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The presence of *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in the Netherlands

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Abstract

This report describes the studies undertaken to investigate the presence of *Echinococcus multilocularis* in foxes in the Netherlands from 1996 to 1998. Firstly, 272 foxes were tested that were shot close to the border with Germany and Belgium, areas that were considered to be of high risk. This study resulted in the establishment of two distinct areas in the Netherlands where *E. multilocularis* was found. Two positive foxes were found in the northern province of Groningen and 3 positive foxes were found in the southern province of Limburg. In order to investigate the spread of *E. multilocularis*, two other areas were investigated, the Veluwe and the coastal area of North and South Holland. A total of 181 foxes in these areas were tested. *E. multilocularis* was not detected in these animals. This is the first report of *E. multilocularis* in foxes occurring in the Netherlands.

Acknowledgments

This study was carried out on behalf of the Veterinary Inspection, Ministry of Public Health, Welfare and Sports. We are grateful to the participating hunters for collecting foxes. Names of the participating game management units (Wild Beheer Eenheden) are given in the addendum. We thank Margriet Montizaan of the Royal Dutch Shooting Society, Bernard Arends and Gerard Visser of the Veterinary Inspection for their stimulating work in coordinating the fox collection. We thank Jaap Mulder and Arie Swaan and the participating hunters for providing the fox samples from the coastal area. We thank the following organisations for their permission to collect foxes in their territories at the Veluwe: Natuurmonumenten, Het Gelders Landschap, Ministry of Defense, De Koninklijke Houtvesterij. Furthermore, we thank Kirsten Tackman and colleagues for their help with the technical instructions of the microscopical methods and the helpful discussions. Anke Dinkel and Thomas Romig are gratefully acknowledged for providing information on the primers and the confirmation of the DNA samples.

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Samenvatting

Echinococcus multilocularis is de kleine lintworm van de vos, die in Centraal Europa reeds lang bekend is. De eitjes van deze lintworm, die in het volwassen stadium in de dunne darm van zijn eindgastheer voorkomen, worden met de ontlasting uitgescheiden en kunnen toevalligerwijze ook door mensen worden opgenomen. Bij de mens kunnen zich na een lange incubatieperiode van meerdere jaren weinig typische klinische verschijnselen voordoen, die terug te voeren zijn op een gestaag doorgroeiend proces in de lever. Deze ziekte, alveolaire echinococcose genoemd, is één van de ernstigste parasitaire zoönosen. Recent onderzoek naar het voorkomen van deze parasiet bij vossen in Duitsland heeft aangetoond dat deze parasiet niet alleen in Zuid-Duitsland voorkomt, maar ook in de meer noordelijke deelstaten. In Noordrijn-Westfalen en Nedersachsen, deelstaten die grenzen aan Nederland, zijn prevalenties van 17% beschreven. Gezien de ernst van de aandoening is het belangrijk om te weten of deze parasiet ook in Nederland voorkomt en hoe de eventuele verdere verspreiding is.
In dit rapport worden de gegevens gepresenteerd van het onderzoek naar het voorkomen van *Echinococcus multilocularis* bij vossen in Nederland. In eerste instantie zijn door de aldaar aktieve jagers, vossen verzameld langs de grens met Duitsland en Belgie. Deze zijn naar het RIVM gestuurd en hier onderzocht op het voorkomen van volwassen parasieten in de dunne darm. Hiervoor zijn naast het door de WHO geadviseerde microscopisch onderzoek van dunne darm schraapsels, ook twee verschillende moleculaire detectie methoden geëvalueerd met als doel om deze op hun waarde te testen op materiaal uit gebieden waarvan het niet bekend is of de parasiet er voorkomt. In totaal zijn in dit grensgebied 272 vossen onderzocht. Bij 5 van deze dieren is een infectie met *E. multilocularis* vastgesteld. Verder bleek dat de twee moleculaire detectiemethoden onderling evengoed waren, maar vergeleken met het microscopisch onderzoek twee meer geïnfecteerde vossen scoorden. De in totaal 5 besmette vossen werden gevonden in twee gebieden in Nederland. Eén gebied was gelegen rond Midwolda in Groningen en een ander gebied rond Gulpen in Zuid-Limburg. De bevinding van meerdere dieren in twee ruimtelijk gescheiden gebieden maakt het minder waarschijnlijk dat de besmette dieren toevallige grenspassanten zijn en geeft een indicatie dat de parasiet zich mogelijk kan handhaven in Nederland d.w.z. zijn levenscyclus kan voltooien. Op grond van deze bevindingen is de geschatte prevalentie in Groningen 5.5% (0-15.9%, 99% betrouwbaarheids-interval) en in Zuid-Limburg 13.6% (4.8-22.3%, 99% CI). Echter om een beter inzicht in het aantal besmette dieren te krijgen, zullen meer dieren uit deze gebieden onderzocht moeten worden.
Om de mogelijkheid van verdere verspreiding in Nederland van deze parasiet te onderzoeken zijn vossen afkomstig uit de Veluwe en de Hollandse duingebieden onderzocht. In totaal zijn 72 vossen onderzocht afkomstig van de Veluwe. Deze dieren waren random verzameld en ook afkomstig uit gebieden, die anders nooit bejaagd worden zoals de Kroondomeinen, Park de Hoge Veluwe en oefenterreinen van het Ministerie van Defensie. Bij geen van deze dieren is een besmetting met *E. multilocularis* geconstateerd. Op grond van deze bevindingen wordt het risico

voor de volksgezondheid in dit voor receatieve doeleinden zeer belangrijke deel van Nederland, zeer klein ingeschat. Ook bij de 109 vossen afkomstig uit het Hollandse duingebied, zijn geen besmette vossen gevonden. Op grond van deze bevindingen kan geconcludeerd worden dat een verdere verspreiding van *E. multilocularis* in Nederland niet is aangetoond en dat het risico op besmetting voor de mens zeer klein kan worden ingeschat. Voor de twee besmette gebieden in Groningen en Zuid-Limburg geldt dat een prevalentieonderzoek zal moeten worden uitgevoerd om een betere inschatting van de risico's voor de volksgezondheid te kunnen geven.

