with year i of the study, β_j the mean effect associated with month j of the year and γ_{ij} is an interaction effect. For g, measuring the modelled discrepancies, we applied the Pearson transformation

$$g(\pi, p) = (\pi - p)/\mathrm{sqrt}(p).$$

The coefficients ($\mu, \alpha, \beta, \gamma$) in the model show the magnitude of influence of different time intervals (i.e. year, month) and provide a basis for a test statistic. For the latter the coefficients were compared with those resulting from 9999 simulations of the null model thus allowing an analysis on the basis of a Monte-Carlo significance test [21]. Statistical significance was assumed for $P < 0{\cdot}05$.

In order to analyze the spatial infection risk for foxes before and under control conditions, relative risk functions [22] were calculated for different time intervals. Density estimations [23] were performed with the uniform kernel function simulating the home ranges of the foxes. The average radius of a home range was assumed as 2·5 km [15]. To examine whether increases in the relative risk functions for observed values deviated from random, a Monte-Carlo based test on the basis of 1000 simulations was performed under the null hypothesis, i.e. simulated *E. multilocularis* cases were randomly assigned in the total sample. For each of the resulting relative risk functions, the deviations from a relative risk of 1·0 were measured over all distances [24] on a logarithmic scale

$$\mathrm{d}_i = \sum_i \{\log \mathrm{RR}_i(x_i)\}^2; \quad i = 1, \ldots, 1000.$$

The resulting set of d_i ($i = 1 \ldots 1000$) determined the test distribution. The observed value (d_0) was then compared to the distribution, thus allowing a decision regarding statistical significance.

Computing

A program written in CLIPPER (Computer Associates International Inc., New York, USA) was used for the documentation of the data in a dBASE file (5.0 for Windows, Borland International Inc., Scotts Valley, CA, USA). Epi-Info 6.03 (Centers for Disease Control and Prevention, Atlanta, GA, USA, and World Health Organization, Geneva, Switzerland) was used for performing χ^2 and Fisher's exact test, and for calculating odds ratios (OR) and relative risks (RR). The mean polishing statistics and the Monte-Carlo test were implemented in C^{++} (Borland International Inc., CA, USA) code following [21] to support the necessary calculations. Harvard Graphics 3.0 (Software Publishing Corporation, Santa Clara, CA, USA), RegioGraph 2.1 (Macon, GmbH, Waghäusel, Germany), S-Plus 2000 (Mathsoft Inc., Seattle, USA) and ArcView (ESRI, Redlands, USA) were used for geographical and graphical documentation and analysis of the results.

RESULTS

For the evaluation of the control study, data on a total of 9387 foxes were available. The data sets include information on 4375 foxes investigated before

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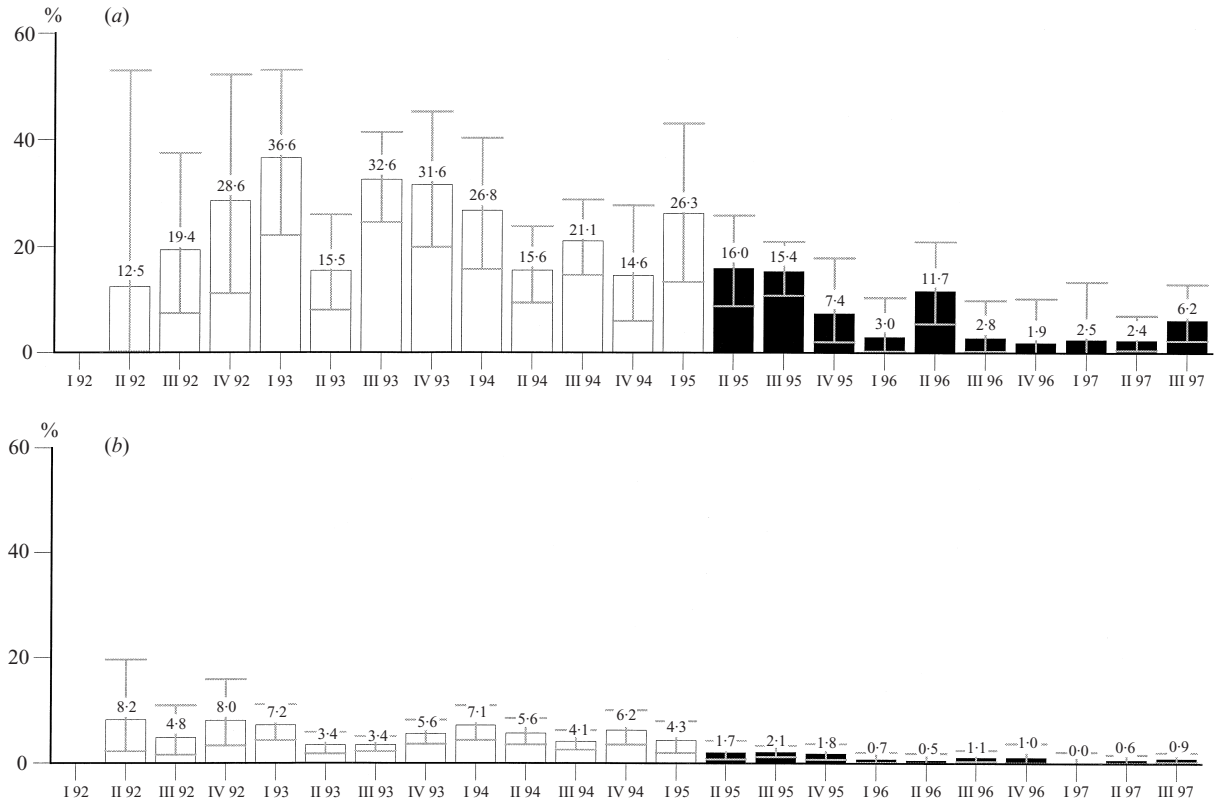

