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Short Communication

Echinococcus multilocularis in red foxes in North Belgium: Prevalence and trends in distribution

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ABSTRACT

A cross-sectional systematic sampling was carried out during three consecutive winters from 2012 to 2015, to update the knowledge on the fox tapeworm (*Echinococcus multilocularis*) distribution in the red fox (*Vulpes vulpes*) in Flanders. Earlier studies reported the low endemicity status of this tapeworm in the northern region of Belgium, in contrast to the south of the country and neighbouring countries. Using a modified Segmental Sedimentation and Counting Technique, followed by PCR-RFLP and sequencing, 923 foxes' intestines were examined for the presence of *E. multilocularis*. Based on microscopic examination, 38 out of 923 foxes were suspected to be infected with either *E. multilocularis* or *Amoebotaenia* spp., of which 19 were molecularly confirmed to be *E. multilocularis,* 18 were found positive for *Amoebotaenia* spp. and one was negative. The overall prevalence for *E. multilocularis* of 2.1% confirms the low endemicity of the fox tapeworm in Flanders. However, in one area in the most eastern part of Flanders (Voeren), neighbouring the Netherlands and Wallonia, a prevalence of 57% (12/21) was observed. Continuous monitoring of the fox tapeworm remains needed to assess spatio-temporal trends in distribution and to assess the risk of this zoonotic infection in Europe. The challenging differential diagnosis of *E. multilocularis* and *Amoebotaenia* spp. based on microscopic examination calls for attention.

1. Introduction

Echinococcus multilocularis is a small cestode, of which the red fox acts as the main definitive hosts and several species of rodents as intermediate hosts in Europe (Eckert et al., 2001; Hanosset et al., 2008; Romig et al., 2017). Humans may become accidental dead-end intermediate hosts upon ingestion of eggs. Human alveolar echinococcosis (AE) caused by infection with the metacestode stage of *E. multilocularis*, is a potentially lethal zoonosis (Oksanen et al., 2016).

Although the incidence of human infection is low in Europe, 0.02–0.18 cases/100,000 inhabitants (Torgerson et al., 2010), the zoonotic potential of the fox tapeworm, in terms of persistence and pathogenicity, poses a major threat to human health (Combes et al., 2012).

In Belgium, the incidence of AE has remained stable, around one case per year during the last two decades, with most cases originating from the Southern part of Belgium (Detry et al., 2005; Kern et al., 2003; Keutgens et al., 2013; Litzroth and Truyens, 2015). However, one case was found in an urban dweller of the capital city of Brussels (Landen et al., 2013). In red foxes, *E. multilocularis* was first reported in the most southern province of Belgium (Brochier et al., 1992). Losson et al. (1997) reported that 51% (95%CI: 42.6–59.4%) of the red foxes in the same province were infected, while more recent studies in the same region conveyed a prevalence ranging between 20.2% (95%CI:

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17.3–23.3) and 24.6% (95%CI: 22.4–27.9%) (Hanosset et al., 2008; Losson et al., 2003). In Flanders, the northern region of Belgium, a first screening in 1996–1999 reported a much lower prevalence of 1.7% (95%CI: 0.5–4.3%) (Vervaeke et al., 2003) and a study in 2007–2008 in the Brussels and Flanders regions showed that none of the 187 sampled foxes were positive (Van Gucht et al., 2009).

Since Flanders is a potential expansion region for the fox tapeworm in Europe, close monitoring of trends in distribution and prevalence is needed. The objective of this study was to map the presence of *E. multilocularis* in the red fox in Flanders.

2. Materials and methods

The study region, Flanders (i.e. northern Belgium), covers an area of 13,522 km^2 with a population density of 484 people/km^2 (https://www.statistiekvlaanderen.be/nl/bevolking-omvang-en-groei). Flanders has a maritime temperate climate with significant precipitation in all seasons. Since 2000, the whole territory of Flanders is colonised by foxes (Vervaeke et al., 2003). The average density of the red fox population is estimated at 1–2 adults/km^2 in Flanders (Van Den Berge, K., personal communication).

