Detection of *Echinococcus multilocularis* in red fox (*Vulpes vulpes*) in The Netherlands at the border with Germany

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April 2011

Abstract
This report describes a part of the follow-up study on field studies conducted in 1996/1997. In this study 252 red foxes(*Vulpes vulpes*) were examined for the presence of *Echinococcus multilocularis.* This investigation was done over the time of October 2010 until April 2011. The foxes that were examined were shot close to the German border. From north Limburg up to Drenthe. In the 252 foxes no positive foxes for *E. multilocularis* were found with microscopical examination. Only microscopic examination of mucosal smears was described in this report, although in the project PCR techniques are also used to detect whether foxes where infected.

1. Introduction

Echinococcus multilocularis is parasite, causing a serious parasitic zoonosis. In humans, the larval stage of this two host tapeworm causes alveolar echinococcosis(AE). *Echinococcus multilocularis* is a small tapeworm of 1-4 mm in size. The life-cycle of this tapeworm is mainly sylvatic, specifically involving wild carnivores as definitive hosts, mainly red fox(*Vulpes vulpes)* and several species of small rodents as intermediate hosts(fig 1)
Dogs and to a lesser extend cats are also seen as a possible definitive hosts, and they can be a threat for humans when they excrete *E. multilocularis* eggs. (Kapel et al, 2006).
Infected dogs and cats might also contaminate their fur with eggs, excrete eggs in their feces or when dogs are used for hunting in fox holes they can pickup eggs.

In accidental cases humans can get infected by uptake of the eggs which are shed by the definitive host. The infection route to humans is through oral uptake of the eggs from the environment. This is a life-threatening infection in human beings. *Echinococcus multilocularis* infection in humans has a incubation time of 5-15 years and the clinical signs are non-specific to the disease. When humans are infected infestation of the liver and even metastasis in the body can be a serious problem. In this stage, treatment may be impossible and the disease might become fatal. In untreated or inadequately treated AE patients the mortality rate can be up to 100% after 15 years.(Ammann and Eckert, 1996)

The risk of exposure for humans is enhanced by several factors like an increasing prevalence and increasing number of infective eggs shed in the environment by the definitive

host. In several regions in western en central Europe, most of the northern en central Eurasia and parts of North America *E. multillocularis* is endemic. The distribution of the parasite in Europe might have been extended due to the increase in red fox populations. This might be due to the successful rabies control campaigns resulting in reduced mortality in foxes. Due to a lower mortality rate and a high reproduction rate the fox population expanded over new regions in Europe.