Fig. 1. Prevalence of infection with *Echinococcus multilocularis* in foxes. Estimated prevalence and the respective two-sided 95% confidence intervals are shown for the period before (white bars) and during (black bars) the control trial for each quarter of the year in (*a*) the endemic and (*b*) the low-endemic area.

control [15]. For 9330 foxes, at least the quarter of the year when they had been sampled was known, and 476 (5·1%) of these animals were found infected. For 8478 foxes, the age class was known (for the respective strata $N_{JE} = 569$, $N_{JL} = 2{,}427$, $N_{AE} = 944$, $N_{AL} = 4{,}538$). Finally, for 8475 foxes the complete data set (region, quarter and age) was available (i.e. for 1992–7: 199; 1830; 1432; 1894; 1624; 1496 foxes).

The investigation density calculated for the total study area (4450 km²) was 2.1 foxes per km², or 3.5 (E: 432 km²) and 1.7 (L: 4018 km²) for the two regional strata, respectively. For consecutive years from 1992–7 the respective annual investigation density values were 0·1, 0·5, 0·4, 0·5, 0·4 and 0·3 when the sample was evenly broken down over the total investigation area. The stratified annual investigation densities in the endemic focus E were 0·1, 0·6, 0·8, 0·8, 0·6, 0·6 and $< 0{\cdot}1$, 0·4, 0·3, 0·4, 0·3, 0·3 in the low-endemic periphery L.

During the control study (April 1995–August 1997), 5012 foxes fulfilled the requirements for examination as outlined in the sampling plan.

Prevalences were estimated on the basis of the random sample for the strata E and L, for each quarter of year, and the control status (Fig. 1). In both regions, E and L, a distinct reduction of prevalence could be observed for the period of the control study. The decrease of the prevalence seemed to be slightly lower in E as the estimated prevalence persisted rather unchanged during the first two quarters of control (II and III/95), rose again in II/96, and to a lesser extend in III/97. Eventually the estimated prevalence in E had decreased below 3% (except for quarter III/97 with 6·2%) and below 1% in L.

To separate time-related influences on the prevalence that had already been effective before the on-set of the control measures from those directly attributable to the control period, the prevalence curves of the various strata were analysed for regular temporal patterns ([21]; Table 1). Seasonality which could have overlaid the control-related dynamics was not detected in most of the strata. Before control, the seasonal factor 'month' was statistically significant only in the stratum 'JE'. However, with the data set for the entire study period, the factor 'year' was statistically significant in all strata (JE; JS; AE; AS), whereas the factor 'month' was only statistically significant in a single stratum (AL). In all strata except

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Table 1. *Analysis of the influence of the factor 'time' (month, year) on the prevalence of* Echinococcus multilocularis *in foxes of the random sample stratified according to region and age before the control trial and during the entire study period*