A cross-sectional systematic sampling was carried out in the Flanders region by the Agency for Nature and Forests, from September to January during three consecutive winters in 2012–2015 (fox hunting season from October to February) with the aim to collect 900 foxes in total. Networks of hunters were involved in the collection of culled red foxes and a total of 925 fox carcassess were collected in the study period, with a range of 303 to 316 foxes per winter (evenly sampled over every hunting area). Each fox carcass was assigned an identity number and sealed in a double plastic bag. Within 24 h, the carcass was frozen at −80 °C for at least one week to inactivate the parasite's eggs and reduce the risk of infection during handling and examination (Eckert et al., 2001). Next, after thawing, necropsy was performed and the intestinal pack was ligated at both ends, removed from the carcass, properly identified and kept at −20 °C until examination.

The Segmental Sedimentation and Counting Technique (SSCT) validated by the French National Reference Laboratory (NRL) for the detection of *Echinococcus* spp. (Umhang et al., 2011), was adapted and optimized to reduce debris, concentrate the sample and reduce microscopic reading time. Compared to the SSCT, an additional sieving step was included at the beginning of the protocol, employing a 50 µm mesh (procedure validated by the Belgian National Reference Laboratory (NRL) and confirmed by the European Reference Laboratory for Parasites (EURLP) through participation in proficiency testing). The material collected after rinsing the fox intestines is collected, poored through the 50 µm mesh sieve and rinced througoughly. The content of the sieve, together with the remaining and additional rinsing water of the sieve is collected in Erlenmeyer flasks and afterwards examined with a stereoscope.

Differential diagnosis with *Amoebotaenia* spp. was done based on morphological characteristics: worm size, the shape and number of hooks on the scolex and the number and shape of the proglottids (Fig. 1) (French National Reference Laboratory (NRL) for *Echinococcus* spp. (Anses)).

A maximum of five cestodes per fox (depending on the number of cestodes found), morphologically identified as *E. multilocularis*, as well as *Amoebotaenia* spp. and doubtful findings, underwent DNA extraction using the Boom extraction method (Boom et al., 1990). In the pre-PCR, DNA extract was added to Promega PCR master-mix and amplification of a mitochondrial 12S rDNA fragment was done utilizing a semi-nested PCR (Geysen et al., 2007). Restriction fragment length polymorphism (RFLP) (*Alu* I and Hinf I) was used to differentiate *Echinococcus* spp. (Tigre et al., 2016). Samples with non-specific bands were considered suspect of *Amoebotaenia*. Three PCR-RFLP products were sent for sequencing to VIB Genetics Service Facility: one with a pattern specific for *E. multilocularis*, one with a small deviation in the pattern for *E. multilocularis* and one with a pattern specific for *Amoebotaenia*. Results were processed using GeneStudio and blasted with NCBI. All data were entered and analysed in Microsoft Excel. Distribution maps were drawn using QGIS.

3. Results

From the 925 collected fox carcasses, two were excluded from further analysis due to degradation. In 38 out of 923 foxes, small cestodes were observed upon microscopic examination (Table 1).

Of the 19 (out of 38) microscopically identified *E. multilocularis* infected foxes, only 17 could be confirmed molecularly (Table 2). The number of worms found in one infected fox varied from only one to over 10,000. One of the two remaining samples showed an *Amoebotaenia* spp. suspect molecular profile (see further). For the second one, neither *Echinococcus* spp. nor *Amoebotaenia* spp. could be confirmed.

Upon microscopic examination, seven of the 38 foxes appeared to have a mixed *E. multilocularis/Amoebotaenia* spp. infection, but this could not be confirmed by molecular analysis. Yet, PCR-RFLP confirmed the presence of *E. multilocularis* in two (2 and 33 worms found) and an *Amoebotaenia* spp. suspected profile in five of these samples (Table 2).

