Epidemiology of *Echinococcus multilocularis* in red foxes in Belgium and Germany

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1. Introduction

Echinococcus (E) multilocularis is a tapeworm of carnivores with a serious zoonotic risk. The parasite has a two-host lifecycle. The main definitive host in Europe and also in the Netherlands is the red fox (*Vulpes vulpes*) and the intermediate hosts are predominantly small rodents and accidentally humans. In definitive hosts, the tapeworm develops in its adult stage in the small intestines and eggs of the tapeworm are shed by the feces and develop, after ingestion by small rodents, to larval or metacestode stages (Van Der Giessen, Rombout et al. 1999, Conraths, Deplazes 2015). Infection with the larval stage of *E. multilocularis* causes alveolar echinococcosis (AE) in humans, of which symptoms develop after 5-15 years and which can be lethal if left untreated (Torgerson, Schweiger et al. 2008). AE is considered to be the most serious helminthic zoonotic disease in the northern hemisphere (Conraths, Deplazes 2015). Humans can become infected by direct contact with infected definitive hosts or by oral uptake of contaminated food and water (Deplazes, van Knapen et al. 2011).

The presence of *E. multilocularis* in the Netherlands was first described in a study in 1999 (Van Der Giessen, Rombout et al. 1999), which was initiated due to detection of infected foxes in adjacent areas in Germany and Belgium. This was considered a potential risk for the introduction of the parasite in the Netherlands, because the fox population in the Netherlands is considered to be continuous with the population in the adjacent countries (Mulder 1985). For this reason, between October 1996 and March 1997, 272 foxes were hunted and examined for *E. multilocularis* in the Netherlands, using the intestinal scraping technique (IST) and PCR. Five foxes were found positive. Two infected foxes were found in the northern province of Groningen, adjacent to the German border and three foxes in the southern part of the province Limburg. Since *E. multilocularis* was not found in a study to detect intestinal parasites in 1984, it was assumed that the parasite was spreading from Belgium and Germany into the Netherlands (Van Der Giessen, Rombout et al. 1999). After that finding, more studies were carried in the Netherlands to determine the prevalence of *E. multilocularis* in foxes. In Groningen the prevalence was almost 10% (10/106 examined) (Van Der Giessen, Rombout et al. 2004) and in South Limburg the prevalence increased from 13% in 2004 to 59% (22/37 examined) in 2013 (Maas, Dam-Deisz et al. 2014).
These findings illustrate the importance of knowledge about the epidemiology of *E. multilocularis* in adjacent countries, to better predict potential changes in the situation in the Netherlands. For this reason, a literature review is carried out, to get a better view of the prevalence and distribution of *E. multilocularis* in Belgium (especially in Flanders, northern Belgium) and Germany (especially the most western part, adjacent to the Netherlands).

2. Historic situation of *Echinococcus multilocularis* in Europe

Until the beginning of the 1990s, the endemic area for *Echinococcus multilocularis* in Europe was believed to be restricted to southern Germany, western Austria, northern Switzerland and the central and eastern

regions of France. The presence of the parasite was determined by examination of foxes in response to the occurrence of human cases of *E. multilocularis.* In France, the parasite was first detected in the 1970s in the east and central part of the country. Prevalences up to 47% were found (Craig, Pawlowski 2002, Fesseler 1990). The parasite was thought to be concentrated in colder climates of the world, including the mountainous areas in Europe. However, subsequent research showed that the range of *E. multilocularis* was not limited to this area, and a spread into areas not known to be endemic was recognised (figure 1). At current, the parasite is present in at least 21 countries of Europe, including Germany, Belgium and the Netherlands (Oksanen, Siles-Lucas et al. 2016, Deplazes 2006).

