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Taxonomy, phylogeny and molecular epidemiology of *Echinococcus multilocularis*: From fundamental knowledge to health ecology

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ABSTRACT

Alveolar echinococcosis, caused by the tapeworm *Echinococcus multilocularis*, is one of the most severe parasitic diseases in humans and represents one of the 17 neglected diseases prioritised by the World Health Organisation (WHO) in 2012. Considering the major medical and veterinary importance of this parasite, the phylogeny of the genus *Echinococcus* is of considerable importance; yet, despite numerous efforts with both mitochondrial and nuclear data, it has remained unresolved. The genus is clearly complex, and this is one of the reasons for the incomplete understanding of its taxonomy. Although taxonomic studies have recognised *E. multilocularis* as a separate entity from the *Echinococcus granulosus* complex and other members of the genus, it would be premature to draw firm conclusions about the taxonomy of the genus before the phylogeny of the whole genus is fully resolved. The recent sequencing of *E. multilocularis* and *E. granulosus* genomes opens new possibilities for performing in-depth phylogenetic analyses. In addition, whole genome data provide the possibility of inferring phylogenies based on a large number of functional genes, i.e. genes that trace the evolutionary history of adaptation in *E. multilocularis* and other members of the genus. Moreover, genomic data open new avenues for studying the molecular epidemiology of *E. multilocularis*: genotyping studies with larger panels of genetic markers allow the genetic diversity and spatial dynamics of parasites to be evaluated with greater precision. There is an urgent need for international coordination of genotyping of *E. multilocularis* isolates from animals and human patients. This could be fundamental for a better understanding of the transmission of alveolar echinococcosis and for designing efficient healthcare strategies.

1. Introduction

Echinococcus multilocularis Leuckart 1863, commonly known as the fox tapeworm, is a cestode from the family Taeniidae, which contains four genera: *Taenia* Linnaeus 1758 and *Echinococcus* Rudolphi 1801, *Hydatigera* Lamarck, 1816 and *Versteria* Lavikainen, Iwaki, Haukisalmi, Konyaev, Oku, Okamoto and Ito, 2013 (Nakao et al., 2013). Using a geological-event calibration point and relaxed-clock approach, Knapp et al. (2011) suggested that taeniids started to diverge in the late Miocene (11.2 million years ago: Ma), while the genus *Echinococcus* began to diversify somewhat later, about 5.8 Ma, at the very end of the Miocene (Fig. 1). In humans, the metacestode stage of *E. multilocularis* causes an infectious disease called alveolar echinococcosis, which has a high mortality rate and is one of the most threatening emerging zoonoses in Eurasia. *E. multilocularis* has a Holarctic distribution, encompassing regions located north of the tropic of Cancer, but is generally not present in polar regions (Davidson et al., 2012; Nakao et al., 2013); polar *E. multilocularis* populations have only been found in the Svalbard Archipelago, Norway (Henttonen et al., 2001). The distribution of the parasite extends longitudinally from North America to Eurasia and includes several highly endemic areas in Asia (Russia, China).

E. multilocularis is characterised predominantly by its sylvatic life cycle, which involves two mammalian hosts: rodents as intermediate hosts (IH), and wild canids as definitive hosts (DH). In Europe, the red fox (*Vulpes vulpes*) is one of the main definitive hosts, while various *Microtus* and other arvicolid rodents, but occasionally also lagomorphs, act as intermediate hosts (Davidson et al., 2012; Vuitton et al., 2003). Regionally, *E. multilocularis* can reach relatively high prevalences in red foxes, recorded also in newly-detected areas in the last decade (Bagrade et al., 2009; Bruzinskaite et al., 2007; Moks et al., 2005). Following the relatively recent urbanisation of some red fox populations, *E. multilocularis* has also