The remaining 12 samples (out of 38), on microscopic examination being suspected as infected with *Amoebotaenia* spp., showed non-specific bands in RFLP. Eleven of these had a profile similar to that of *Amoebotaenia* spp.. However, we were not able to molecularly confirm this as no 12S sequences of *Amoebotaenia* are deposited in Genbank. One sample gave unclear results upon molecular testing, yet, sequencing revealed a moderate agreement with *Mesocestoides corti* genome assembly (query cover 99%, identical 79%) although the sequence itself was not known in NCBI.

Therefore, overall, 19 samples were confirmed as *E. multilocularis* (6 in 2012–2013, 8 in 2013–2014 and 5 in 2014–2015), resulting in an overall prevalence of *E. multilocularis* for all examined samples of 2.1% (95%CI: 1.2–3.2%).

The geographical distribution of the negative and positive samples with respect to the commune of origin during the entire study period is presented in Fig. 2. For 8 of the 923 foxes the commune of origin was unknown (all negative for *E. multilocularis*). Many positive foxes were recorded in Voeren, a commune in the most eastern part of Limburg province, bordering Wallonia (i.e. southern Belgium) and the Netherlands. Here, 12 of the 21 investigated foxes were positive (57.1%, 95%CI 34–79.2%) (Zoomed area in Fig. 2). When excluding the results of this one commune, the overall results for the Flanders Region yield a prevalence of 0.8% (95%CI: 0.3–1.6%).

4. Discussion

This is the first and largest study on *E. multilocularis* prevalence in systematically sampled red foxes in Flanders with molecular confirmation of positive cases. As compared to the study of Vervaeke et al. (2003), our results confirm that Flanders is a region of low endemicity and no increase in prevalence of *E. multilocularis* in red foxes has occurred over the past ten years. We found an overall prevalence of 2.1% for *E. multilocularis* in red foxes in Flanders, with mostly single infections per commune except for one commune with a high prevalence in the most eastern part of the region (Voeren). In neighbouring regions, *E. multilocularis* has been detected in red fox (prevalence in Southern Belgium 19%, Northern France 7%, Luxembourg 17% and the Netherlands 18% and no data from Germany) (data from 2000 onwards) (Oksanen et al., 2016). In foxes east of Maastricht, The Netherlands, a very high prevalence of 59% was detected in 2013 (van der Giessen and Claes, 2016). From these data, it is clear that Belgium has a lower, stable prevalence of *E. multilocularis* compared to the neighbouring regions (Oksanen et al., 2016). But there is evidence of the emergence of a new hot spot area of *E. multilocularis* in the eastern border zone of