	Year				Month				Interaction			
	Before control		Entire study period		Before control		Entire study period		Before control		Entire study period	
Stratum†	*U*‡	*P*§	*U*‡	*P*§	*U*‡	*P*§	*U*‡	*P*§	*U*‡	*P*§	*U*‡	*P*§
AE	1·16	n.s.	11·78	***	1·00	n.s.	1·73	n.s.	0·75	n.s.	2·67	***
JE	1·62	n.s.	2·93	*	2·54	*	1·67	n.s.	1·39	n.s.	2·02	**
AL	2·07	n.s.	18·36	***	1·16	n.s.	1·83	*	1·31	n.s.	4·34	***
JL	0·63	n.s.	2·77	*	0·28	n.s.	0·44	n.s.	0·59	n.s.	1·06	n.s.

† AE, adult foxes shot in endemic region; JE, juvenile foxes shot in endemic region; AL, adult foxes shot in low-endemic region; JL, juvenile foxes shot in low-endemic region.
‡ *U* statistics. n.s., nonsignificant.
§ Based on 9999 runs of a Monte-Carlo simulation [21].

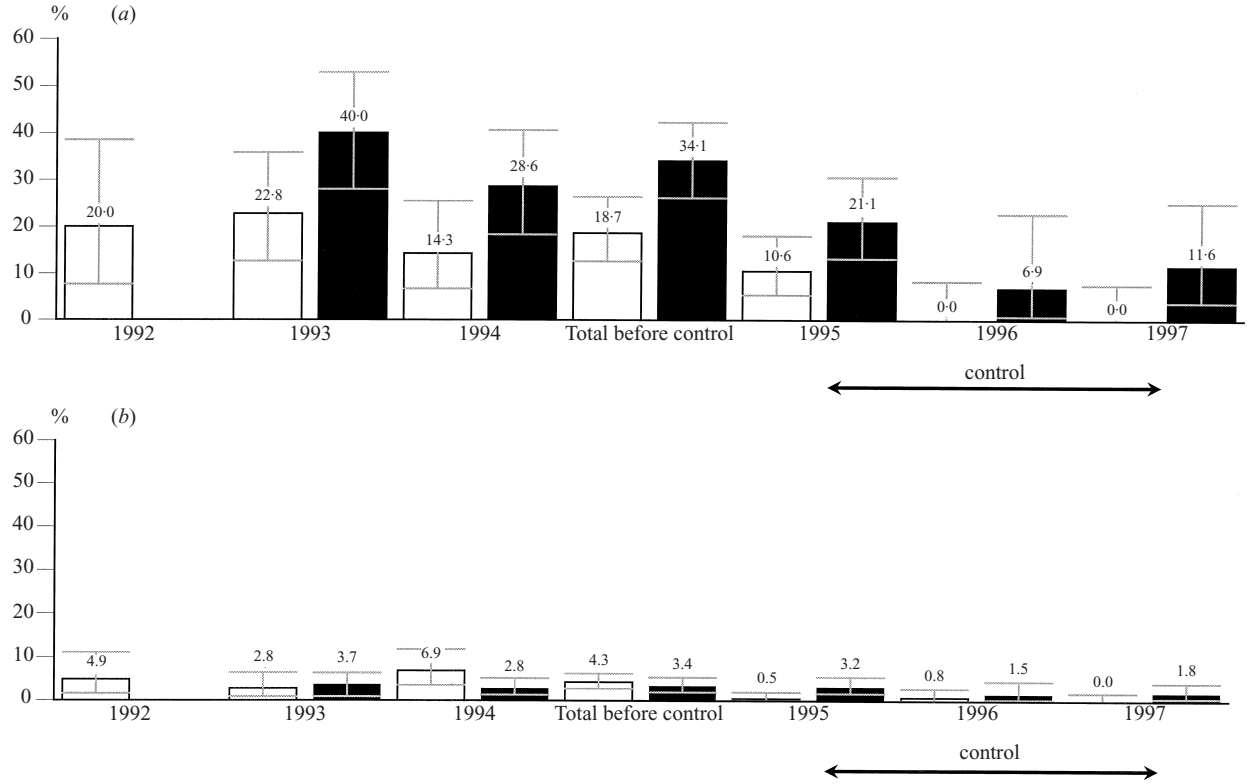

Fig. 2. Prevalence of infection with *Echinococcus multilocularis* in foxes for the interval July–September* of each year. Estimated prevalence and the respective two-sided 95% confidence intervals are shown for adult and juvenile foxes for the period before and during the control trial for each quarter of the year in (*a*) the endemic and (*b*) the low-endemic area (white bars, adult foxes; black bars, juvenile foxes). * in 1997: July–August only.