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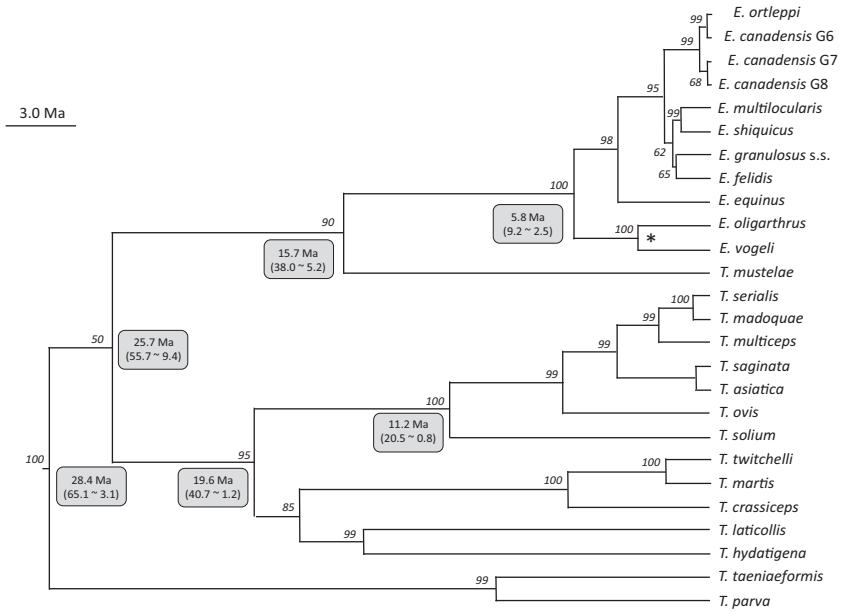

Fig. 1. A relaxed-clock chronogram of taeniid tapeworms reconstructed by Bayesian inference (BEAST) using an exon data set from *rpb2*, *pepck* and *pold* genes. Values at each node are posterior probabilities (%). Grey rectangles indicate estimated ages of representative nodes (Ma, for million years ago), with the 95% HPD (the highest posterior density) shown in parentheses. An asterisk denotes the node representing the most recent common ancestor of *Neotropical Echinococcus* spp., used as a calibration point for time estimates (Knapp et al., 2011).

been reported from a number of European cities, prompting considerable concern for public health (e.g. Deplazes et al., 2004; Hofer et al., 2000; Plumer et al., 2014; Laurimaa et al., 2015).

The intermediate hosts of *E. multilocularis* become infected by ingesting parasite eggs, after which the parasite larvae colonise internal organs, usually the liver, where lesions develop. The parasite lesions can cause severe health problems due to their tumour-like growth if they are not diagnosed and treated early. While rodents are the main IH, humans represent accidental intermediate hosts, as do certain other species, such as captive monkeys, beaver, muskrat and nutria (Gottstein et al., 2014; Rehmann et al., 2005; Umhang et al., 2013; Vuitton et al., 2003).

The diversity of mammalian hosts and its large geographical distribution have often inspirited taxonomists, who have historically and more recently proposed different species denominations or sub-species descriptions. Here, we review the current state of knowledge concerning the taxonomy and phylogenetic position of *E. multilocularis* within its genus. Empirical observations were first based on morphological criteria, but have more recently also included genetic data. The genetic diversity of the parasite as an indicator of its spatial and temporal dynamics is also discussed.

2. What defines *E. multilocularis* as a species?

The study of taxonomy is an essential discipline permitting the identification, naming and classification of taxa – organisms with common characters – and species is the most fundamental taxon in the systematic classification. The question of whether the two types of echinococcosis, cystic and alveolar, were caused by the variants of the same or two different species, remained a mystery for a long period of time (Tappe et al., 2010). In the middle of 19th century, Rudolf Virchow described the morphological features of the *E. multilocularis* larval stage in human patients (Virchow, 1856). It differed from *Echinococcus granulosus* in the appearance of the alveolar colloid, its small vesicles containing gelatin, and the presence of relatively few protoscoleces, named in Virchow's description "young animals". The "multitude of small echinococcal vesicles" observed by Virchow provided the basis for the term "multilocular" to describe the appearance of the lesions and contrast with the unilocular metacestode stage development in *E. granulosus*. However, at that time, alveolar echinococcosis was suspected to be caused by an aberrant *Echinococcus* sp., and was only described as a distinct species in 1863, thanks to Leuckart's investigations, which provided the name *E. multilocularis*.