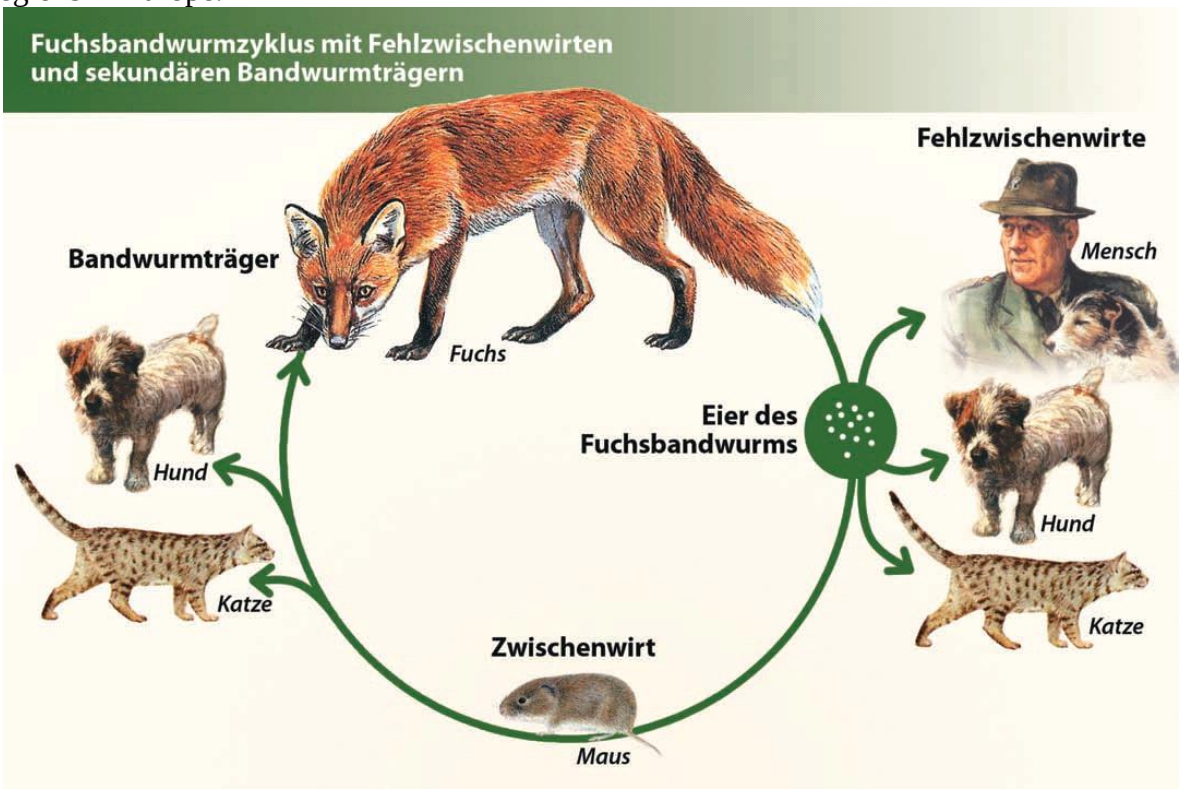


Fig. 1: Lifecycle of *Echinococcus multilocularis.* (Wild und Hund,2010)

Foxes have been present in the Netherlands for centuries in the eastern and southern parts of the country and the populations here are considered to be continuous with those of Germany and Belgium. Since 1970, the fox spread all across the Netherlands and is now definitely settled in the coastal areas and in cities. (Mulder Natuurlijk) *Echinococcus multilocularis* has never been found in the Netherlands (Borgsteede et al, 1984) until the study which started October 1996 and lasted till March 1997. Infected foxes with *E. **m**ultilocularis* were found in the northern part of Groningen en the southern part of Limburg.(van der Giessen et al., 1998) Until now it cannot be determined whether *E. multillocularis* was undetected until the study of 1996/1997 or had recently extended its range, although previous studies in foxes did not show positives. (Borgsteede, 1984).

Until now, some human cases of alveolar echninococcosis have been diagnosed in the Netherlands. All except for one human case, reported in 2008 in the southern part of Limburg, which might be traced back to endemic regions where the patient lived and might have obtained the infection. The human case reported in 2008 might be the first autochthonous human case in the Netherlands. In Switzerland human alveolar echinococcosis cases doubled in the last 10 years, which follows a fourfold increase in the fox population. (Schweiger et al., 2007)

There are different methods to detect *E. multilocularis* in definite hosts. The two classical methods are the microscopic examination of either mucosal smears taken from the small intestine or the sedimentation counting technique of the small intestines after necropsy. Another method that can be used for detecting *E. multilocularis* is a PCR based detection on

colon content (van der Giessen et al., 1998). Also a copro-antigen ELISA can be used to detect *E. multilocularis*, especially in high endemic areas were the egg shed is high. (Desplazes et al. 1999)**.**

In this study, we analyzed the presence of *E. multillocularis* in an area in the Netherlands, adjacent to Germany. The aim of the study is to compare the results of the studies performed 15 years ago with the current situation of *E. multilocularis* in this region. In this report, the results of the microscopic examination of mucosal scrapings of foxes to detect *Echinococcus multilocularis* was described. During this project, I participated in the necropsy and collected the samples. Furthermore, I prepared the mucosal scrapings. Moreover, I contacted hunters to collect more foxes.

2. Materials and methods

2.1 Study Area and Animals

This study was conducted between October 2010 until 31 March 2011.

The area that this study covers is 15 km inland along the German border from northern Limburg until Coevorden (Drenthe). (fig 2). In this area, the fox population was estimated to be 4000 animals and a sample size of 288 foxes was calculated to detect infected foxes (Takumi PVA, 2009). Foxes were collected by asking 65 WBE's to participate in this study. The WBE's that were involved in this study are colored yellow in fig. 2. Hunters were asked through the WBE to send shot red foxes to the RIVM. To do this they received a package from the RIVM. This package included a cadaver bag, instructions and a questionnaire.

After shooting a fox, hunters filled out the questionnaire describing the location where the fox was shot, at what time and with which hunting method. Hunters could then call the GD Deventer and GD Deventer picked up the package the next day.