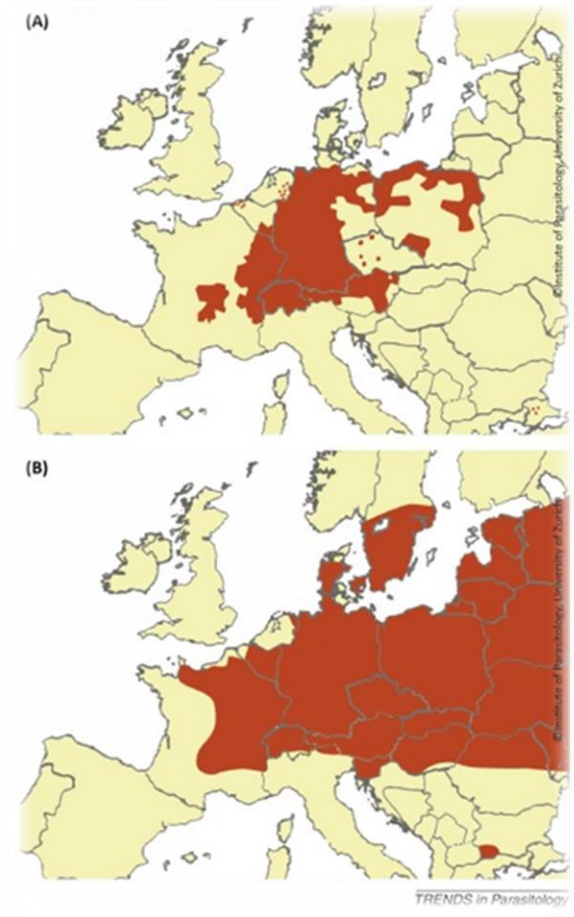

Figure 1: *Geographical distribution of Echinococcus multilocularis in red foxes in Europe.* ***(A)*** *Known distribution in 1997.* ***(B)*** *Update of known distribution in 2015. Reprinted from: Gottstein, B., Stojkovic, M., Vuitton, D.A., Millon, L., Marcinkute, A., Deplazes, P. Threat of alveolar echinococcosis to public health – a challenge for Europe. Trends Parasitol., 31 (2015), pp. 407-412.*

However, the lack of information about the presence of the parasite in the previous decades, makes it impossible to determine whether the range of *E. multilocularis* has increased or whether the parasite was already present in these areas but was never detected before. The increase of the fox population in Europe in the past decades could have played an important role in the extension of the range of the parasite. This increase is thought to be the result of reduced mortality in foxes as a consequence of successful rabies control programs, restrictions on fox hunting, nature conservation measures and the opportunistic behaviour (adaptation) of foxes (Vervaeke, Dorny et al. 2003).

3. *Echinococcus multilocularis* in red foxes in Belgium

3.1. Wallonia

In 1992, *E. multilocularis* was first recorded in the southern part of Belgium called Wallonia. In a study in the south-eastern part of the country, a prevalence of 15.3% (13/85 examined) was found in foxes from the Ardennes, a mountainous region in the province of Luxembourg (maximum attitude of 700 meter above sea level). Research done in the same region in 1997 showed an increased prevalence of 51% (74/145 examined) in the fox population (Vervaeke, Dorny et al. 2003).
In a subsequent study between 1998 and 2002 in Wallonia, 709 red foxes were collected and examined for *E. multilocularis*, using the intestinal scraping technique (IST). The attitude in this region varies between 80 meter above sea level in Hesbaye and 700 meter above sea level in the Ardennes. *E. multilocularis* was observed in 20.2% (143/709 examined) of the foxes and ranged from 1.6% (2/126 examined) in the Hesbaye low plateau, over 12.6% (24/190 examined) and 17.2% (5/29 examined) in Condroz and Fagne-Famenne respectively (middle central plateaus) to 23.1% (6/26 examined) and 33.1% (106/320 examined) in Lorraine and the Ardennes (southern high plateau). However, no positive foxes were examined in Plateau de Herve, in the middle

central plateaus. This study suggest a decreasing prevalence of *E. multilocularis* together with the attitude in westward direction (Losson, Kervyn et al. 2003).