In 1954, Rausch and Schiller described a new species which infected sledge dogs and polar foxes (*Vulpes lagopus*), and microtine rodents on Saint Lawrence Island (Alaska) in the Bering Sea (Rausch and Schiller, 1954). The authors named this new species *E. sibiricensis* and suggested that as the larvae resembles closely the alveolar larva of *Echinococcus* sp. (=*E. multilocularis*) described in southern Europe and Russia. The taxon was later relegated by Vogel to the status of a possible sub-species *E. multilocularis sibiricensis*, due to slight differences in morphology and life cycle (Vogel, 1957). However, Rausch and Vogel were later able to prove the conspecific status of *E. multilocularis* and *Echinococcus sibiricensis* by exchanging eggs of the parasite and infecting the natural intermediate hosts of their research areas. The name introduced by Leuckart for the larval stage was given priority (reviewed in Tappe et al., 2010). However, molecular analysis of isolates from metacestodes originating from adult worms infecting arctic foxes in Alaska and Svalbard (see part 4) have again raised the possibility of a "polar species" (Knapp et al., 2007, 2012).

In 1961 Shults described another subspecies *Alveococcus* (=*Echinococcus*) *multilocularis kazakhensis*, from Kazakhstan and Russia, which exhibited multilocular development but infected the domestic dog as DH, and sheep and pigs as IH, thus differing from the nominal *E. multilocularis* which infects rodents as IH (from Kumaratilake and Thompson, 1982). However, Rausch and Nelson (1963) suggested that the parasite could be *E. granulosus*.

More recently, a further subspecies, *E. m. russicensis,* was described in corsac foxes (*Vulpes corsac*) from Inner Mongolia, China (Tang, 2007), exhibiting slight morphological differences to the uterine structure in comparison with the nominal *E. multilocularis*.

Proposals have been made to place *E. multilocularis* into a separate genus *Alveococcus*, but these have not gained gen-

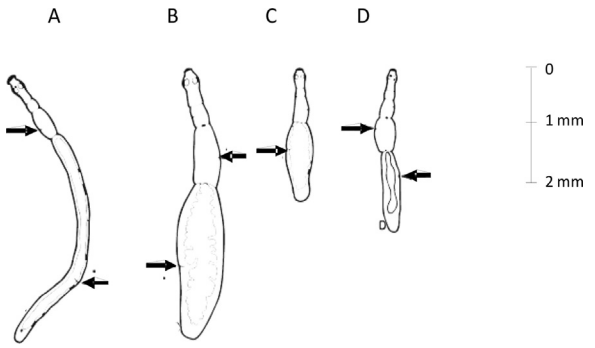

Fig. 2. Morphological differences between four *Echinococcus* species, (A) *E. vogeli*, (B) *E. granulosuss.s.*, (C) *E. multilocularis* and (D) *E. ortleppi*. The arrows indicate the genital pores (adopted from Thompson and McManus, 2002).

eral acceptance; the following reasoning is cited from Rausch and Nelson (1963): "Because of the unusual morphological and biological peculiarities of this cestode, a separate genus, *Alveococcusabuladze,* 1959, has been proposed (Lukashenko and Zorikhina, 1961). While this proposal would seem to have at least as much merit as the establishment of separate genera for species of Taenia s.l., it can be rejected on similar grounds (Rausch, 1963). Morphological differences in the strobilar stage are not significant above the species level; the structure of the larva is distinctive, but, in view of the pleomorphism exhibited by the larval *E. granulosus* and of the inadequate knowledge of the larval stages of some other species of *Echinococcus*, it seems premature to erect a new genus on this basis." Nonetheless, the name *Alveococcus multilocularis* has not vanished completely and has been used in a few publications.