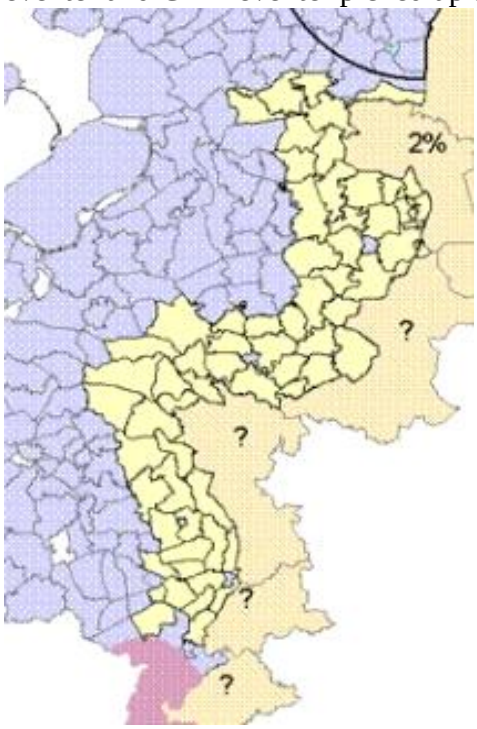


Fig 2: Cooperative WBE's (given in yellow)(Takumi and van der Giessen, 2009)

During the project, we noticed that the questionnaire wasn't specific enough with regard to the question of the hunting method. This was not a problem for the investigation of echinococcus but was important for the ecological study done by Mulder Natuurlijk. To solve

this problem, hunters who already had send foxes were called by me to further explain their hunting method and the information was included in the corresponding questionnaire.

The geographical distribution of the foxes collected was not evenly spread during the study. There were more foxes collected from the region of Limburg and Noord-Brabant and only a low number of foxes were coming from the Achterhoek (East Gelderland) and Zuid-Twente (Southeast Overijssel) To obtain more foxes from these region, I actively called hunters in these regions and I asked them to send shot foxes to the RIVM. I also asked the reason why they did not send foxes yet. The hunters were not very clear why they did not participate. After this telephone action, a few more foxes were send in from these regions.

During this study only one of the two Dutch hunting organization was directly involved in the project and was provided with information during the project. This was the KNJV. The KNJV informed their members through their magazine. The other hunting organization, the NOJG, did also inform their members through their magazine but they were not directly involved in the project. Do to the lack of information during the project to the NOJG members it is possible that there were less foxes coming from certain areas in the research area. It is known that in these regions a lot of hunters are members of the NOJG and not connected to a WBE. (NOJG administration)

Foxes were transported to RIVM by the GD and were frozen for at least 1 week at -80°C prior to necropsy. This was done to inactivate the possible eggs of *E. multilocularis* present in and on the carcass to lower the infection risk for the examiners according to the methods described by WHO.

Necropsy was performed at the Dept. of Pathology at the Faculty of Vet. Medicine in Utrecht**.** During necropsy small intestine and colon contents were removed for the detection of *E. multilocularis*. Also carpal muscles from the front paws, liver, spleen and lung biopsies and thorax fluid were removed for other studies. Measurements and the weight of the foxes were taken and the age was determined for ecological studies.

2.2 Parasitological examination

For the parasitological examination, mucosal smears of the small intestine were examined microscopically. This is according to the recommendation of the WHO collaborating Center for Parasitic Zoonosis in Zürich (van der Giessen et al., 1998). The smears were made by dividing the small intestine in 6 to 8 parts and every part was slit open with scissors in full length. The debris was removed with a microscopic slide. Parasites present in the debris e.g. Toxocara spp. and Taeniae spp.were semiquantitative recorded. Scrapings were taken of the deep mucosa of the intestine using microscopic slides. The mucosal smear was transferred to a square petri dish and squashed on the bottom. In total 18-24 smears were taken of the small intestine and were examined microscopically with a magnification of 7x to 50x. Colon contents were used for PCR detection of *E. multilocularis*, these results are not included in this report.

3. Results

In total, 252 foxes were included in the study. Of these, 94 foxes were more than one year old (adults) and 185 foxes were less than one year of age (juveniles) as shown in table. 1. The distribution and number of foxes in the different regions is shown in fig. 3. Of the 252 foxes , 72 foxes were originating from Drenthe and Northeast Overijssel , 44 foxes were originating from South Twente and the Achterhoek and 136 foxes were originating from the southern part of the investigated area. In the southern part most foxes were collected and investigated, whereas in the central part foxes were limited.