Summary

Alveolar echinococcosis is a serious parasitic zoonosis, caused by the larval stage of *Echinococcus multilocularis*. The adult stage of this small tapeworm of 1-4mm length occurs in the small intestines of a canid endhost, which in Europe is mainly the fox. Eggs shed by this tapeworm are ingested by arvicolid rodents and develop in this intermediate host to the larval of the metacestode stage. Although the two host parasitic life cycle of this tapeworm is mainly sylvatic, sometimes humans get infected by oral uptake of eggs shed in the feces of the endhost and this may lead after an incubation period of many years to a serious disease called alveolar echinococcosis. *Echinococcus multilocularis* has been known for a long time in Central Europe including the southern states of Germany. However recent studies have shown that the parasite is also present in more northern states of Germany. Prevalences of 17% have been reported in Nordrhein-Westfalen and Niedersachsen, states close to the border with the Netherlands. Considering the serious disease in humans, it is important to know whether or not this parasite also occurs in the Netherlands.

In this report the results of several studies are presented to investigate the presence and spread of *E. multilocularis* in the Netherlands. Firstly, foxes were collected in the border area with Germany and Belgium. Microscopical examinations of the small intestines and two different PCR based assays were used to detect the presence of *E. multilocularis*. A total of 272 foxes were examined in this study and 5 of these foxes were positive for *E. multilocularis*. Three foxes were found positive by microscopy and an additional 2 by each of both PCR assays. These foxes were found in two distinct areas, one close to Midwolda in Groningen and one area close to Gulpen in the south of Limburg. The estimated prevalence in Groningen is 5.5% (0-15.9%, 99% CI), and in Limburg the prevalence is 13.6% (4.8-22.3%, 99% CI).

To get a better insight into the spread of *E. multilocularis* to other areas in the Netherlands, another 72 foxes, randomly sampled at the Veluwe were examined. This area is located in the center of the country and has an important recreational function, because it contains the largest forest area of the country. No positive foxes were found in this area. Also in the most western part of the country, the coastal area at North- and South-Holland, foxes were collected. Again no positives were found out of the 109 foxes examined.

In summary, the results of these studies show that *E. multilocularis* was found in the Netherlands, in two distinct areas close to the border with Germany and Belgium. Until now, there is no indication of spread of *E. multilocularis* throughout other areas in the Netherlands. Based on these results, the risk for public health is considered to be very low However, to get a better insight in the public health implications in infected areas, more detailed prevalence studies are needed.

1. Introduction

Echinococcus multilocularis is a small taeniid cestode of 1 to 4 mm length. The adult stage of the parasite can infest the small intestines of its definite host, primarily foxes or other canids and rarely cats. Eggs shed in the environment by the adult parasite will develop to the larval or metacestode stage after uptake by arvicolid rodents that serve as intermediate hosts (Ammann and Eckert, 1996). The parasite is the causative agent of alveolar echinococcosis, a very serious life threatening disease in humans, who may acquire the infection after accidental oral uptake of the eggs. The incubation time of this disease can be as long as 10 to 15 years and even then clinical signs are not typically related to the disease. At that time infestation of the liver and even metastasis in the body can be so serious that treatment is difficult or even impossible and consequently the disease becomes fatal (Amman and Eckert, 1996). Hence, prevention of this life-threatening infection in humans is of major importance. An important parameter to estimate the potential infection risk of humans in endemic areas is the determination of the prevalence of *E. multilocularis* in definite hosts (Deplazes and Eckert, 1996). The classical and still most reliable method for detection is microscopical examination of mucosal smears derived from the small intestines after necropsy. However, this method is laborious and can only be carried out post mortem (Deplazes and Eckert, 1996). More recently, other diagnostic assays have been described like serological assays (Gottstein et al.,1991; Deplazes et al., 1992), a coproantigen ELISA detection method (Deplazes et al., 1990; Deplazes and Eckert, 1996), and a PCR based detection of *E. multilocularis* eggs in fecal samples of foxes (Bretagne et al., 1993; Mathis et al., 1996; Monnier et al.,1996, Dinkel et al.,1998). PCR based methods have the advantage that they can be used on feces of living animals, even after freezing the fecal material to reduce infection risks (Veit et al., 1995). However, their usefulness for the screening of fox populations needs to be evaluated under field conditions in comparison with microscopical examination of intestinal smears.

The most important endemic areas of *E. multilocularis* are Alaska, Siberia, and Northern China, generally the colder climates of the world (Schantz et al., 1995). In Europe, a relatively small endemic area is located in Germany, Switzerland, Austria and France, where alveolar echinococcosis is known since 1855 (Virchow). Here, mainly the red fox (*Vulpes vulpes*) and sometimes other canids are the natural endhosts of *E. multilocularis* (Eckert, 1989).Although it seems that the distribution pattern of *E. multilocularis* is concentrated in the colder climates of the world including the mountain areas in middle Europe, a possible spread of the parasite into regions formerly not known to be endemic areas, is recognized (Lucius and Bilger, 1995; Malczewsky et al., 1995). Studies in Belgium showed a prevalence of 51% in the province of Luxembourg (Losson et al., 1997) and in states in Germany close to the border areas with the Netherlands an average prevalence of 17.7% in Nordrhein-Westfalen, 27.8% to 33.1% in

Rheinland and 17.8% in Lower Sachsen were reported (Lucius and Bilger, 1995). *Echinococcus multilocularis* has never been found in the Netherlands in foxes, although no studies were carried out since 1984 (Borgsteede). Recently, the first human case has been diagnosed in the Netherlands, although it seems likely that this patient was infected in Switzerland, the country where he was born and lived for 20 years (Raasveld et al., 1997).
In this report the presence of *E. multilocularis* in the Netherlands was investigated. Three distinct areas, the border area with Germany and Belgium, the central area of the Netherlands, the Veluwe and the coastal area were selected. To detect the parasites in foxes, the classical mucosal scraping method was used and compared with two PCR based methods. The PCR assays were developed to detect DNA of eggs of *E. multilocularis* in fecal material of foxes. The usefulness of these PCR assays was evaluated. This is the first report of the presence of *E. multilocularis* in the Netherlands.