This was confirmed in a subsequent study between January 2003 and December 2004, where 990 foxes in Wallonia were examined for *E. multilocularis*, using the intestinal scraping technique. In this study, an average prevalence of 24.55% was found (248/990 examined). The prevalence in the southern high plateau was 33.8% (48/142 examined) and 40.84% (87/213 examined) in respectively Lorraine and the Ardennes. However, the highest prevalence was detected in Fagne-Famenne-Calestienne (61.8% (21/34 examined)). The lowest prevalences were found in the areas where the altitude is below 100 meter above sea level. In the Condroz and the Hesbaye area, prevalences of 24.86% (45/181 examined) and 10% (42/420 examined) respectively were recorded. The prevalence decreased in north-westward direction, probably related with the decreasing altitude in westward direction. The surprising high prevalence of *E. multilocularis* in Fagne-Famenne-Calestienne (61.8%) could be explained by the presence of sporadic hyper-endemic foci in this region (Hanosset, Saegerman et al. 2008). Overall, the prevalence of *E. multilocularis* in foxes had increased in these areas of Wallonia, compared to the previous study.

3.2. Flanders

However, the range of *Echinococcus multilocularis* was not restricted to the hilly southern region of Belgium. Also in Flanders, where the altitude is below 100 meter above sea level, infected foxes were detected. As mentioned, this could be a result of the increased fox population since 1980 as a consequence of successful rabies control programs (Vervaeke, Dorny et al. 2003).
Between 1996 and 1999, the first infected were detected in Flanders. In this study, 237 foxes were examined, using the intestinal scraping technique. Four foxes (1.7%) were infected with *E. multilocularis*. One of these infected foxes was hunted in northern part of the study area, close to the Dutch border (Vervaeke, Dorny et al. 2003, Vervaeke, Brandt et al. 1997).
As mentioned earlier, the presence of *E. multilocularis* was described in this same period for the first time in the Netherlands in the northern part of the province Groningen and the southern part of the province Limburg. In the province of Limburg, situated adjacent to the border with Belgium, the prevalence was estimated at 13.6%. The foxes were examined using the intestinal scraping technique and two different PCR assays, based on DNA of the parasite in colon content (Van Der Giessen, Rombout et al. 1999).
It was hypothesised that these first detections of infected red foxes in the northern part of Belgium and the southern part of the Netherlands were the result of an extension of the range of *E. multilocularis* in these areas (Vervaeke, Dorny et al. 2003).

However, the prevalences in the Netherlands and Flanders were not as high as the prevalence in Wallonia. Separated studies, done between 1996 and 2003 in Wallonia, Flanders and the Netherlands showed that the prevalence of the parasite in red foxes is much higher in the southern part of Belgium, than in the northern part and in the Netherlands. This conclusion was solidified by an analysis of several studies in Belgium and the Netherlands in which a total of 1202 foxes were tested, using the intestinal scraping technique (184 from the Netherlands, 200 from the northern part of Belgium and 818 from the southern part of Belgium). This analysis shows a decreasing prevalence of *E. multilocularis* in north-western direction. The model, based on these results, estimates that the epicentre of the *E. multilocularis* infection is located in south-eastward direction, i.e. in Germany. It analyses that the prevalence is 40% in the southernmost part of Belgium and decreases to approximately 10% at the northern border of Belgium, adjacent to the province Limburg (the Netherlands) (Vervaeke, van der Giessen et al. 2006).

The presence of *E. multilocularis* in Flanders has some special consequences for the

zoonotic risk of the parasite, because of the relative high grade of urbanisation in this part of Belgium (including the regions of Brussels and Flanders), in contrast to Wallonia. In a few decades, the fox population has increased significantly. The average fox density in Flanders increased to 1-2 adults/km^2. The foxes often dwell at short distance to domestic houses. This increases the risk of transmission of zoonoses (including *E. multilocularis*) because more inter- and intra- species contacts (between humans, pets and potential infected foxes) can occur. Subsequent studies illustrated more dynamics of *E. multilocularis* in the relatively densely urbanized regions Brussels and Flanders (Brochier, De Blander et al. 2007, Van Gucht, Van Den Berge et al. 2010).