Based on morphology, four species are currently recognised in the genus *Echinococcus*: *E. multilocularis*, *E. granulosus*, *E. vogeli* and *E. oligarthrus* (Eckert et al., 2001). Additional species currently proposed are *E. shiquicus* (Xiao et al., 2006), and *E. felidis*, *E. ortleppi*, *E. equinus*, *E. canadensis* and *E. intermedius*, which are currently classified as *E. granulosus sensu lato* (*s.l.*) (Thompson and McManus, 2002a,b; Hüttner et al., 2008; Thompson 2008; Saarma et al., 2009; Nakao et al., 2010; Nakao et al., 2013). Morphologically, the *Echinococcus* species have relatively similar adult stages. However, the adult stage of *E. multilocularis* is smaller than that of *E. granulosus sensu stricto* (s.s.), and *E. vogeli* (Fig. 2), though *E. oligarthrus*, and the *E. multilocularis* both exhibit a large number of segments and a vaselike uterus. The larval or metacestode stage allows *E. multilocularis* to be more easily distinguished from other species. *E. multilocularis* has a multilocular development, *E. granulosus* a unicystic development and *E. vogeli* and *E. oligarthrus* (the Neotropical species) a polycystic development (Eckert et al., 2001). In contrast to *E. granulosus,* which only has endogenous proliferation of the germinal layer of the metacestode, *E. multilocularis* has both endogenous and exogenous proliferation (Eckert et al., 2001; Kumaratilake and Thompson, 1982). In summary, the characteristics that separate *E. multilocularis* from other *Echinococcus* species are: its large number of segments and a vaselike uterus in the adult stage; a multilocular or multivesicular metacestode development; an endogenous and exogenous proliferation of the germinal layer; and a life cycle involving wild rodents.

To conclude, one can say that the first taxonomy considerations about *E. multilocularis* were established on morphological criteria. Only a century after Virchow's description, *E. multilocularis* was fully recognised as the causative agent of alveolar echinococcosis and the experiments conducted by Rausch and others on the different stages of *Echinococcus* species have permitted to avoid multiplication of species descriptions. Thus, the taxon status of *E. multilocularis* is undeniable.

3. Phylogenetic position of *E. multilocularis* in the genus

Next to taxonomy, phylogenetics is another, more recent discipline investigating relationships among organisms. Both disciplines have largely converged by now. Phylogenetic reconstruction is an essential tool for understanding parasite evolution and genetic diversity, and for recognizing and diagnosing different *Echinococcus* parasites.

The major question concerning the position of *E. multilocularis* in the genus is whether it is a separate entity from the *E. granulosus* complex (i.e. genotypes G1-G10) – as suggested by taxonomic studies – or not. To date, there is no unequivocal answer to this question. On one hand, a number of studies have placed it separately from the *E. granulosus* complex and are thus in accordance with classical taxonomy. On the other hand, some studies have placed *E. multilocularis* in the midst of the *E. granulosus* genotypes, rendering the *E. granulosus* complex paraphyletic (Fig. 3A–C) and contradicting the classical taxonomy of the genus. In the early days of *Echinococcus* phylogenetics, analyses using mitochondrial DNA (mtDNA) data (Bowles et al., 1992; Bowles and McManus, 1993) indicated that *E. multilocularis* was different from *E. granulosus*. Many recent studies based on mtDNA (e.g. Lavikainen et al., 2006; Le et al., 2002; McManus, 2002; Moks et al., 2008) and nuclear data (Bart et al., 2006; Haag et al., 2009; Lavikainen et al., 2003; Saarma et al., 2009) support this view. However, there are also studies that postulate a paraphyletic relationship between *E. multilocularis* and *E. granulosus* based both on mtDNA (Hüttner et al., 2008; Lavikainen et al., 2003; Nakao et al.,2007; Nakao et al., 2013; Obwaller et al., 2004; Thompson et al., 2006), and nuclear data (e.g. Knapp et al., 2011). Even analyses relying on data derived from a large portion of the mitochondrial genome (Nakao et al., 2007), and more than five thousand basepairs of nuclear DNA (Saarma et al., 2009) have been unable to resolve the conflict. Based on analysis of 12 mitochondrial genes (Nakao et al., 2007) *E. multilocularis* was positioned, together with *E. shiquicus*, in the middle of different *E. granulosus* genotypes (Fig. 3A). Paraphyly has also been observed in other studies involving nuclear genes (Knapp et al., 2011; Nakao et al., 2013). However, a different phylogeny was inferred by Saarma et al. (2009) based on 5 nuclear genes (5086 bp) which placed *E. multilocularis* clearly separate from the *E. granulosus* complex (i.e. not paraphyletic) (Fig. 3B). Thus, it appears that further genetic analysis is needed to resolve the phylogeny of *Echinococcus* and the position of *E. multilocularis* in the genus.