2. Material and Methods

2.1 Animals

Hunters of game management units (Wild Beheer Eenheden) of three selected areas were asked to participate in collecting foxes. Red foxes shot by hunters were sent to the National Institute of Public Health and the Environment within 24 h. Carcasses were frozen at -20^{0}C followed by deep freezing at -80^{0}C for 1 week, prior to necropsy. At necropsy, small intestines and content of colon were removed and refrozen again at -80^{0}C for at least 1 week prior to parasitological examination. Strict safety precautions were taken during handling of the animals, necropsy and parasitological examinations to avoid or exclude an infection risk (Eckert et al., 1991; Eckert and Desplazes, 1996).

2.2 DNA isolation from fecal colon content

One gram of fecal colon content of foxes was suspended in 1 ml of 50 mM Tris-HCl, 10 mM EDTA, pH 8 buffer ($T_{50}E_{10}$), 0.5% SDS and boiled for 10 minutes. After proteinase K treatment (1mg/gram feces) the suspension was extracted by phenol, phenol chloroform and chloroform. Afterwards, DNA was precipitated with isopropanol as described previously (Sambrook et al., 1989). The DNA pellet was washed in 70% ethanol and dissolved in 100 µl 10 mM TrisHCl 1 mM EDTA pH 8 ($T_{10}E_1$). DNA was purified from inhibitors by the guadinine/celite method described by Boom et al. (1990). The DNA/celite suspension was put in 100 µl $T_{10}E_1$ onto a 2 ml Sephacryl S500 spin column equilibrated in $T_{10}E_1$. After spinning the column for 5 min at 800g, 1 ml of the DNA solution was precipitated with isopropanol (Sambrook et al., 1989). The DNA was dissolved in 50µl $T_{10}E_1$. Five µl of this solution was used in the PCR.

E. multilocularis DNA derived from *Microtis arvalis*, kindly provided by Dr. Bretagne, France, was used as positive control DNA. *E. multilocularis* positive fox faeces with known numbers of worm burdens were kindly provided by Dr. K. Tackman, Germany, and were used as positive controls for the sample preparation method.

2.3 Primers and amplification

For the detection of *E. multilocularis* DNA a fragment of the 12S mitochondrial rRNA gene was amplified by a single tube nested PCR assay. The method was a modification of the nested PCR described by Dinkel et al. (1998). Primers were: outer primers Em-1 and -2, 5'TAAGATATATGTGGTACAGGATTAGATACCC3' and 5'GGTGACGGGCGGTGTTGTA3', respectively, and inner primers Em-3 and -4, 5'ATATTACAACAATATTCCTATC3' and 5'ATATTTTGTAAGGTTGTTCTA3', respectively. PCR was optimized for pH, Mg and the concentrations of outer and inner primers. PCR was performed in 50 µl containing 60 mM Tris-HCl pH 9, 15 mM $(NH_4)2SO_4$, 4 mM $MgCl_2$, 100 µM of each dNTP, 8 nM of each primer Em-1 and -2, 0.5 µM of each primer Em-3 and -4 and 0.5 U SuperTaq (Sphearo-Q, Leiden, the Netherlands). The PCR cycle program consisted of an initial incubation at 94^0C for 5 min followed by 20 cycli of 1 min 94^0C, 1 min 65^0C and 1 min 72^0C to amplify a fragment by the outer primers followed by 35 cycli of 1 min 94^0C, 1 min 55^0C and 1 min 72^0C to amplify the fragment flanked by the inner primers. After the last elongation step of 72^0C for 10 min, the samples were stored at 4^0C.
A second PCR based on the amplification of U1snRNA was essentially performed as described by Bretagne et al. (1993) with the following modifications: the PCR mixtures (50 µl) contained 60 mM Tris-HCl pH 10, 15 mM $(NH_4)_2SO_4$, 2mM $MgCl_2$, 100 µM of each dNTP, 0.5 µM of each primer and 1.0 U Taq polymerase (Perkin Elmer). Samples were run in duplo and one part of the samples was routinely spiked with 100 fg of *E. multilocularis* DNA to detect inhibition. For the detection of PCR fragments, 10µl of PCR product was electrophoresed on 1.5% agarose gels in ethidium bromide as described previously (Sambrook et al., 1989).

2.4 Parasitological examination

Parasitological examination of the small intestines was carried out according to the methods recommended by the WHO Collaborating Center for Parasitic Zoonoses in Zurich (Eckert et al.,1991; Deplazes and Eckert, 1996). Small intestines were slit open in full length with a scissor and the debris was removed with a forceps. Deep mucosal scrapings were taken using microscopic slides. Mucosal material was transferred to a square plastic petri dish and squashed on the bottom of the dish. About 5 smears of the proximal, middle and posterior part of the small intestines were microscopically examined (enlargement between 7-70X). *E. multilocularis* was identified according to the size of the whole adult cestode and the relative size of the last proglottid as compared to the whole size. In rare cases only typical proglottids with oncospheres inside were identified (Tackman et al., 1998).