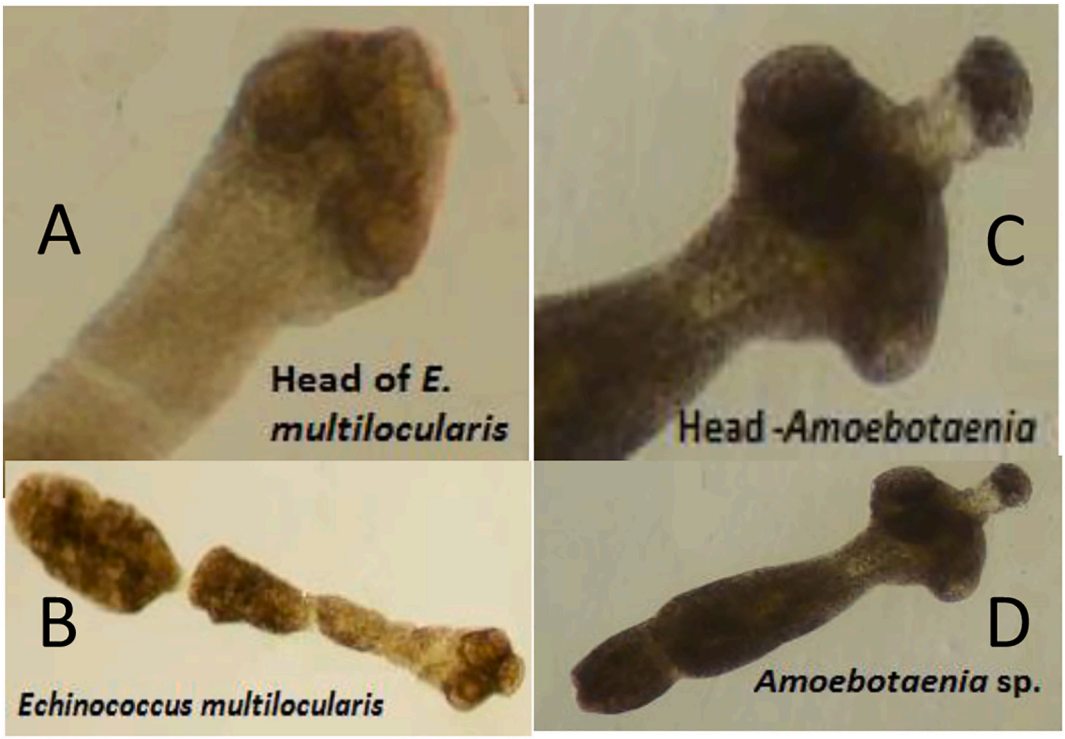

Fig. 1. Morphological differentiation between *Echinococcus multilocularis* (A,B) and *Amoebotaenia* sp. (C,D) based on worm size, the shape of and the number of hooks on the scolex and the number and shape of the proglottids (source: French National Reference Laboratory (NRL) for *Echinococcus* spp.)

Table 1
Microscopic and molecular identification of *Echinococcus multilocularis in* fox intestines collected in Flanders from September 2012 to January 2015.

Province	N samples	N suspected*	N confirmed**	% infected
Limburg	165	17	14	8.5 (95%CI: 4.7–13.8%)
Flemish Brabant	146	8	2	1.4 (95%CI: 0.2–4.9%)
Antwerp	197	2	0	0
West Flanders	212	7	2	0.9 (95%CI: 0.1–3.4%)
East Flanders	195	3	1	0.5 (95%CI: 0.1–2.8%)
Unknown	8	1	0	0
Total	923	38	19	2.06 (95%CI: 1.2–3.2%)

* Suspected by microscopy, also including samples morphologically identified as *Amoebotaenia* spp.
** Molecular confirmation by PCR-RFLP.

Table 2
Diagnostic agreement between morphological and molecular identification of *Echinococcus multilocularis* in fox intestines collected in Flanders from September 2012 to January 2015.

Test results		
Microscopy	Molecular examination	*n*
E. multilocularis	*E.multilocularis*	177
	Negative	1
	Amoebotaenia spp.	1
Amoebotaenia spp.	*Amoebotaenia* spp.	11
	Mesocestoides corti genome assembly (moderate agreement)	1
Mixed infection *E. multilocularis* and *Amoebotaenia* spp.	*Amoebotaenia* spp.	5
	E.multilocularis	2

Belgium with the Netherlands.

We therefore conclude that the parasite is not spreading from the highly endemic South and East, which contrasts the predictions made by Vervaeke et al. (2006). Furthermore, prevalance is increasing in other European countries such as Germany (pooled prevalence of 13.8% from 1973 to 2000 and 29.2% from 2000 to 2012) and Poland (pooled prevalence of 2% from 1994 to 2000 and 14.8% from 2000 to 2014) (Oksanen et al., 2016). Only in the most eastern part of Flanders (Voeren), a high prevalence was observed. This area borders directly to highly endemic regions of Wallonia and the Netherlands and is very close to the German border.

