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Elucidating the spread of the emerging canid nematode *Angiostrongylus vasorum* between Palaearctic and Nearctic ecozones

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ABSTRACT

Angiostrongylus vasorum is an emerging parasite that is currently distributed through Western Europe and parts of South America. An isolated population is also present in Newfoundland, Canada. This presents a risk of onward spread into North America, but its origin is unknown. To ascertain the phylogeographic relationships and genetic diversity of *A. vasorum* within the western Palaearctic and eastern Nearctic ecozones, a total of 143 adult and larval nematode specimens were collected from foxes (*Vulpes vulpes*) and dogs (*Canis lupus familiaris*) in Canada, Denmark, France, Germany, Ireland, the Netherlands, Portugal and the United Kingdom, and a coyote (*Canis latrans*) in Canada. DNA was extracted and the second internal transcribed spacer and two mitochondrial loci were amplified and sequenced. Multiple haplotypes ($n = 35$) based on combined mitochondrial sequences (1078 bp) of the partial cytochrome oxidase subunit I (COI), large subunit ribosomal RNA (rrnL) and the complete nicotinamide adenine dinucleotide dehydrogenase 3 (NADH3) sequences, were observed throughout the Palaearctic countries sampled; however, only a single haplotype was observed for the Canadian *A. vasorum* population. The likely origin of *A. vasorum* in Newfoundland is therefore inferred to be within the western Palaearctic. There was no evidence of genetic segregation of parasites in dogs, foxes and coyotes, supporting the hypothesis that transmission occurs between wild and domestic canids. The transmission dynamics and population structure of this nematode are further discussed.

1. Introduction

Angiostrongylus vasorum (Nematoda; Metastrongyloidea) is a parasite of the heart and pulmonary circulation of domestic and wild canids, associated variously with respiratory disease, haemorrhage, and other signs, which can be severe or even fatal (Bourque et al., 2005; Koch and Willesen, 2009). Infection has also been reported in non-canid definitive host species (Torres et al., 2001; Dias et al., 2008; Patterson-Kane et al., 2008). This parasite is of increasing concern to the veterinary profession as clinical cases are reported in dogs in new geographic areas, especially given the difficulty in treating the more severe disease outcomes, and the limited options for prevention (Helm et al., 2010).

Within countries endemic for *A. vasorum*, the frequency of clinical disease in dogs appears to show great local variation, perhaps due to spatio-temporal heterogeneity in climatic conditions that influence larval and intermediate host dynamics (Morgan et al., 2009). For example, in the United Kingdom, focal populations of *A. vasorum* have historically been reported in Cornwall (Simpson and Neal, 1982), southern Wales (Patteson et al., 1987) and the South-east of England (Chapman et al., 2004). More recent geographic spread has been associated with both local expansion of known endemic foci, and sudden appearance in areas previously believed to be free of infection (Morgan et al., 2008; Helm et al., 2009; Yamakawa et al., 2009). Infected dogs imported into non-endemic countries such as Australia and the USA (Williams et al., 1985; Tebb et al., 2007) also pose a risk of long distance spread of this emerging disease (Morgan et al., 2005). The current known worldwide distribution for *A. vasorum* is broad and includes parts of Europe, Africa, and South America, but within this range occurrence also appears to be very patchy (Bolt et al., 1994; Morgan et al., 2005). This raises questions about the biogeographic relationships for this nematode between different continents. Phylogenetic analysis of *A. vasorum* specimens from Europe and South America revealed each to belong to distinct lineages, with the potential for these to be different species (Jefferies et al., 2009). *A. vasorum* from Uganda remains genetically uncharacterised (Bwangamoi, 1972).

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Interestingly, a very restricted distribution of *A. vasorum* has also been reported in North America, currently comprising only the eastern part of the Canadian island of Newfoundland (Smith and Threlfall, 1973; Jeffery et al., 2004). Suitable definitive and intermediate hosts are widely distributed across much of the Nearctic ecozone of North America, and the reason for such a restricted distribution remains unknown. A range of factors including increased pet travel and the capability of coyotes to act as reservoir hosts present considerable risks for the future spread of *A. vasorum* within Canada and potentially other regions of North America (Bourque et al., 2008). Establishment of effective management programmes to limit further spread of angiostrongylosis is therefore pertinent and overdue.

Currently, knowledge of the population dynamics and epidemiology of *A. vasorum* is very limited. Mitochondrial (mt) gene-based analyses have been reported to offer useful insights into the population structure and transmission of various nematode species (Hu et al., 2004), and may be useful in determining the phylogeographic relationships of *A. vasorum* populations, and also whether there is transmission between foxes and dogs. This study aimed to test the hypothesis that *A. vasorum* recently became established in Canada by determining the phylogeographic relationships among populations in the Nearctic (Newfoundland, Canada) and Palaearctic (various countries, Europe) using ribosomal and mt DNA sequence analysis. Additionally, genetic overlap between *A. vasorum* populations in dogs and foxes was used to assess the likelihood of interspecies transmission.