The availability of nearly complete genomes of *E. multilocularis* and *E. granulosus* (Tsai et al., 2013) offers the possibility of inferring the *Echinococcus* phylogeny with a much larger number of genes than has previously been used. The 115 megabase genome of *E. multilocularis* comprises 9 chromosomes, of which 10345 genes have been identified. As in other sequenced tapeworms such as *E. granulosus*, *Taenia solium*, *Hymenolepis microstoma* and the trematode *Schistosoma mansoni*, the gene repertoire for metabolic functions is highly reduced in *E. multilocularis*, whereas the capacity to absorb nutrients from the host is increased. Concerning the process of reproduction in cestodes and trematodes, the two chromosomes of *E. multilocularis* correspond to the *Z* sex chromosome in *S. mansoni* (a species representing unique sexual dimorphism in the flatworm group), prompting discussion about the divergence and origin of sexual dimorphism and hermaphroditism in flatworms.

With the availability of next generation sequencing technologies (NGS), huge amounts of genetic data can be obtained as never before. Future sequencing projects focusing on other *Echinococcus* species genomes and groups of targeted genes (e.g. genes involved in parasite development, survival and metabolic features) can open the way to deeper investigations of *Echinococcus* phylogeny and many other aspects of the evolutionary history of these parasites.

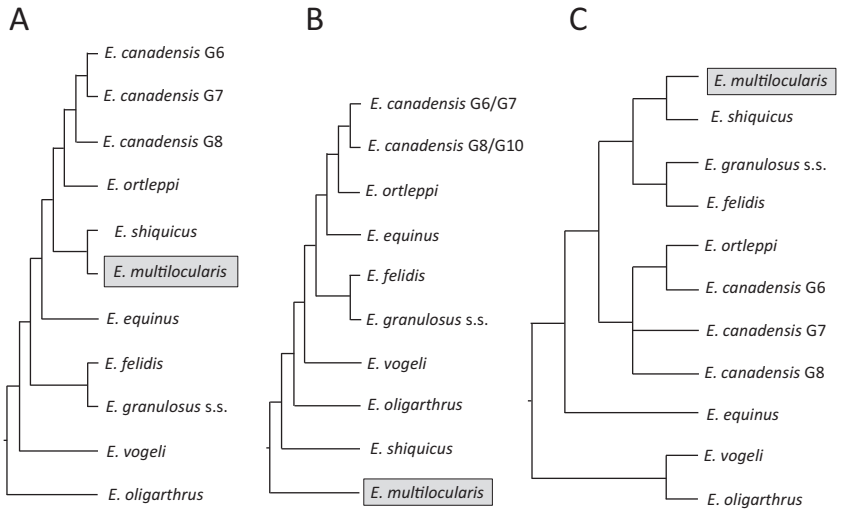

Fig. 3. The summary of recent publications describing phylogenetic relationships among *Echinococcus* spp. (A) A phylogeny based on the DNA sequences of mitochondrial genes (Nakao et al., 2007; Hüttner et al., 2008). (B) A phylogeny based on the DNA sequences of nuclear protein-coding genes (Saarma et al., 2009). (C) A phylogeny based on exon sequences of nuclear protein-coding genes (Knapp et al., 2011).