We hypothesize that the persisting low prevalence of *E. multilocularis* in northern Belgium, despite a stable fox population (personal communication Van Den Berge, Koen, INBO (Research Insitute Nature and Forest) and a highly endemic neighbouring region, is due to a combination of geographical, climatic and biological factors, affecting both the survival of parasite eggs in the environment and the transmission. In contrast to the highly endemic Wallonia region, Flanders has a lower altitude (0–100 m above sea level vs. 400–700 m), milder climatic conditions, less rainfall, less coverage by forest and a different nature and utilization of the soil. These factors may be responsible for a shorter survival time of eggs in the environment (Eckert et al., 2001). Within the southern part of Belgium, a higher fox tapeworm prevalence was reported in dense forested and high-altitude areas in the Ardennes as compared to lower altitude areas (Hanosset et al., 2008; Losson et al., 2003).

Another reason for the disparity in prevalence in the Belgian regions may be related to the presence and densities of the natural intermediate hosts and the predator-prey relationship between red foxes and those

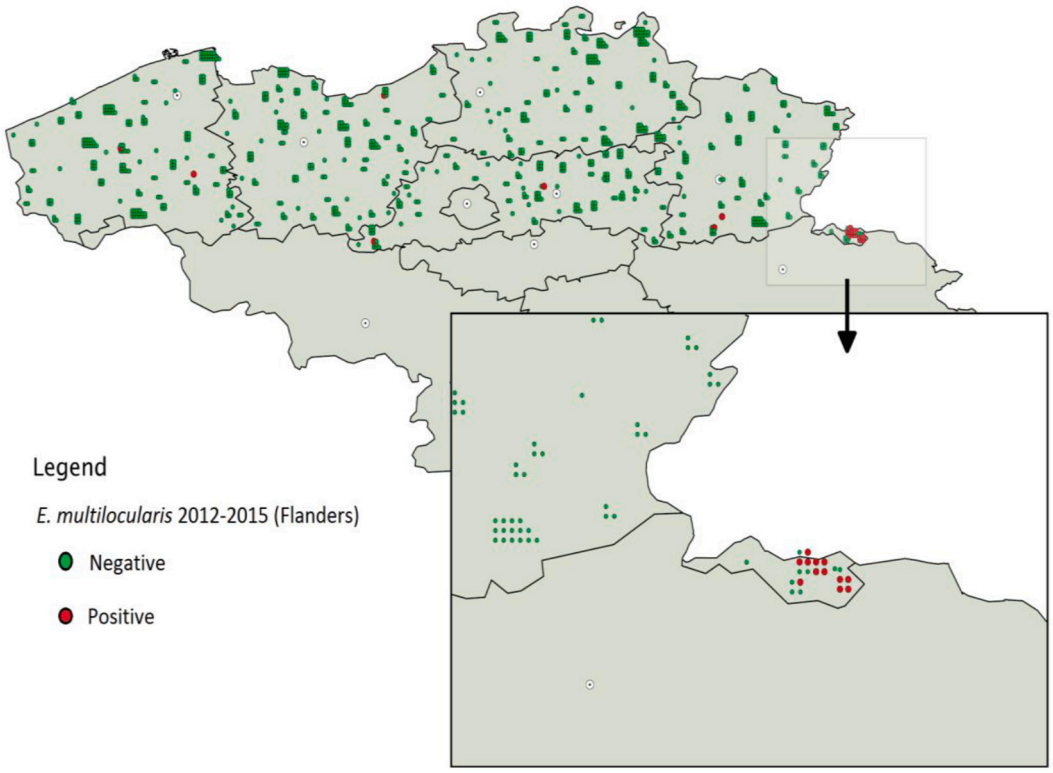

Fig. 2. Map showing red fox sample locations in the Flanders region: red and green dots present intestinal samples of foxes sampled in the period 2012–2015, with positive and negative findings for *Echinococcus multilocularis*, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

intermediate hosts. These two factors differ greatly according to the level of urbanization (Otero-Abad and Torgerson, 2013) and depend on many interacting factors such as landscape character, biotope, climatic conditions, movement of foxes and availability of prey (Eckert et al., 2000). The main intermediate host species of *E. multilocularis* are voles (e.g. *Microtus* spp., *Arvicola* spp., *Myodes* spp.). The European stable endemic region is coincident with the known distribution of *Microtus arvalis*, but not with other vole species, giving evidence that this *M. arvalis* plays a crucial role in the maintenance of the parasite's life cycle and that its absence could be a limiting factor for the spread of *E. multilocularis* in a region (Guerra et al., 2014).