JL an interaction between the factors 'year' and 'month' was observed for the entire study period, but not for the period before control.

Comparing age classes, the prevalence was estimated for the time interval July–September in each year under study (i.e. quarter III; Fig. 2). In the second and third year of control no infected adult fox was detected in region E during this interval, while 6·9% (CI: 0·8, 22·9) and 11·6% (CI: 3·8, 25·1) juvenile foxes were found infected in the respective strata. More pronounced, in region L all infected foxes were juveniles from the last year of the control trial,

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Table 2. *Differences in the prevalence of* Echinococcus multilocularis *infection between adult and juvenile foxes in the interval July–September in years before and during the control trial*

Region	Control status	Year	*N*	χ^2	*P*	Odds ratio (OR)			Relative risk (RR)		
						OR	95%	CI	RR	95%	CI
Endemic	No	1993	122	4·13	*	2·26	0·96	5·45	1·75	1·00	3·08
Endemic	No	1994	133	3·97	*	2·40	0·93	6·54	2·00	0·98	4·07
Endemic	No	Total	285	8·77	**	2·25	1·27	4·04	1·83	1·21	2·75
Endemic	Yes	1995	199	4·14	*	2·25	0·96	5·53	1·99	1·01	3·93
Endemic	Yes	1996	71	2·98	n.s.†		u.d.				
Endemic	Yes	1997	98	6·74	*†		u.d.				
Low endemic	No	1993	647	0·47	n.s.						
Low endemic	No	1994	351	3·07	n.s.						
Low endemic	No	Total	1100	0·59	n.s.†						
Low endemic	Yes	1995	739	7·04	**	6·02	1·32	55·62	5·86	1·32	25·98
Low endemic	Yes	1996	452	0·60	n.s.†						
Low endemic	Yes	1997	556	4·87	*†		u.d.				

† Fisher exact *P*-value. n.s., nonsignificant; u.d., undetermined.