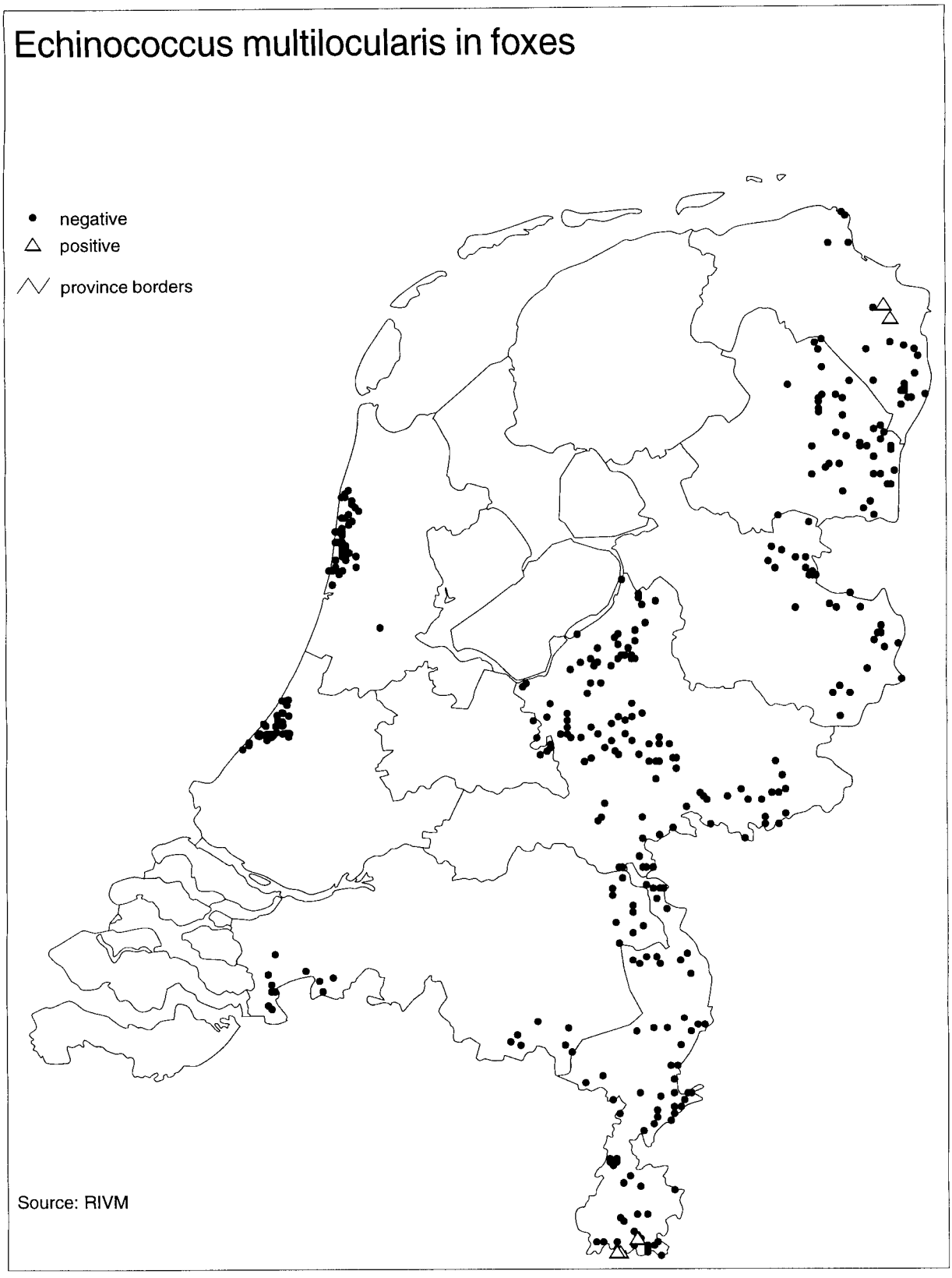


Fig. 1. Overall spatial distribution pattern of foxes examined for E. multilocularis in the Netherlands. The origin of each fox is plotted on a map of the study area. Foxes found negative for E. multilocularis are represented by a dot (•). Foxes found positive for E. multilocularis are represented by a triangle (Δ).

3. Results

A total of 453 foxes was examined by microscopical examination of intestinal smears and of 396 foxes the intestinal contents were also tested by each of both PCR assays. Of these 453 foxes, 272 were derived from the border areas, 72 foxes from the Veluwe and 109 foxes from the coastal area. To analyse the geographical distribution pattern of the foxes examined, the location of each fox was plotted on a map (Fig. 1).
Of these 453 foxes examined, only 5 foxes were positive for *E. multilocularis* (Table 1).

Table 1. Numbers of foxes investigated in the different areas and the results of the different tests.