Mucosal smears of six foxes were at first classified as suspicious after microscopic examination. Parasites were taken out of the Petri dishes and examined in more detail by microscopy the next day. All six parasites examined turned out other than *E. multilocularis*. In addition, suspicious samples will be confirmed by PCR typing techiniques. Sofar, no positive foxes were found.

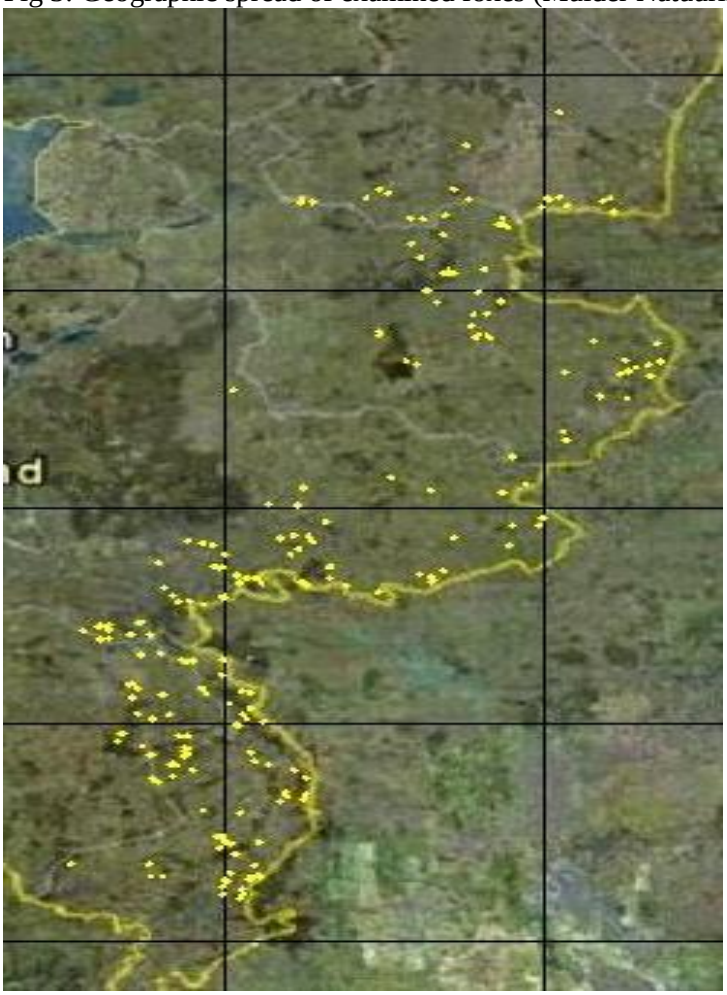
Table 1 Number of adults and juveniles

	Adults	**Juvenile**
Number of Foxes	94	185

Table 2 Number of foxes in different areas, North=Drenthe+Northeast Overijssel, Mid= Zuid Twente+Achterhoek, South=Mid Gelderland+Brabant+Limburg

Location	**Number of Foxes**
North	72
Middle	44
South	136
Total	252

Fig 3: Geographic spread of examined foxes (Mulder Natuurlijk)



4. Discussion

For this study, a sample size of 288 foxes was determined based on a population of 4000 foxes in this area before the study started (Takumi and van der Giessen, 2009). Of the 252 foxes already tested, no positives were identified, therefore it was concluded that the prevalence was < 1.15% with a 95% Confidence Interval. It was concluded that given the number of animals tested, this is suitable for this investigation.(J.W.B. van der Giessen pers. comment).

The microscopic examination alone is not a very sensitive method to determine whether a fox is infected with *E. multilocularis.* Previous studies demonstrated that a PCR-test on the colon content has a higher sensitivity then the microscopic examination (Takumi et al., 2007; van der Giessen, et al, 1998). Especially in foxes with low worm burdens the sensitivity of detecting *E. multilocularis* with PCR is higher. In 1998, 3 of the 272 foxes were found positive on microscopic examination although 5 of the 272 foxes were found positive with PCR. (van der Giessen et al., 1998) In the previous study in the border region with Germany, using microscopic examinations, the presence of *E. multilocularis* was not found. In the study described here, we expected a low or sporadic occurence of the parasite based on negative results in previous studies (van der Giessen et al., 1998). Hence, the microscopy could be not sensitive enough, to identify low worm burdens in foxes and therefore colon contents of these foxes will also be examined with PCR as described before. These results are not included in this report. No further spread of *E. multilocularis* was obtained in this study although the number of foxes in some areas especially in the Achterhoek is low.