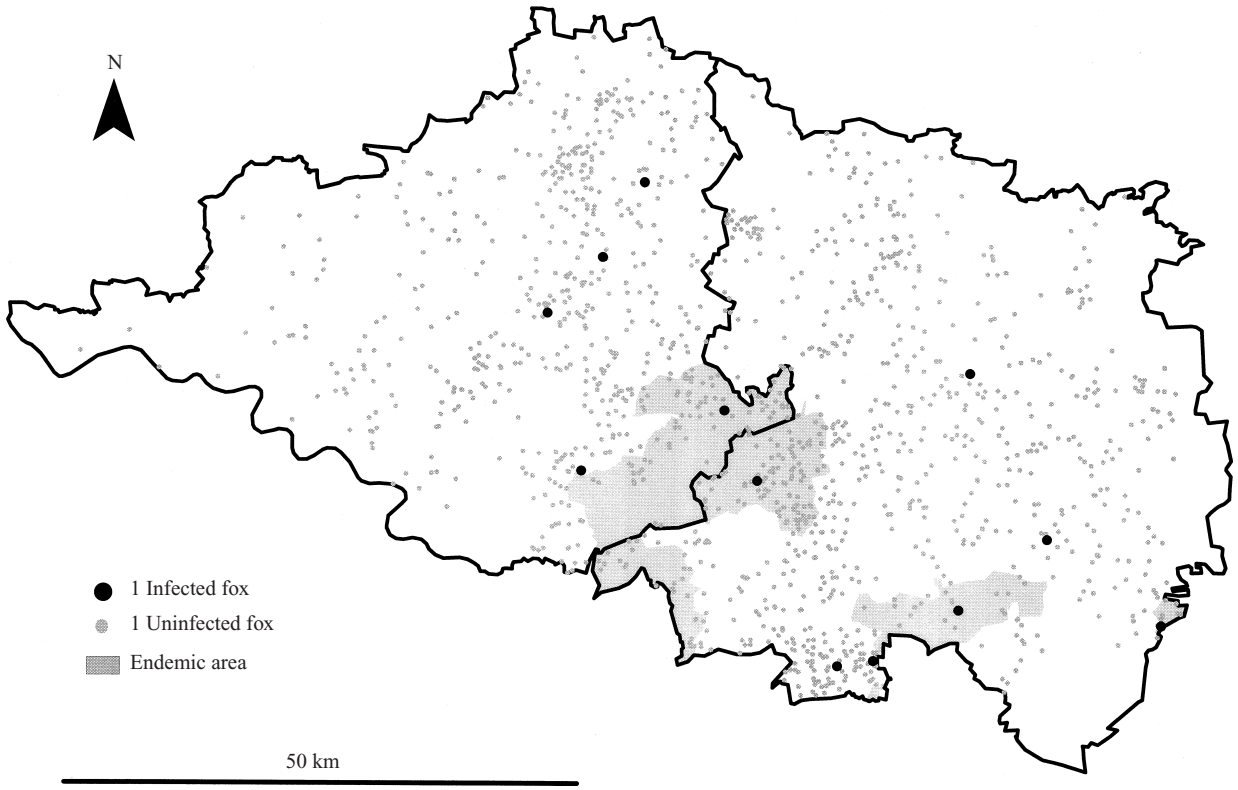

Fig. 3. Spatial distribution of *Echinococcus multilocularis*-infected foxes. Geographic positions of foxes found infected with *E. multilocularis*-infected (black dots) foxes or uninfected with this parasite (grey dots) between July 1996 and June 1997 are shown.

resulting in an estimated prevalence of 1·8% (CI: 0·6, 4·1). In the region E, the prevalence in adult and juvenile foxes is significantly different both, before and under control, except for 1996, while in region L, the two age classes differ significantly only under control conditions, again with the exception of 1996 (Table 2).

The spatial distribution of infected versus non-infected foxes under control was visualized on a map for the time interval following campaign 10 until

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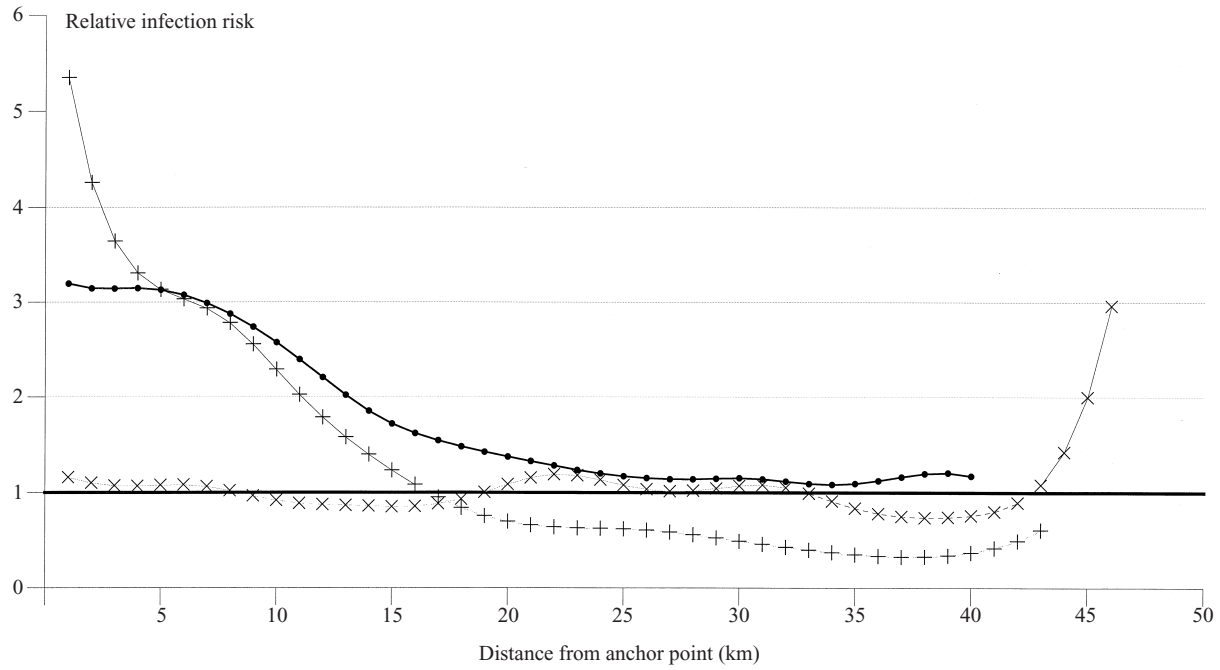