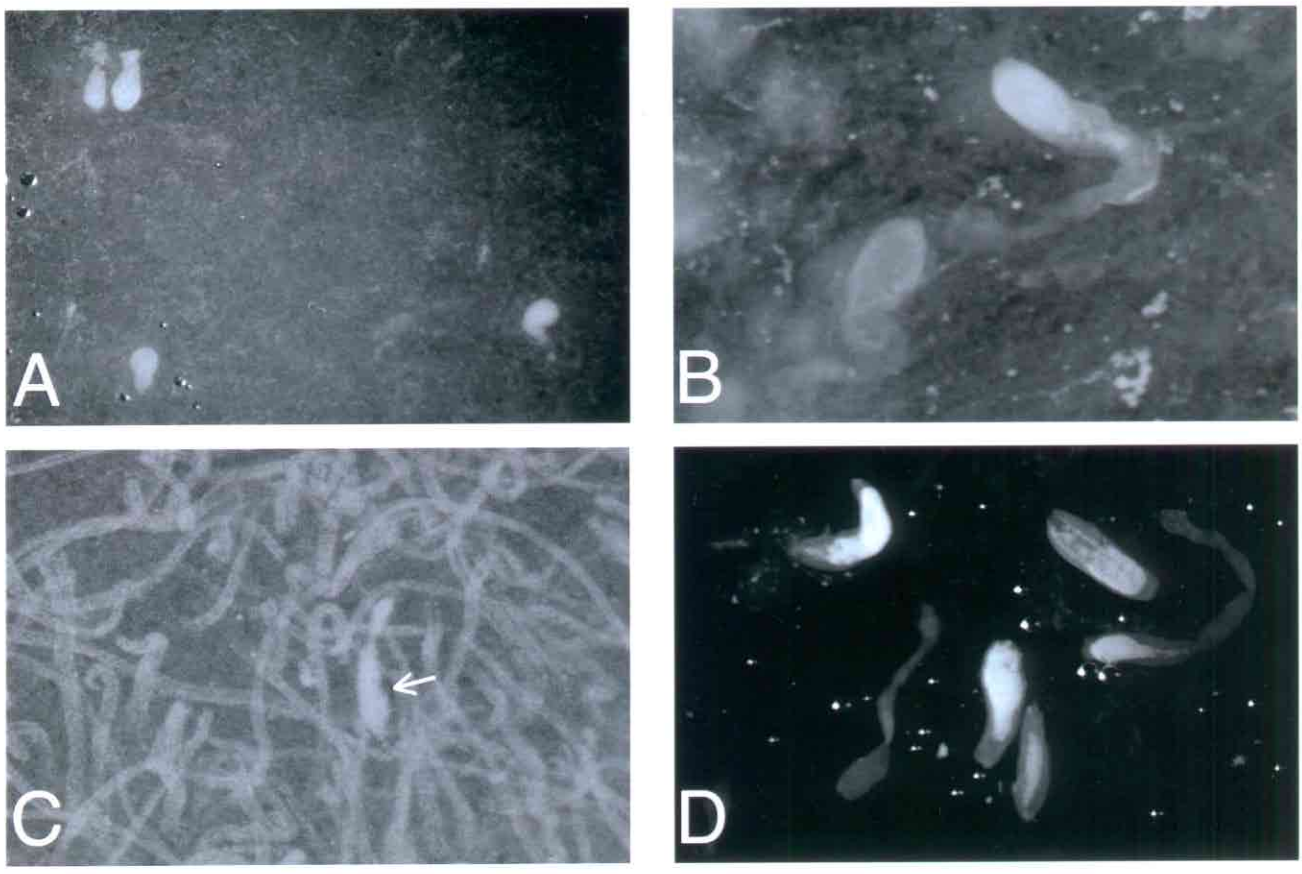
Area	Number of foxes examined	Microscopy positive/n	Nested PCR positive/n	U1snRNA PCR positive/n
Border area	272	3/272	5/272	5/272
Veluwe	72	0/72	0/71	0/71
Coast (northern)	68	0/59	0/44	0/44
Coast (southern)	41	0/40	0/9	0/9
Total	453	3/443	5/396	5/396

The positive foxes were located at the border area with Germany and Belgium (Fig. 1). Of these 5 positive foxes, 3 animals were positive by microscopical examination of the intestinal smears (Fig. 2), while the other 2 were detected by each of the PCR assays only (Table 2). Intestinal smears of *E. multilocularis* positive foxes, kindly provided by K. Tackman, were used as positive control smears (Fig. 2). The sensitivity and the specificity of both PCR assays used in this study was described by Giessen et al. (submitted).

Table 2. Results of the Echinococcus multilocularis positive foxes derived from the border area.

Fox number	Age	Sex	Location	Microscopy (worm burden)	Nested PCR	U1snRNA PCR
1. (49)	Juvenile	Male	Limburg	-	+	+
2. (59)	Adult	Male	Limburg	-	+	+
3. (156)	Juvenile	Male	Groningen	+ (30)	+	+
4. (232)	Juvenile	Female	Limburg	+ (100)	+	+
5. (255)	Adult	Female	Groningen	+ (1)	+	+

Fig. 2. Microscopical examination of the intestinal smears of 3 positive foxes originating from the border area (A, B, C). For the positive control an intestinal smear of an E. multilocularis positive fox (D) was used as kindly provided by K. Tackman.



DNA samples, which were positive by the nested PCR were confirmed by southern blot hybridization using an internal probe by Romig and coworkers in Stuttgart by the method described by Dinkel et al. (1998).
The *E. multilocularis* positive foxes were found in two geographical distinct areas in the Netherlands. One area in the northern province of Groningen and one area in the most southern part of the province of Limburg. In Groningen the size of the investigated area was about 1100 km^2. The estimated prevalence in this area is 5.5% (range 0-15.9% with 99% confidence interval (CI)) with the assumption of a population of 1320 animals in this area. The size of the area in the south of Limburg is about 768 km^2 and the estimated prevalence of *E. multilocularis* is 13.6% (range 4.8 to 22.3%, 99% C.I.).
The geographical distribution patterns of the foxes examined at the Veluwe are shown in detail in Fig. 3. The foxes were randomly distributed, however no *E. multilocularis* positive fox was found in this area (Table 1). In addition, 41 foxes from the southern coast area and 68 foxes from the northern coast area were examined. None of these animals were found positive for *E. multilocularis.* The geographical distribution pattern of these foxes are shown in detail in Fig. 4 and Fig 5.