4. Geographical genotypes and molecular epidemiology

4.1. Diversity of mtDNA

The intra-specific diversity of *E. multilocularis* was first described by sequencing coding or non-coding fragments of mtDNA. These analyses revealed very low diversity amongst geographically distinct isolates: nucleotide diversity in *E. multilocularis* was estimated to be about 10 times lower than in *E. granulosus* s.l. (Haag et al., 1997). From early mitochondrial studies, two geographical genotypes (haplotypes) were described among European isolates (M1) and Chinese, Japanese, Alaskan and North American isolates (M2) (Bowles et al., 1992; Bowles and McManus, 1993; Okamoto et al., 1995). Based on sequencing of 3 mitochondrial targets, Nakao and co-workers have since described 17 regional haplotypes from 76 isolates sampled in Europe (5 haplotypes), Asia (10 haplotypes) and North America (2 haplotypes) (Nakao et al., 2009). Moreover, one North American and two Asian haplotypes co-exist on St Lawrence Island in the Bering Sea; a geographical border between Asia and North America that could be considered a passageway for *E. multilocularis*. Such geographical diversity and differentiation amongst *E. multilocularis* populations could be explained by the movements of mammals during the Pleistocene along with local or regional isolation of the parasite in glacial refugia during multiple glaciation events (Nakao et al., 2009) and bottleneck effects (observed for *E. shiquicus* on the Qinghai-Tibetan Plateau) (Ma et al., 2012). Nakao and co-workers suggested an introduction of *E. multilocularis* by foxes into Europe in the late Pleistocene period, between 130,000 and 10,000 years ago and its spread in Asia and North America across the Bering Sea was estimated to have occurred during the Holocene period (<10,000 years ago). In Europe the core area of *E. multilocularis* is in the Alpine arch region, comprising Switzerland, southern Germany, eastern France and Austria and this has been proposed as the parasite's historical endemic area (Eckert and Deplazes, 2004). However, to trace back the spatial dynamics of the parasite from this historical endemic region, more discriminant molecular markers are required.

4.2. Diversity according to nuclear data: the EmsB microsatellite

The EmsB microsatellite was selected from among 17 *E. granulosus* loci to assess genetic diversity in *E. multilocularis* (Bart et al., 2006). This multi-locus microsatellite is highly polymorphic among geographically distinct samples (Fig. 4) compared to other markers described, providing a distinct $(CA)_n$ $(GA)_n$ pattern. EmsB and the single-locus EmsJ, EmsK and NAK1 microsatellites were assessed for polymorphism using 76 *E. multilocularis* isolates from different locations (Europe, North America and Asia) (Knapp et al., 2007; Nakao et al., 2003). Highest polymorphism was obtained with EmsB (29 profiles) in comparison to NAK1 (7 genotypes) and EmsJ/EmsK combined together (3 genotypes). The discriminatory power of EmsB was assessed at different spatial scales (micro-local, local and regional scales). The circulation of a single profile was described among rodents from size-limited fields, in Switzerland and Alaska (Knapp et al., 2007), allowing conclusions to be drawn about the discriminatory power of the marker without overestimating diversity at this spatial scale.

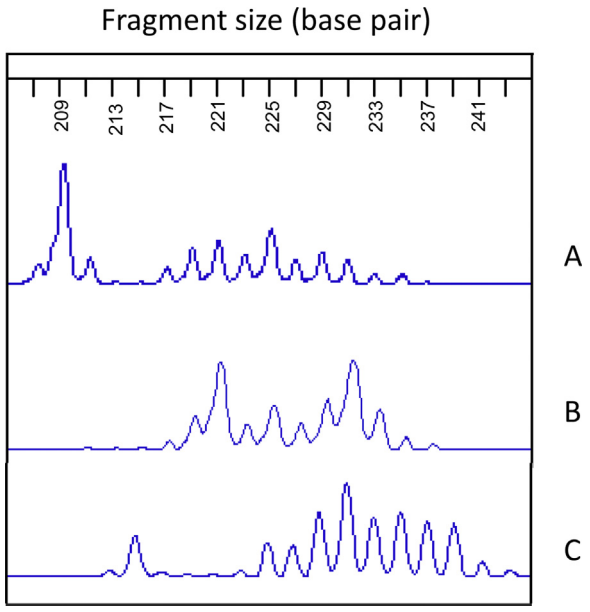

Fig. 4. Electropherogram of the microsatellite EmsB fluorescent PCR products, with profiles from (A) an Alaskan sample, (B) a Chinese sample and (C) a French sample.