In a study in the Brussels-capital region, 160 foxes were collected and examined between January 2000 and April 2004. None of the foxes was detected to be infected with *E. multilocularis*. The foxes were examined using the intestinal scraping technique and the result of 20 negative tested foxes was confirmed, using the sedimentation and counting technique (SCT) (Brochier, De Blander et al. 2007). In another study in 2007 and 2008, 187 red foxes were sampled in Flanders and Brussels, the southern part of the study area being located near to the endemic region in Wallonia. However, none of the foxes tested positive for *E. multilocularis,* using the intestinal scraping technique. This result suggested, in contrast with other studies (Vervaeke, van der Giessen et al. 2006) that the parasite was not present in the northern region of Belgium. It was unclear why the parasite was not detected in this study despite the relatively high prevalence in the southern region of Belgium (Van Gucht, Van Den Berge et al. 2010).
One of the possible explanations for the lower prevalence in this region, is the diet of the foxes. Stomach content analyse shows that the content in foxes in Flanders consist, in contrast to studies in other regions, for a relatively small part out of *Arvicolidae*, the rodent family that is considered to be the major intermediate hosts of *E. multilocularis* (Eckert, Deplazes et al. 2001). This difference could be explained by landscape ecological factors. As a result of very intensive agriculture in the Flemish area, the region, the habitats are probably less suitable for *Arvicolidae.* Also muskrats appeared rarely in the diet of foxes in Flanders (Van Gucht, Van Den Berge et al. 2010). In a recent study in Wallonia, a significant correlation between the prevalence of *E. multilocularis* in foxes and muskrats was detected. In this study, the role of other rodents in the transmission of *E. multilocularis* was considered as very small, because of the low prevalence of the parasite in this species, in contrast to the muskrats (Hanosset, Saegerman et al. 2008). The reduction of the muskrat population in large parts of Flanders in the last decades, could have resulted in the low prevalence of *E. multilocularis* in foxes in this region, as a consequence of the lack of important intermediate hosts (Van Gucht, Van Den Berge et al. 2010).

One of the explanations for the low prevalence in the Brussels-capital regions and other densely urbanized regions is that the proportion of rodents in the diet of foxes living in urbanized regions is lower than the proportion in rural foxes. Small rodents, the key intermediate host, play an essential role in the lifecycle of *E. multilocularis* (Conraths, Deplazes 2015, De Blander, Kervyn et al. 2006). In urbanized regions, anthropogenic food resources are very important to feed urban foxes. Food from households, public areas and allotment gardens is enough to feed them. A study in Zurich shows that more than 50% of the stomach content of urban foxes consists of anthropogenic food (Contesse, Hegglin et al. 2004, Deplazes, Hegglin et al. 2004). The low prevalence of *E. multilocularis* in the urbanised Brussel-capital region decreases the risk of zoonoses (Brochier, De Blander et al. 2007).

Another possible explanation for the differences between Wallonia and the northern parts of Belgium, are the differences in attitude and climatic conditions. The milder climate and the limited rainfall in the northern part (800 mm/ year compared to 1400 mm/ year in Wallonia) possibly plays a role in the preservation of *E. multilocularis* eggs in the environment, because low temperature and moist environment are the best conditions for

the eggs to remain infective (Eckert, Deplazes et al. 2001, Van Gucht, Van Den Berge et al. 2010). Furthermore, because the southern part of Belgium is more forested then the northern part, the *E. multilocularis* eggs probably are better protected against heating and desiccation (Van Gucht, Van Den Berge et al. 2010, Veit, Bilger et al. 1995)

In the most recent study, performed in Flanders in the three consecutive winters 2012 – 2015, about 900 red foxes were hunted and examined for *E. multilocularis*, using the modified Segmental sedimentation and counting technique (SSCT). This study showed that the prevalence and spread remained very low (0.8%) in most of this region, with occasional infections. However, in the region of Voeren, at the eastern border with Wallonia and South Limburg in the Netherlands, a much higher prevalence (57%, 12/21) was found (personal communication Marleen Claes, Institute of Tropical Medicine, Belgium). Together with the high prevalence that was found in 2012-2013 in the area East of Maastricht in the Netherlands, this suggests the existence of a hotspot in this region.