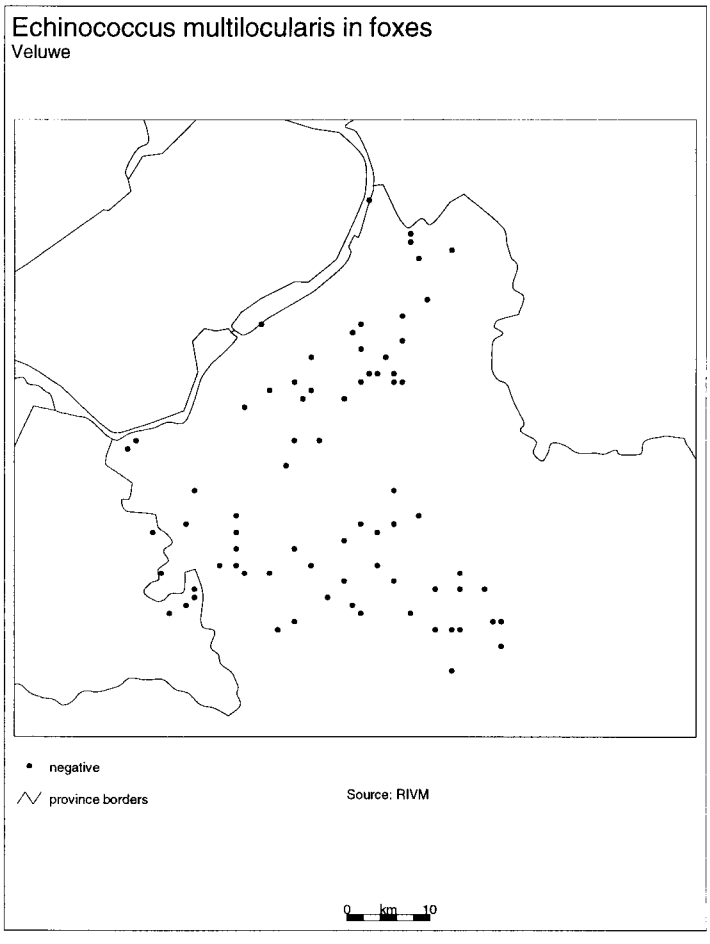


Fig. 3. Geographical distribution of negative (•) foxes for E. multilocularis originating from the Veluwe.

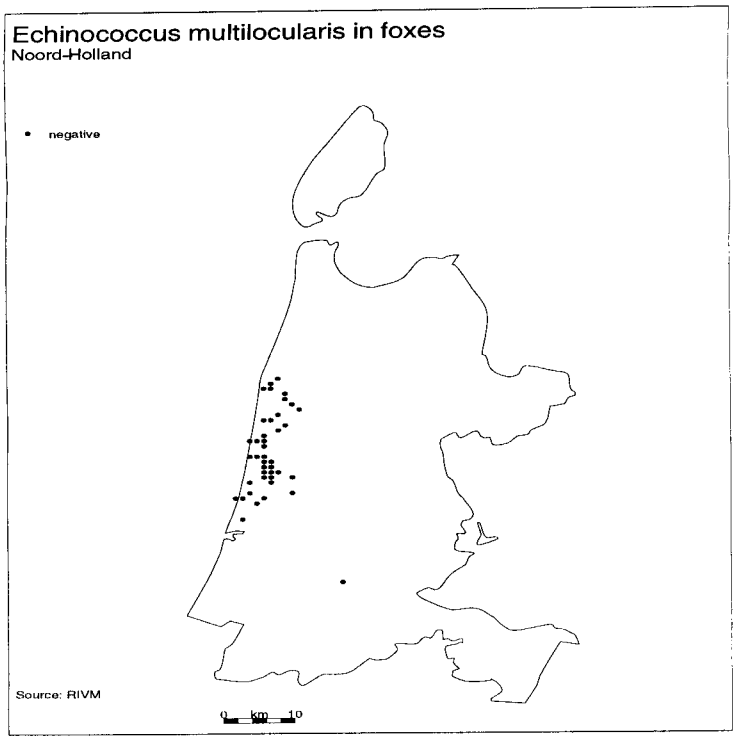


Fig. 4. Geographical distribution of negative (•) foxes for E. multilocularis originating from the northern coastal area.

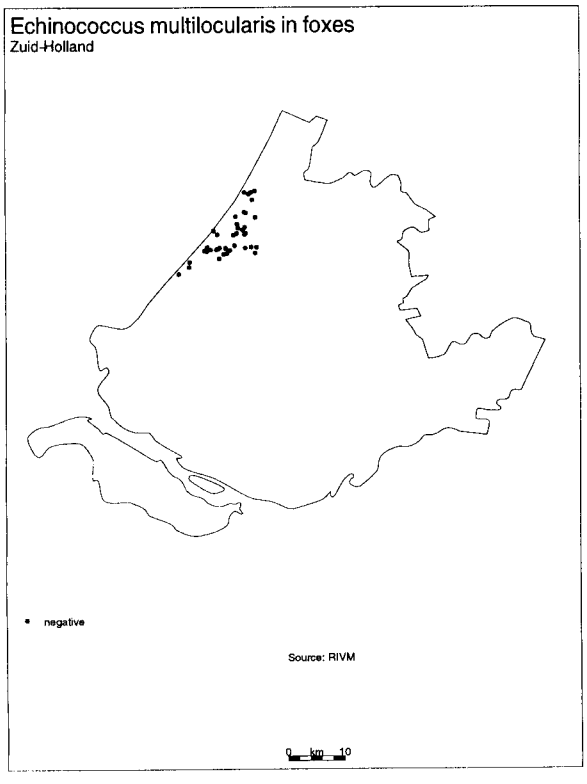


Fig. 5. Geographical distribution of negative (•) foxes for E. multilocularis originating from the southern coastal area.