For future projects it may be better to make sure that hunters especially in de Achterhoek are better informed, and personal attention might increase the participation for the future. May be it is better to contact individual hunters instead of contacting the WBE's. For future estimations of the prevalence in the Achterhoek en South Twente, it might be worthwhile to focus a detailed study in this area. In this way the gap might be filled up. It is not clear why there were so few foxes collected from these area's during this study. It might be due to the participation of hunter within the WBEs. When I contacted the hunters in this area it also not became clear why the participation was low. The prevalence of *E. multilocularis* in the same area across the border is not known. (K. Takumi and J.W.B. van der Giessen, 2009).

During my participation in this project, I was frequently involved in the output of the project by helping to develop the new questionnaire and called the hunters for further instructions en information.

5. Acknowledgements

During this field study I experienced that is hard to arrange a field study and lots of people are involved. These studies are important because in this way real-life situations are investigated. Further on it is needed the know how big the health risk is for people living in area's infected with *E. multilocularis.*

I want to thank the RIVM because they give me the opportunity to participate in the study. Especially I want to thank Joke van der Giessen for being my supervisor during the project. Also I want to thank Jaap Mulder from "Mulder Natuurlijk" for lending me his findings and the contact during the study.

6. References: wees consequent met refereren

- R.W. Ammann, J. Eckert; 1996; Cestodes. Echinococcus. Gastroenterol.; Clin. North Am. 25, 655-689
- K. Takumi, A de Vries, M. L. Chu, J Mulder, P Teunis, J.W.B. van der Giessen; 2008 Evidence for an increasing presence of *Echinococcus multilocularis* in foxes in the Netherlands; International Journal for Parasitology 38: 571-578
- J.W.B. van der Giessen, Y.B. Rombout, J.H. Franchimont, L.P. Limper, W.L. Homan; 1998; Detection of *Echinococcus multilocularis* in foxes the Netherlands.; Veterinary Parasitolgy 82 (1999) 49-57
- J.W.B. van der Giessen, Y.B. Rombout, L. Limper, A. van der Veen, C. Molenbeek, H. Franchimont, W. Homan; The presence of *Echinococcus multilocularis* in the red foxes (*Vulpes vulpes)* in the Netherlands; 1998; RIVM report
- K. Takumi and J.W.B. van der Giessen; 2009; Plan voor aanpak: Surveillance voor *Echinococcus multilocularis* in vossen voor 2010;
- F.H.M. Borgsteede; 1984; Helminth parasites of wild foxes (*Vulpes vulpes)* in the Netherlands; Z. Parasitenkd 70, 281-285.
- J.W.B van der Giessen, A. der Vries, M.L. Chu, V.Stortelder, J. Mulder, C. de Lezenne Coulander, P. Teunis; 2004; The prevalence of *Echinococcus miltilocularis* in foxes in Limburg 2002-2003; RIVM report.
- C. M. Kapel, P.R. Torgerson, R.C. Thompson, P. Deplazes; 2006; Reproductive potential of *Echinococcus multilocularis* in experimentally infected foxes, dogs, raccoon dogs and cats.; Int.J. Parasitol. 36, 79-86
- A. Schweiger, R.W. Ammann, D. Candians, P.A. Clavien, J. Eckert, B. Gottstein; 2007; Human alveolar echinococcosis after fox population increase, Switzerland. Emerg. Infect. Dis. 13, 878-882.
- P. Deplazes, P. Alther, I. Tanner, R.C.A. Thompson and J. Eckert; 1999; *Echinococcus multilocularis* coproantigen detection by enzyme-linked immunosorbent assay in fox, dog and cat populations. *Journal of Parasitology* **85** 115-121.
- Nur kein Panik; Wild und Hund; 13; 2010. (http://www.rivm.nl/cib/binaries/Janko2010_Nur_Keine_Panik_tcm92-69867.pdf)
- Mulder Natuurlijk, Jaap Mulder, www.mulder-natuurlijk.nl