2. Materials and methods

2.1. Nematode isolates

A total of 143 nematodes were collected from 44 different hosts in Canada, Denmark, France, Germany, Ireland, the Netherlands, Portugal and the United Kingdom between the years 2005 and 2008. Adult *A. vasorum* were collected post-mortem from the lungs, pulmonary artery and/or right cardiac ventricle of red foxes (*V. vulpes*), dogs (*C. lupus familiaris*) and a coyote (*C. latrans*). First stage larvae (L1) were also collected from dog faeces after concentration using a modified Baermann's protocol (McGarry and Morgan, 2009). A single fifth stage larva (L5) was also isolated from the eye of a dog. Due to the difficulty in obtaining high numbers of nematodes from separate geographic locations, *A. vasorum* specimens were collected on the basis of availability and were therefore not statistically representative of each population sampled. Sampling locations are shown in Fig. 1 and full details of all isolates collected are given in Table 1.

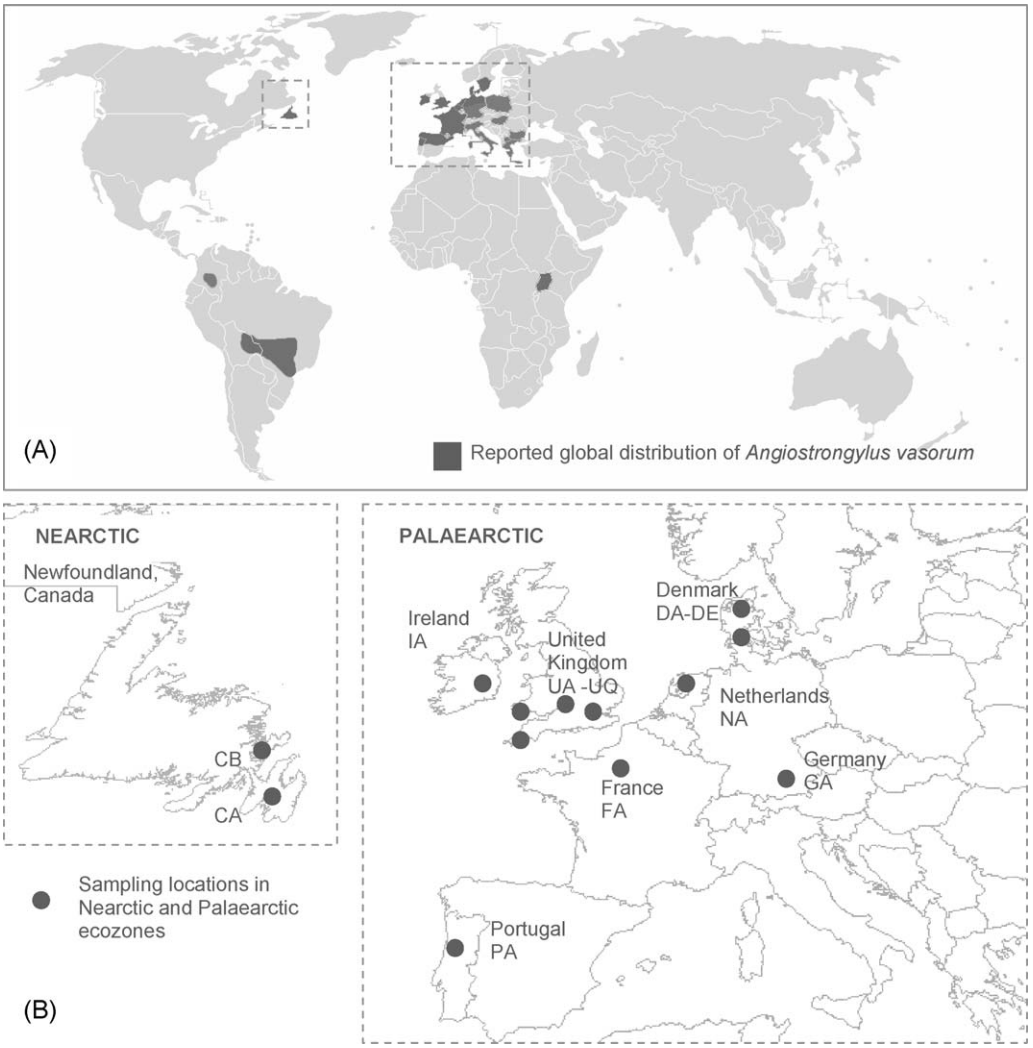

Fig. 1. Schematic representation of the reported worldwide distribution of *Angiostrongylus vasorum* (A) and locations of the main Palaearctic and Nearctic populations sampled for this study (B).