4. *Echinococcus multilocularis* in red foxes in Germany

Echinococcus multilocularis was first detected in Germany in the 1970s. In Tübingen, a district in Baden-Württemberg, a prevalence of 12.4% was founded in red foxes. Subsequent research between 1974 and 1984 estimated the prevalence at 18.3%. Later studies, dating from the beginning of the 1990s, showed an increased prevalence of 55.6% and 58.5% in the district of Tübingen. Also in other districts in the southern part of Germany, the prevalence of *E. multilocularis* in red foxes increased (Muller, Partridge 1974). The high prevalences in the southern federal states motivated other federal states to study the prevalence of several parasites in foxes, including *Echinococcus multilocularis.* In studies in federal states in the western part of the country, near the border with the Netherlands, a significant part of the foxes was found positive for the parasite. In Rhineland-Palatinate, North Rhine-Westphalia and Lower Saxony, prevalences of respectively 27.8-33.1%, 17.7% and 17.8% were found. Several studies suggest that *E. multilocularis* was spreading into previously non-endemic areas (Lucius, Bilger 1995).

However, these studies were done over short time periods of just a few months and restricted study areas. To get a better overview of the changes in prevalences in certain area, analyses of a longer period are needed. Studies in Lower Saxony, a province in the northern part of Germany, adjacent to the province of Groningen in the Netherlands, found an increasing prevalence of *E. multilocularis* in red foxes. The red foxes were examined using the intestinal scraping technique. The provincial prevalence increased from 12.1% between 1991-1994 to 14.9% between 1994-1997 in a sample size of respectively 2748 and 2617 foxes. In some specific areas, the prevalence increased from 50% to 60% in this period. To prove this trend, a new study was done between 2003 and 2005 with a sample size of 3094 foxes, sampled from 41 regions of Lower Saxony (Germany). An average provincial prevalence of 19.7% was found. So, the prevalence in Lower Saxony increased from 12.1% (1991-1994), to 14.9% (1994-1997) to 19.7% (2003-2005). This increase demonstrates the emergence of the parasite in this region (Berke, Romig et al. 2008).
No data is available from this region after 2005. However, a few studies in adjacent federal states can provide a few information about the development of the situation of *E. multilocularis* in the region

Recent research, done in Saxony-Anhalt, situated on the centre of Germany adjacent to the eastern border of Lower Saxony, showed an increasing prevalence of *E. multilocularis*. The foxes were examined parasitologically, using the intestinal scraping technique. The prevalence increased from 13.6% (1882 foxes examined) in 1998-2005 to 23.4% (2307 foxes examined) in 2006-2010. The spatial movement of the parasite during the study period was estimated at 20.7 km in north-north-eastern direction. This suggests a spread of 3.2 km per year. The increased prevalence of

E. multilocularis was thought to be correlated with the increasing fox population density in this region (Denzin, Schliephake et al. 2014).

In accordance with the increased prevalence in Lower Saxony between 1991 and 2005, the prevalence in red foxes in the federal state Thuringia also increased. This federal state is located south of Lower Saxony and Saxony-Anhalt. Between 1990 and 2009, 26,220 foxes were examined for infection with *E. multilocularis*, using the intestinal scraping technique. Spatial analyses of the data showed an expansion of the range of the parasite in Thuringia. In 1990, the highest prevalence (> 40%) was detected in the north-western part of the federal state, whereas in the eastern districts no infected foxes were found or low prevalences were detected. However, the prevalence in Thuringia increased from 10-15% in the beginning of the 1990s to 30-40% in the last decade, with a peak of 42% in 2005. The increasing prevalence of *E. multilocularis* together with the simultaneously increasing fox population density, may have resulted in an increased biomass of *E. multilocularis* (Staubach, Hoffmann et al. 2011).

5. Conclusion

The epidemiology of *E. multilocularis* in Belgium and Germany is very important to predict potential changes in the situation in the Netherlands, because the fox population in the Netherlands is considered to be continuous with the population in the adjacent countries. This literature review shows that the prevalence in Belgium and Germany has increased in the past decades. The range of the parasite has also expanded. This may suggests that the prevalence of *E. multilocularis* in the Netherlands also will increase and that the parasite will expand its range in de coming decades in Groningen, South- Limburg but probably also in areas which are not known to be an endemic at current. This emphasizes the importance to monitor the spread of the parasite in the Netherlands in the coming years and the relevance of the knowledge about the epidemiology of *E. multilocularis* in adjacent countries.

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