On a studied area of 900 m^2, located in an endemic region in France, where fox density is 3 to 4 individuals per km^2 (win-

ters 2003–2004 and 2005–2006), 6 EmsB profiles were described among 79 *E. multilocularis* positive foxes (Knapp et al., 2008). At the time, this was the first description of genetic diversity in *E. multilocularis* worms isolated at a local scale.

The genetic diversity of the parasite has also been assessed more widely in Europe using EmsB (Knapp et al., 2009). This allowed the expansion of the parasite to be traced from its historical endemic area (in the Alpine Arch), where genetic diversity is relatively high, to newly described endemic areas (e.g. northern Poland, Slovakia, Czech Republic), where diversity is lower. Five foci in the historical endemic areas and 4 foci in the new endemic areas were investigated by genotyping 571 worms from 123 red foxes, resulting in the description of 32 EmsB profiles. This study revealed a predominance of certain EmsB profiles at local scales – mostly in new endemic regions – due to founder events.

The contamination of new endemic regions could thus be investigated thanks to the data collected on EmsB polymorphism by Bart et al. (2006) and the different research teams using the EmsB marker. On the Svalbard archipelago, where the parasite was described in 1999, a circumpolar origin – presumably due to the introduction of a Russian rodent in the 1960s (Henttonen et al., 2001) – rather than a European origin involving arctic fox was first highlighted by EmsB data (Knapp et al., 2012). In Canada, a metacestode lesion in a dog was genotyped, and a European origin for the parasite was suggested by analysis of mtDNA (Jenkins et al., 2012). However, an EmsB-based genotyping study should be performed to confirm these findings. In France, which is an endemic area in Europe, a total of 383 worms from 128 foxes were analysed in the historical endemic eastern part and 2 new foci in the northern and the western parts (Umhang et al., 2014). The authors hypothesised that the range of the parasite may have expanded in two episodes of dispersal: first from the eastern part to the north, and subsequently from the western part to the west.

Thanks to these studies, a collection of EmsB data has been established, and a large number of genotyped and geo-localised samples are now available for further investigation. Studies of genetic diversity among strains infecting different intermediate hosts – primarily humans, but also rodents and aberrant hosts such as captive monkeys or dogs – are urgently required in order to better understand routes of contamination and to link pathogenic strains with dominant profiles. To complement the large number of genotyping studies already performed, further in vitro and in vivo studies on different *E. multilocularis* strains sampled from humans and other animals, representing various pathogenic phenotypes, should be performed to shed light on classifications according to pathogenicity (Bartel et al., 1992).

The epidemiological contrast between areas known to be hyper-endemic, such as China (Torgerson et al., 2010), and those with low human infection rate, such as North America (Yamasaki et al., 2008) provides a good opportunity to investigate differences in *E. multilocularis* pathogenicity. Epidemiological studies should be based on the establishment of national reference centres to record detailed information for all alveolar echinococcosis cases, perform systematic strain genotyping and epidemiological investigations such as establishing the risk of infection associated with activities such as agriculture, owning a domestic garden, contact with wild animals, domestic animal deworming and others (Kern et al., 2003).

4.3. Copro-sample analysis

EmsB genotyping has usually been performed on adult worms or metacestode samples. However, this requires hosts sampling and necropsy, which is time-costly, administratively laborious and often considered to be unethical. *E. multilocularis* is relatively easy to detect in faecal samples of definitive hosts using various molecular techniques, e.g. standard-PCR (Laurimaa et al., 2015), multiplex-PCR (Trachsel et al., 2007), real-time PCR (Knapp et al., 2014) or real-time multiplex-nested PCR (Dinkel et al., 2011). Recently, a newly developed non-invasive genetic method was published by Laurimaa et al. (2015) which allows both *E. multilocularis* and its host species to be identified from carnivore faecal samples. The method is also highly sensitive: the presence of *E. multilocularis* can even be detected when only a single parasite egg is present in the sample.