Fig. 4. Relative risk for infection with *Echinococcus multilocularis* as a function of distance from the centre of the endemic focus before and during the control trial. The relative risk for infection with *E. multilocularis* was calculated on the basis of a density estimation for the period before (●–●) control, and during control campaigns 1–5 (+) and 10–13 (×).

campaign 13 (i.e. July 1996–June 1997) depicting the last phase of control (Fig. 3). Infected foxes appear randomly distributed throughout the study area when compared to the situation before control [15] and regional clustering is no longer apparent. The less focal distribution of the parasite could be demonstrated by relative risk functions estimated for increasing distance from the anchor point within one of the endemic foci [22], by comparing data obtained before control [15] with the results achieved under control. To this end, periods following representative campaigns (1–5, beginning of intervention; 10–13, end of intervention) were chosen (Fig. 4). When the relative risk of infection in space (calculated for the distance to a hypothetical anchor point in the centre of the endemic focus as described, [15]) is compared for the situation before control and for the early campaigns 1–5 (Fig. 4), the increase in relative infection risk near the anchor point is still present ($p_{\text{before}} = 0{\cdot}001$; $p_{[1-5]} < 0{\cdot}001$). However, the relative risk of 1 is approached in a distinctly shorter distance already after the first campaigns. At the end of the intervention (campaigns 10–13), an increase in the relative risk could no longer be observed ($p_{[10-13]} = 0{\cdot}78$) and the graph remained at a relative risk of approximately one regardless of the distance from the anchor point.

None of the dogs and cats examined was found infected with *E. multilocularis*.

DISCUSSION

Successful control of *E. multilocularis*-infection in foxes may provide a significant contribution towards the reduction of the infection risk for humans in regions endemic for the parasite. It must be noted, however, that the identification of risk areas for human AE where a control programme can be taken into consideration is difficult since the true risk factors for human infection are still controversial. It is not even clear whether a direct relationship exists between the regional prevalence in foxes and human infections, although there is some evidence in favour of this hypothesis [25]. In Germany, the regional distribution of infected foxes and known cases of human AE is surprisingly different at present [26]. This may at least in part be due to imprecise knowledge about the true numbers of AE cases and their geographical distribution, since reporting of AE is not mandatory. Moreover, it is not clear whether the 'new' endemic areas for *E. multilocularis* in foxes in Germany are really 'new' or were only overlooked until the last decade. Due to the long incubation period of AE in

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humans (up to 15 years), possible changes in exposition will be reflected by alterations in the incidence of AE only with considerable time delay. Nevertheless, projects evaluating control strategies under field conditions, are an important source of information for decisions about the practicability and efficacy of intervention measures.

Control measures against another parasite of the genus *Echinococcus*, *E. granulosus*, have already been carried out successfully in different areas of the world [27–36]. They concern livestock kept under pastoral conditions (especially the dog–sheep–cycle of *E. granulosus*). Control is based on anthelminthic treatment of dogs and preventing the contact of dogs with infected offal. More recently, a vaccine was evaluated in control trials [37, 38].

Praziquantel has proved highly effective against various cestode parasites including *E. multilocularis* [39]. Rausch et al. [40] effectively used it for the first time to control *E. multilocularis* in dogs in an endemic area in Alaska, where dogs played a crucial role in the parasitic cycle and in transmission of the infection to humans. The changes in the prevalence of the parasite during the control measures was monitored by investigating the regional population of intermediate hosts.

In regions such as central Europe, where control has to focus on the silvatic cycle of *E. multilocularis*, intervention measures are more difficult to develop [32, 41]. Praziquantel can only be delivered to foxes as a component of a bait, but the proportion of animals successfully treated by offering baits is much lower than by direct application of the drug. Moreover, the treatment does not influence the susceptibility of the host population since foxes can be re-infected within a few hours after uptake of a bait, as praziquantel just clears the worm-burden but has no lasting effect. Therefore, baits have to be distributed frequently which makes the strategy expensive and can cause logistic problems.