In Flanders, a considerable part of the fox diet consists of brown rats (*Rattus norvegicus*) (Van Gucht et al., 2009) and these rodents are likely less suitable hosts for *E. multilocularis* (Oksanen et al., 2016; Romig et al., 2017). Since the abundance of rodents is affected by the land use pattern, the intensive nature of agricultural practices in the highly urbanized northern region of Belgium may result in less suitable habitats for the Arvicolidae, with an overall increase in brown rats.

However, other rodents, such as the Nutria (*Myocastor coypus*) and the muskrat (*Ondrata zibethicus*) are also presumed to be suitable intermediate hosts in Europe (Conraths and Deplazes, 2015; Oksanen et al., 2016; Umhang et al., 2013). In Wallonia, a positive correlation was seen between the prevalence of *E. multilocularis* in the muskrat and in the red fox, with 11.18% of muskrats infected (Hanosset et al., 2008). Along the Ourthe river, 22.2% of 657 muskrats were infected with *E. multilocularis* (Mathy et al., 2009). Neighbouring countries have also detected the parasite in muskrat populations with a prevalence of 0.1% in Limburg and Groningen in the Netherlands (Borgsteede et al., 2003), 4.1% in Lower Saxony in Germany (Baumeister et al., 1997) and one muskrat found positive in 12 examined in France (Boussinesq et al., 1986). In Flanders, 82 out of 9425 muskrats (0.87%) analysed between 2008 and 2017 were infected with *E. multilocularis* (Cartuyvels et al., 2020). This indicates that the spread of *E. multilocularis* from the Southern and Eastern regions has not occurred in muskrats, either. This can be due to the low presence of muskrats because of ongoing pest control activities, creating large areas free of muskrats, except for the border regions where their density is still high (Cartuyvels et al., 2020; Van Gucht et al., 2009).

This study identified difficulties in the morphological differentiation of *E. multilocularis* and *Amoebotaenia* spp., specifically on damaged worms. Confirmation of *E. multilocularis* by molecular tests was needed and, in some cases, even showed misdiagnosis by morphology. Differential diagnosis of both species requires special attention in future studies. *Amoebotaenia* spp. are cestodes that mainly parasitize poultry and use earthworms as intermediate hosts in which the cysticercoid larval stage develops. The finding of an *Amoebotaenia* sp. in red foxes was first reported in France and it was identified as *Amoebotaenia paradoxa* (Pétavy et al., 1990).

In conclusion, our data show that the prevalence of *E. multilocularis* in the red fox is not increasing in the overall northern region of Belgium. The finding of *Amoebotaenia* spp. in red foxes in Flanders indicates that a differential diagnosis is needed in future *E. multilocularis* prevalence studies in Belgium and other European countries. These findings call for further studies, in the final as well as in the intermediate hosts. Collaboration with bordering regions and countries, and notification of survey results, are important to monitor trends in distribution.

Ethical statement

No laboratory test animals were used for this study.

Declaration of Competing Interest

None.

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References

Baumeister, S., Pohlmeyer, K., Kuschfeldt, S., Stoye, M., 1997. Prevalence of *Echinococcus multilocularis* and other metacestodes and cestodes in the muskrat (*Ondatra zibethicus* LINK 1795) in Lower Saxony. Dtsch. Tierarztl. Wochenschr. 104, 448–452.

Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., van der Noord, J., 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. 28, 495–503.

Borgsteede, F.H., Tibben, J.H., van der Giessen, J.W., 2003. The musk rat (*Ondatra zibethicus*) as intermediate host of cestodes in the Netherlands. Vet. Parasitol. 3, 29–36.