4. Discussion

The last years several studies have been carried out to estimate the presence of *E. multilocularis* in areas in Europe where the parasite was not identified before. Also in areas in Germany close to the border with the Netherlands, *E. multilocularis* was found (Lucius and Bilger, 1995). In order to estimate the presence of *E. multilocularis* in the Netherlands, firstly, foxes originating from the border area with Germany and Belgium were collected. The intestinal scraping method used in this study (Eckert and Deplazes, 1996) is generally accepted as the gold standard method. We also incorporated PCR based methods to evaluate their use in an area where the presence of *E. multilocularis* is not known. Of the 272 foxes examined from the border area, 5 foxes were found positive for *E. multilocularis*. The sensitivity of both PCR assays was higher compared to microscopical examination, although the number of positive foxes was very low. Beside the 5 positive fecal samples, all other fecal samples were negative by PCR. Of these foxes at least 20% was also infected with other Taeniid species (data not shown). This indicates a good specificity of both PCR methods, confirming the specifcity test performed with DNA from closely related parasites (Van der Giessen et al., submitted). Using our fecal sample preparation method, we experienced no inhibition in our PCR assays. Summarizing, we conclude that the PCR based methods described in this study are suitable for epidemiological surveys in areas where the parasite occurs only sporadically.

Although the whole border area was incorporated in the study and foxes were obtained randomly, the 5 positives were only found in two distinct areas. Two foxes were detected in Groningen close to Midwolda. The other 3 positive foxes were found in the south of Limburg around Gulpen. Theoretically it is possible that these 5 positive animals migrated from outside the Netherlands indicating that the life cycle of the parasite is still not established in the Neherlands. However, if positive foxes migrated from the endemic areas opposite the border in Germany it is more likely that infected foxes were found more randomly across the border area instead of two distinct areas in the Netherlands. Findings of epidemiological studies *of E. multilocularis* in foxes in Germany indicate limited migration patterns. Most foxes seem to migrate no more than 5 to 10 km. Only a few animals tend to migrate over 50 to 70 km (Tackman et al., 1998). The finding of infected intermediate hosts, which tend to migrate only a few km, should give more certainty about the establishment of the life cycle. However, it will be difficult and laborious to find infected rodents, especially in sporadic areas (Tackman, pers. comment).

In order to study the spread of *E. multilocularis* in the Netherlands, foxes from three other areas were collected. The Veluwe is located in the middle of the Netherlands and contains the largest forest area of the country which has an important recreational function. Of this area 72 animals were examined and no positives were found. Also from two other areas in the most western part of the Netherlands, the coastal area of North- and South Holland, foxes were offered for

examination. Of the 109 animals examined here, none was found positive *for E. multilocularis*. Although these results can not exclude the presence of *E. multilocularis* in these areas, it is an indication that the risk for public health is limited.
The study described here was designed to detect the presence of *E multilocularis* in the Netherlands. Heterogeneous spatial distribution patterns of *E. multilocularis* are well known, The finding of small high endemic foci are recognized within sporadic endemic areas (Tackman et al., 1998). These finding are of major importance to design studies to estimate the prevalence of *E. multilocularis*. Based on the data obtained in the study at the border area, the fox population densities and the prevalence of *E. multilocularis* in the Netherlands and adjacent states in Germany were comparable. However, to obtain more precise information about the prevalence of *E. multilocularis* in these areas and thus get a better insight in the potential risk for public health, another study will be undertaken. This study will be carried out in Groningen, one of the two areas where *E. multilocularis* was found.
In conclusion, this is the first report describing the presence of *E. multilocularis* in foxes in two distinct areas in the Netherlands, one spot is located in Groningen the other in the south of Limburg. Further epidemiological studies will be undertaken to obtain a better estimate of the associated human infection risk.

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Addendum

Names of game management units (wildbeheer eenheden (WBE)) participating in border area study.

Name WBE	Residence	Potential hunting field (hectare)	Real hunting field (hectare)
WBE Vlagtwedde Noord	Veendam	5000	4000
WBE Meeden	Wildervank	nk	6000
WBE Bellingwedde	Blijham	11.000	8500
WBE Bierum Fivelingo	Zandeweer	11.000	5332
WBE Bargerveld	Zwartemeer	9000	5600
WBE Westerwolde	Ter Apel	7000	nk
WBE De Grensstreek	Schoonebeek	5600	4400
WBE 't Scholtensveld	Emmen	8500	5146
WBE De Wieken	Hollandscheveld	5000	2500
WBE Ellertsveld	Assen	8500	8355
WBE De Hondsrug	Gasselternijveen	10.500	10.500
WBE De Monden	Buinerveen	9500	8098
WBE Stadskanaal	Onstwedde	12.000	7500
WBE De Vechtstroom	Hardenberg	10.000	8113
WBE Veald	Overdinkel	3200	1295
WBE Zuid Oost Tubbergen	Albergen	5190	5190
WBE Ootmarsum	Geesteren OV	4100	3400
WBE Meerlose Baan	Broekhuizenvorst	8355	6345
WBE Bergen Limburg Noord	Siebengewald	8000	6162
WBE Maas en Niers	Ven Zelderheide	7500	5000
WBE Moerstraten	Heerle	7000	6500
WBE Dalgronden	Eerste Exloermond	8500	8000
WBE Mars en Westerstroom	Meppen	13.000	9200
WBE Oostermoer Noord	Annen	5000	3500
WBE Zuidplantage	Huijbergen	4700	4500
WBE Roosendaal	Rucphen	10.000	6000
WBE Baronie v.Cranendonck	Leende	11.200	11.200
WBE Oosterkempen	Valkenswaard	7500	4700
WBE Grens Vecht Akkerland	Bruchterveld	8000	nk
WBE Tussen Vecht/Dedemsvaart	Ommen	10.000	7900