The first study to use praziquantel treatment to control *E. multilocularis* in foxes was carried out between December 1989 and February 1991 in the Swabian Jura, southern Germany [16]. This region has been known as highly endemic for at least 150 years. In Germany, most known human AE cases occur in this region. Schelling and colleagues [16] temporarily observed a distinct reduction of the prevalence when baits containing praziquantel were distributed at intervals of 8–14 weeks. The study area of 566 km^2 was situated within a highly endemic region. Thus influences on the study area from its surroundings could not be excluded.

Based on the results of Schelling et al. [16], a large-scale application of praziquantel in the fox population was planned and started in Brandenburg in 1995. In view of lessons learnt from the German rabies control programme, a large control area was chosen (5000 km^2). The application regime was adapted to specific epidemiological conditions in Brandenburg which are different from those in southern Germany. The focal endemic situation in the Northwest of Brandenburg represents a typical area for the rather low-endemic eastern part of Germany [14]. The region is characterized by local endemic foci with a low-endemic periphery [15]. This initial status provides advantages for the evaluation of the control programme: (i) the effect of control can be studied under both, endemic and low-endemic conditions in a single project; (ii) since the study area is surrounded by a low-endemic region, interfering influences from outside the study area were negligible; (iii) finally, public expectations regarding the success of the project were low since no human AE cases have been reported from this region so far. Nevertheless, a control study in a wild living population cannot fulfil all requirements for demonstrating the causal relationship between the control measures and the observed effects in a strict sense. The aim of such a study is to detect effects, for instance on the prevalence of the infectious agent, which are not random and are associated with the control measures in a plausible way.

The expected dynamics of parasite abundance under the supposed effect of control measures can be derived from the life-cycle of the parasite in the following manner. At the beginning, before control, the area was to a certain extent contaminated with oncospheres (generation O_0) and there was a population of infected intermediate hosts (silvatic rodents, especially *Microtus arvalis* and *Arvicola terrestris*; generation R_0). Thus, the re-infection risk for foxes was almost unchanged in the first phase of control. The persistingly high level of estimated prevalences in the endemic area for the first two quarters (II and III in 1995) after the beginning of control clearly indicated the presence of this residual infection pressure to foxes. These re-infections had to be terminated by applying praziquantel during the prepatent period to prevent further contamination of the control area with infectious oncospheres (generation O_1). If every praziquantel-treated fox in the control area was likely to get re-infected shortly after

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treatment, the prepatent period (approximately 28 days) would be the optimal interval between individual treatments to prevent the shedding of oncospheres (O_1). Intervals of 6 weeks between the campaigns were chosen in the first year because it was assumed that the risk of immediate re-infection was low, especially in the low-endemic periphery.

It can be assumed that oncospheres of generation O_0 (shed by foxes before control and as a result of the first campaign; praziquantel does not kill oncospheres) will lose their infectivity within a few months. Since oncospheres are sensitive to higher temperatures and dryness, their survival will be shorter (approximately 2 months), if control measures begin in spring or summer, while they will remain infectious for up to 8 months if shed in autumn or winter [42]. Depending on the survival function for O_0 within the control area and if no oncospheres of generation O_1 are shed by re-infected foxes due to on-going control measures interrupting prepatent infections, the exposure for rodents will decrease. Naturally, the re-infection risk for foxes will also drop as a consequence. In the trial, the prevalence in foxes distinctly decreased after the sixth campaign in November 1995. At this time, a complete turn-over of the *Microtus arvalis*-population could be assumed while overwintering animals of R_1 (born under control conditions) consisted mostly of young, non-reproductive individuals presumably already born under lower exposure. When it was safe to assume that the re-infection pressure for foxes had decreased, the intervals between individual campaigns could be expanded to 12 weeks.

The analysis of the effect of the factor time on the prevalence before control showed that a seasonal effect ('month') was only detectable in the sub-population of juvenile foxes under endemic exposure. This effect is caused by the very low or lack of exposure of unweaned cubs and the high susceptibility of juvenile foxes when they are first exposed [15]. Since it takes longer for a fox to be confronted with the parasite under low-endemic conditions, many foxes in the low-endemic area are already adult before they are infected. A true seasonal effect in adult foxes could not be observed in this study before control, either under endemic or under low-endemic exposure. Before control, an effect of the factor 'year' or interactions between 'year' and 'month' were not observed. We thus consider the variation in the annual prevalence observed particularly in the endemic area (non age-stratified random sample) in this regard as random. By contrast, after the onset of control measures, an effect of the factor 'year' could be detected in all strata, which indicates a strong influence during the control measures in the fox sub-populations, both, in the initial endemic area and in the surrounding low-endemic periphery. A significant impact of the factor 'month' was only observed during control in the adult sub-population in the area of initially low endemic exposure. It may be related to dispersal effects, which could only be detected when the prevalence was very low, i.e. under control conditions.