Boussinesq, M., Bresson, S., Liance, M., Houin, R., 1986. Ann. Parasitol. Hum. Comp. 61, 431–434.

Brochier, B., Coppens, P., Pastoret, P.P., 1992. A programme of eradication of rabies by vaccination of the fox. Pathol. Biol. 40, 899–909.

Cartuyvels, E., Baerts, K., Van Den Berge, K., Berlengee, F., Stuyck, J., 2020. Het voorkomen van de vossenlintworm (*Echinococcus multilocularis*) in de muskusrat (*Ondatra zibethicus*) in Vlaanderen: Screening op de aanwezigheid van cysten in muskusratten gevangen door VMM in kader van bestrijding. https://inbo.github.io/fis-reports/Reports/Fox%20tapeworm%20in%20muskrats/index.html.

Combes, B., Comte, S., Raton, V., Raoul, F., Boué, F., Umhang, G., Favier, S., Dunoyer, C., Woronoff, N., Giraudoux, P., 2012. Westward spread of *Echinococcus multilocularis* in foxes, France, 2005–2010. Emerg. Infect. Dis. 18, 2059–2062.

Conraths, F.J., Deplazes, P., 2015. *Echinococcus multilocularis*: Epidemiology, surveillance and state-of-the-art diagnostics from a veterinary public health perspective. Vet. Parasitol. 213, 149–161.

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

Eckert, J., Conraths, F.J., Tackmann, K., 2000. Echinococcosis: an emerging or re-emerging zoonosis? Int. J. Parasitol. 30, 1283–1294.

Eckert, J., Deplazes, P., Craig, P.S., Gemmell, M.A., Gottstein, B., Heath, D., Jenkins, D.J., Kamiya, M., Lightowlers, M., 2001. Echinococcocis in animals: Clinical aspects, diagnosis and treatement. In: Eckert, J., Gemmel, M.A., Meslin, F.-X., Pawlowski, Z.S. (Eds.), WHO/OIE Manual on Echinococcosis in Humans and Animals: A Public Health Problem of Global Concern. France, Paris, pp. 73–100.

Geysen, D., Kanobana, K., Victor, B., Rodriguez-Hidalgo, R., De Borchgrave, J., Brandt, J., Dorny, P., 2007. Validation of meat inspection results for *Taenia saginata* cysticercosis by PCR-restriction fragment length polymorphism. J. Food Prot. 70, 236–240.

Guerra, D., Hegglin, D., Bacciarini, L., Schnyder, M., Deplazes, P., 2014. Stability of the southern European border of *Echinococcus multilocularis* in the Alps: evidence that *Microtus arvalis* is a limiting factor. Parasitology 16, 1–10.

Hanosset, R., Saegerman, C., Adant, S., Massart, L., Losson, B., 2008. *Echinococcus multilocularis* in Belgium: prevalence in red foxes (*Vulpes vulpes*) and in different species of potential intermediate hosts. Vet. Parasitol. 151, 212–217.

Kern, P., Bardonnet, K., Renner, E., Auer, H., Pawlowski, Z., Ammann, R.W., Vuitton, A.D., Kern, P., Registry, European Echinococcosis, 2003. European echinococcosis Registry: human alveolar echinococcosis, Europe, 1982–2000. Emerg. Infect. Dis. 9, 343–349.

Keutgens, A., Simoni, P., Detrembleur, N., Frippiat, F., Giot, J.-B., Spirlet, F., Aghazarian, S., Descy, J., Meex, C., Huynen, P., Melin, P., Müller, N., Gottstein, B., Carlier, Y., Hayette, M.-P., 2013. Fatal alveolar echinococcosis of the lumbar spine. J. Clin. Microbiol. 51, 688–691.

Landen, S., Van de Sande, J., Berger, P., Ursaru, D., Baert, J., Delugeau, V., 2013. Alveolar echinococcosis in a Belgian urban dweller. Acta Gastroenterol. Belg. 76, 317–321.