Current PCR-based detection methods only permit the presence or absence of *E. multilocularis* to be established and do not allow parasite diversity to be investigated. However, studies based on isolation of eggs and total parasite copro-DNA extraction can provide information about parasite diversity and this is of importance because worms with different EmsB profiles have been described from the intestines of individual foxes (Knapp et al., 2009; Knapp et al., 2008). Micromanipulations are necessary to isolate individual and intact eggs using microscopy. Subsequent DNA amplification followed by genetic analysis on this small quantity of DNA is currently the only way to obtain accurate genetic diversity data from copro-samples. In such analyses, the identity of the parasite host should also be evaluated, especially in cases of positive copro-samples from domestic animal end-hosts living in close contact with humans. In addition to the PCR-based methods described above, microsatellite analysis and DNA-barcoding can also be performed in this context (e.g. Benson et al., 2012; Galan et al., 2012).

5. Conclusion

While classical taxonomy places *E. multilocularis* clearly separate from other species in the *Echinococcus* genus, including the *E. granulosus* complex, its phylogenetic position in the genus requires further studies with an extended repertoire of nuclear markers and sub-species have to be further studied in this way with comparison to ecological and epidemiological data to validate them as valid taxa or not. The recently published nearly complete genome sequences of *E. multilocularis* and some of its relatives, such as *E. granulosus* and *T. solium* (Tsai et al., 2013), hold great promise for fine tuning its phylogenetic position, and better understanding its evolutionary history, including specific adaptations to its host species. Detoxification mechanisms and the capacity to evade intermediate host immune systems with partial control of the infection represent interesting targets for finding better solutions for therapy (Vuitton and Gottstein, 2010). From an epidemiological perspective, it is interesting to note that *E. multilocularis* is a taxon with relatively low genetic diversity. This characteristic is due on one hand to the species' recent evolutionary history and on the other to its hermaphrodite reproduction, which is common to the entire Taeniidae family and reflects self-fertilisation with autogamy or geitonogamy, when gametes are from separated but genetically identical organisms (Haag et al., 2007). The species also presents contrasting epidemiological characteristics throughout its range, with a hyper-endemic focus in China and rare occurrence in North America in humans, even though the adult tapeworms are highly prevalent in carnivores in both areas (Storandt et al., 2002; Vaniscotte et al., 2011). Even though genotyping is easily performed on the EmsB microsatellite by fragment size analysis, additional highly polymorphic microsatellites or SNP markers are needed to assess genetic diversity. The methodology for analysis of these loci should be standardised, so that data from different sources can be easily compared. Moreover, accumulated data especially on EmsB, should be gathered into a common public database in order to advance international cooperation in epidemiological studies of this dangerous zoonotic disease. On top of genotyping studies, epidemiological data should be collected for each human

case, based on the rules set by the existing national reference centres. The FrancEchino-Alveolar Echinococcosis National Reference Centre in Besançon, France, or the Robert Koch Institute in Berlin, Germany have already conducted health monitoring for several decades. These reference centres investigate population risk factors, which were highlighted by European collaboration programs such as the EurEchinoReg project, based on collaboration of western and central European countries (Kern et al., 2003). Moreover, eco-epidemiological projects associated with genotyping programs in Europe (especially in newly endemic areas of Eastern Europe) and Asia are needed to further study the diversity of the parasite in order to investigate its spatio-temporal patterns and emerging or re-emerging status. In this way, lesion phenotypes, genetic aspects and epidemiological context could be studied in multidisciplinary and long-term monitoring projects (Lindenmayer et al., 2011), to better understand the epidemiology of alveolar echinococcosis and steadily move toward effective control of the multifaceted tapeworm parasite.

Acknowledgments

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