The analysis of the interval July–September is important, because exposure to *E. multilocularis* is nearly the same for the juvenile and adult fox sub-population at this time and because the prevalence may also be influenced by factors such as partial immunity [15]. Surprisingly, almost all foxes found infected in the second year and all foxes found infected in the third year were juvenile. This result can explain the re-increases of the prevalence observed in the endemic area, estimated for the non-age-stratified random sample (Fig. 1), as these re-increases occurred when the juvenile sub-population came under exposure. While the epidemiological importance of juvenile foxes under endemic exposure was greater than that of adults, even before control, this seems to be true under low-endemic exposure only for the control period. This finding provides evidence that juvenile foxes possibly represent a risk factor for the effectiveness of control. If one assumes that most juveniles were presumably shot near to the place where they became infected, while adult may have migrated farther after infection, the infection in juvenile foxes may indicate that the parasitic cycle persisted in the control area, at least at the regional level. Moreover, the epidemiological importance of juvenile foxes is further supported by the fact, that these animals were not only more frequently but also more intensively infected than adults. High intensities of infection (> 1000 parasites) were more often found in juvenile (20% of all infections) than in adults (5%), both, before and during control. This relation was also independent from the status of endemic or low-endemic exposure and may thus reflect a duration effect with fresh infections of high intensity in juvenile and 'older', less intense or partially cleared infections in adult foxes.

The predominance of juvenile, i.e. less migrating, foxes in the infected sub-population may indicate a low influence from outside the project area during the control measures. This is evident from the spatial distribution of infected foxes and the analysis of the

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relative risk in distance from the virtual anchor point of one of the endemic foci. During the first phase of intervention measures, a progressive reduction of the risk area around this anchor point could be observed, until an increased infection risk for foxes within the focus was no longer detectable at the end of the control study. It needs to be clarified whether future control measures could be restricted to the cores of a risk area.

The validity of the results of this study is based on a largely unbiased implementation of the study design: (i) The sampling density was kept constant in time and space during the project. The hunting pressure did not change due to the control project, since the sampling density was lower than the original annual hunting bag (hunting index approximately one fox km^{-2} in the study area). (ii) To increase the reliability of data on the spatial origin of sampled foxes and the date of sampling, a bonus was paid throughout the State of Brandenburg (i.e. a region of about 30000 km^2 comprising the study area) to minimize false declaration of shooting positions. It must be stressed, however, that the hunting bag is not completely unbiased with regard to the spatial and temporal distribution of the fox population and its age structure. Therefore, any interpretation must account for this limitation. (iii) A homogeneous regional distribution was achieved by establishing six easily accessible collection points in different parts of the study area. Temporal influences of seasonal hunting on the sampling intensity could not be compensated for. (iv) Infected foxes have the same chance of being shot as uninfected animals, since *E. multilocularis*-infections are inapparent in the definitive host. Thus, sampling was random. In a few cases, however, the infection status of sampled foxes may not have been independent. Particularly, cases of familial clustering were observed. Out of 10 foxes which were found infected after the ninth campaign, 6 were from 2 litters with 3 infected and 1 uninfected cup in each litter. Yet, we believe that the random sample fulfils the criteria required for results with a tolerable degree of bias.

The results of this study show a decreasing prevalence of *E. multilocularis* within the project area, which could only be observed during control measures, as the prevalence was higher before the onset of the control measures and started to rise again after the end of the control period (K. Tackmann, unpublished observations). The decrease in prevalence cannot be explained by a random process. Because of the known efficacy of praziquantel against this parasite in definitive hosts and the well-established suitability of the baits in rabies control projects in foxes, an association between these observed effects and the control measures is plausible and very likely. Therefore, in principal, it can be concluded, that the application of praziquantel-containing baits led to a distinct reduction of the prevalence of *E. multilocularis* in foxes under initially endemic and low-endemic conditions. However, the parasite was not eradicated with the chosen strategy. Further studies will have to show how long the reduction of the prevalence will last and why a complete eradication of the parasite was not achieved.

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