WBE Stepelo/Broekelo/Ussel	Enschede	7000	4500
WBE Lonneker Losser	Enschede	9000	6137
WBE Rossum	Beuningen OV	3000	2000
WBE Geesteren	Geesteren OV	nk	3000
WBE Lutte/Beuningen	De Lutte	2600	2300
WBE Savelsbos	Gronsveld	8200	3200
WBE Voerendaal	Gulpen	7000	6600
WBE Grensland Vaals	Lemiers	3259	3259
WBE Heuvelland	Margraten	4000	3600
WBE De Grenskant	Weert	8000	8000
WBE Land van Horne	Horn	6000	3100
WBE Helden	Panningen	5300	5300
WBE Beesel/Swalmen	Beesel	5118	4295
WBE Swentibold	Urmond	2500	1365
WBE Maasland	Stevensweerd	2125	1664
WBE Graetheide	Puth	nk	1350
WBE De hondskerk	Puth	4500	1168
WBE Annendaal	Maria Hoop	6500	5600
WBE De Roerstreek	Vlodrop	5000	4200
WBE Hunsel	Heel	3000	2350
WBE De Overlaat	Beers	6490	5500
WBE 73-77	Rijkevoort	nk	4400
Wbe Oploo	St.Anthonis	7000	5700
WBE Venray	Venray	nk	6210
WBE Reusel	Reusel	5300	5119
WBE Maasterras	Belfeld	7100	2050
WBE Brunsummerheide E.O.	Landgraaf	5100	3816
WBE Dommelvallei	Valkenswaard	5300	2613
WBE Geuldal (Silbe)	Valkenburg-Silbe	4000	4000
WBE Oldambt	Scheemda	nk	6000
WBE Maas en Roer	St.Odilienberg	2285	1960
WBE Everlose Beek	Maasbree	5600	3700
WBE Honesch Langelo Buurs	Haaksbergen	6000	6000
WBE Vriezenveen	Vriezenveen	7000	5986
WBE L'Voorde-Groenlo	Vragender	9000	7262
WBE Groesbeek	Nijmegen	3500	2700
WBE Zelhem Doetinchem	Halle	12.000	10.000
WBE Bevermeer	Wehl	8400	7500
WBE Aalten	Aalten	10.000	7500
WBE Over-Betuwe Oost	Bemmel	7233	4146
WBE Heumen Nijmegen	Malden	2000	1450

WBE Circul Ooy en Mil.	Leuth	3800	3640
WBE Gendringen-Bergh	Ulft	10.000	10.000
WBE Wisch	Terborg	7279	nk
WBE Zundert	Achtmaal	9200	8483

Names game management units (wildbeheer eenheden (WBE)) participating in study the Veluwe

Name WBEe	Residence	Potential (hectare)	Real (hectare)
WBE Lunteren	Ede	5600	3775
WBE Oldenbroek/Oosterwolde	Oosterwolde	5000	4200
WBE N.W. Veluwerand	Doornspijk	5000	4600
WBE De Vale Ouwe	Elspeet	7000	7000
WBE Leefgebied 1	Ermelo	11.500	nk
WBE De Schaffelaar	Harskamp	20.000	20.000
WBE Z.W. Veluwe	Otterlo	8700	7500
WBE Nat.Park de Hoge Veluwe	Hoenderlo	5600	nk
WBE Koninklijke Houtvesterij	Apeldoorn	10.500	nk
WBE N.O. Veluwe	Vaassen	16.000	16.000
WBE Nijkerk	Putten	24.000	14.000
WBE Midden Veluwe	Ugchelen	11.000	9980
WBE Zuid Oost Veluwe	Arnhem	15.000	15.000

Names game management units (wildbeheer eenheden (WBE)) participating in study coastal area (Noord-Holland)

Name Instantie	Potential hunting field	Real hunting field
WBE De Wijckermeer	6000	2000
WBE Uitgeest e.o.	3000	3000
WBE Noordkop	20.000	12.036
WBE Noord Kennemerland	nk	3600

nk: not known

Appendix 1 Mailing list

1	Directeur-Generaal van de Volksgezondheid
2	Inspectie Gezondheidsbescherming Waren en Veterinaire Zaken van het Ministerie van Volksgezondheid, Welzijn en Sport
3	Drs. H. Verburg
4	Dr. J.H.M. Nieuwenhuijs
5	Drs. K. Minderhoud
6	Hoofdinspecteur Gezondheidszorg
7	Plv.Directeur-Generaal Milieubeheer
8-11	Regionale Inspecties Waren en Veterinaire Zaken
12	Hoofdinspecteur voor de Geestelijke Gezondheidszorg en Gehandicaptenzorg
13	Voorzitter van de Gezondheidsraad
14	Depot van Nederlandse Publikaties en Nederlandse bibliografie
15	Vereniging voor Natuur en Milieueducatie, Amsterdam
16	Nederlandse Vereniging voor Preparateurs, Middelburg
17	Vereniging voor Das en Boom, Beek Ubbergen
18	Nederlandse Vereniging tot Bescherming van Dieren, Den Haag
19	Vereniging voor Zoogdierkunde en Zoogdierbescherming, Utrecht
20	Prof. Dr. F. van Knapen, Universiteit Utrecht
21	Prof. Dr. W.C.A.Cornelissen, Universiteit Utrecht
22	Dr. F.H.M.Borgsteede, ID-DLO, Lelystad
23	Dr. H.Schipper, AMC, Amsterdam
24	B.Arends, Inspectie W&V, Groningen
25	Ir.M.Montizaan, KNJV, Amersfoort
26	Ing. G.Visser, Inspectie W&V, Zutphen
27	Drs.A. Swaan, PWN, Castricum
28	Drs.J. Mulder, DZH, Katwijk
29	Dr. F. Niewold, IBN-DLO, Wageningen
30	Drs. J.van Steenbergen, LCI
31	Drs. E. Pierey, LNV, Den Haag
32	Dr. K. Tackman, Germany
33	Dr. T. Romig, Germany
34	Ir. M. Vervaeke, Universiteit Antwerpen, Belgium
35	Dr.E. Pozio, Italy
36	Directie RIVM
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38	Dr. Ir. A.M. Henken
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41	Dr. .J.W. Sprenger
42	Dr. P.G.N. Kramers
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