Litzroth, A., Truyens, C., 2015. Echinococcose (*Echinococcus* spp.), zoönosen en vector-overdraagbare ziekten, Epidemiologische surveillance in België, 2013 en 2014. In: Dienst epidemiologie van infectieziekten, volksgezondheid en surveillance. Brussel, België.

Losson, B., Mignon, B., Brochier, B., Bauduin, B., Pastoret, P.P., 1997. Infestation du Renard roux (*Vulpes vulpes*) par *Echinococcus multilocularis* dans la Province de Luxembourg (Belgique): résultats de l'enquête effectuée entre 1993 et 1995. Ann. Med. Vet. 141, 149–153.

Losson, B., Kervyn, T., Detry, J., Pastoret, P.P., Mignon, B., Brochier, B., 2003. Prevalence of *Echinococcus multilocularis* in the red fox (*Vulpes vulpes*) in southern Belgium. Vet. Parasitol. 117, 23–28.

Mathy, A., Hanosset, R., Adant, S., Losson, B., 2009. The carriage of larval *Echinococcus multilocularis* and other cestodes by the musk rat (*Ondatra zibethicus*) along the Ourthe river and its tributaries (Belgium). J. Wildl. Dis. 45, 279–287.

Oksanen, A., Siles-Lucas, M., Karamon, J., Possenti, A., Conraths, F.J., Romig, T., Wysocki, P., Mannocci, A., Mipatrini, D., La Torre, G., Boufana, B., Casulli, A., 2016. The geographical distribution and prevalence of *Echinococcus multilocularis* in animals in the European Union and adjacent countries: a systematic review and meta-analysis. Parasit. Vectors 9, 519.

Otero-Abad, B., Torgerson, P.R., 2013. A systematic review of the epidemiology of echinococcosis in domestic and wild animals. PLoS Negl. Trop. Dis. 7, e2249.

Pétavy, A.F., Deblock, S., Prost, C., 1990. Epidemiology of alveolar echinococcosis in France. Intestinal helminths in the red fox (*Vulpes vulpes* L.) from Haute-Savoie. Ann. Parasitol. Hum. Comp. 65, 22–27.

Romig, T., Deplazes, P., Jenkins, D., Giraudoux, P., Massolo, A., Craig, P.S., Wassermann, M., Takahashi, K., de la Rue, M., 2017. Ecology and life cycle patterns of *Echinococcus* species. Adv. Parasitol. 95, 213–314.

Tigre, W., Deresa, B., Haile, A., Gabriël, S., Victor, B., Van Pelt, J., Devleesschauwer, B., Vercruysse, J., Dorny, P., 2016. Molecular characterization of *Echinococcus Granulosus s.l.* cysts from cattle, camels, goats and pigs in Ethiopia Vet. Parasitol. 215, 17–21.

Torgerson, P.R., Kelle, K., Magnott, M., Ragland, N., 2010. The global burden of alveolar echinococcosis. PLoS Negl. Trop. Dis. 4, e722.

Umhang, G., Woronoff-Rhen, N., Combes, B., Boué, F., 2011. Segmental sedimentation and counting technique (SSCT): an adaptable method for qualitative diagnosis of Echinococcus multilocularis in fox intestines. Exp. Parasitol. 128, 57–60.

Umhang, G., Richomme, C., Boucher, J.M., Guedon, G., Boué, F., 2013. Nutrias and muskrats as bioindicators for the presence of *Echinococcus multilocularis* in new endemic areas. Vet. Parasitol. 197, 283–287.

van der Giessen, J., Claes, L., 2016. Spread of *Echinococcus multilocularis* in Foxes in the Netherlands and Belgium. Wildlife crossing borders, BWDS and DSWH Symposium.

Van Gucht, S., Van Den Berge, K., Quataert, P., Verschelde, P., Le Roux, I., 2009. No emergence of *Echinococcus multilocularis* in foxes in Flanders and Brussels anno 2007-2008. Zoonoses Public Health 57, 65–70.

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

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