Veterinary Parasitology 212 (2015) 427–430

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Environmental determinants of the spatial distribution of *Mesocestoides* spp. and sensitivity of flotation method for the diagnosis of *mesocestoidosis*

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ARTICLE INFO

Article history:
Received 27 February 2015
Received in revised form 18 June 2015
Accepted 22 June 2015

Keywords:
Mesocestoides
Red fox
Spatial distribution
Geographic information system
Flotation method
Diagnosis

ABSTRACT

Mesocestoides spp. are zoonotic cestodes of wild and domesticated carnivores. Although the adult stages are relatively harmless intestinal parasites, the metacestode stages (tetrathyridia) can be responsible for life-threatening peritonitis and pleuritis in several species including dogs, cats, non-human primates and probably man. The aim of the present study was to reveal the spatial distribution pattern of *Mesocestoides* spp. in the most important final hosts, red foxes (*Vulpes vulpes*), to analyse the relationship of these patterns with landscape and climate by geographical information systems and to evaluate faecal flotation method for the detection of infection in the final host. Fox carcasses, representing 0.5% of the total fox population were randomly selected out of all the foxes of Hungary. The intestinal tract was examined by sedimentation and counting technique. The sensitivity of the flotation method was evaluated by the testing of the faecal samples of 180 foxes infected with *Mesocestoides* spp. The prevalence of infection was high in foxes (45.8%; 95% CI = 41.0–50.6%), and the parasite was detected in all areas of Hungary. The high prevalence of the parasite in foxes suggests that the infection might also be common in outdoor dogs and cats. *Mesocestoides* infection could not be detected in any of the foxes by flotation method indicating that the sensitivity of the method is less than 0.6%. Therefore, almost all canine and feline infections remain undetected in the veterinary practice. Based on the statistical analysis, the altitude was the only determinant of the spatial distribution of *Mesocestoides* spp. indicating that infections in carnivores including dogs and cats can be expected mainly in midland regions (150–750 m above sea level). It might be attributed to the altitude-dependent species richness and abundance of the intermediate and final hosts of the parasite.

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1. Introduction

Despite 60 years of research, the life cycle of *Mesocestoides* spp. remains incompletely described (Loos-Frank, 1991). Most likely, two intermediate hosts are required for completion of the life cycle. The first intermediate hosts are still unknown but might be oribatid mites or other arthropods (Loos-Frank, 1991). The invasive larvae (tetrathyridia) develop in a variety of vertebrates (amphibians, reptiles, birds and mammals). Small rodents are the most important second intermediate hosts in Europe (Loos-Frank, 1991). Ingested tetrathyridia complete the development in the intestine of the final hosts. Nevertheless, tetrathyridia may also penetrate the gut wall in final hosts, thus reaching the peritoneal cavity and causing life-threatening peritonitis (Boyce et al., 2011). Adult *Mesocestoides* spp. tapeworms are intestinal parasites of domestic and wild carnivores and are found globally with the exception of Australia (Boyce et al., 2011). The most important final hosts are red foxes (*Vulpes vulpes*) in Europe (Al-Sabi et al., 2014).

Mesocestoides spp. are zoonotic parasites. Human infections with adult *Mesocestoides* spp. are rare; nevertheless, several cases due to consumption of raw or undercooked snakes, chicken and wild game viscera have been described worldwide (Ohtomo et al., 1983; Eom et al., 1992; Fuentes et al., 2003). Pleural and peritoneal tetrathyridiosis has been described in non-human primates (Reid and Reardon, 1976; Guillot and Green, 1992; Fincham et al., 1995; Tokiwa et al., 2014). Although only three suspected human infections with tetrathyridial stages have been reported in man, human tetrathyridosis might have been erroneously reported as proliferative sparganosis caused *by Spirometra* spp. earlier (Lin et al., 1978; Beaver and Rolon, 1981; LaChance et al., 1983). In contrast with

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http://dx.doi.org/10.1016/j.vetpar.2015.06.021
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intestinal *Mesocestoides* infection, the larval forms can cause serious clinical signs and can lead to death in non-human primates and other animals; therefore, tetrathyridia might be responsible for similar disease in man. Due to globalization, the increased trade of exotic culinary products and the spread of different culinary traditions can be observed worldwide (Cascio et al., 2011). Therefore, the risk of human mesocestoidosis might increase in the future.

The aim of the present study was to reveal the spatial distribution pattern of *Mesocestoides* spp. in the most important final hosts, red foxes, to analyse the relationship of these patterns with landscape and climate by geographical information systems and to evaluate faecal flotation method for detection of infection in the final host.

2. Materials and methods

2.1. Sample collection and parasitological examination

From November 2013 to June 2014, carcasses of red foxes killed by hunters in connection with the rabies, *Trichinella* spp. and *Echinococcus multilocularis* monitoring program in Hungary were sent in individual plastic bags at +4 °C to the National Food Chain Safety Office of Budapest. Carcasses were individually labelled by the hunters with an identification number reporting the information on the nearest place to killing on the topographic map and the date of collection. If the nearest place to hunting was a human settlement, the fox position within a municipality (dots) was randomly chosen. Fox carcasses ($n = 413$), representing 0.5% of the total fox population of each county, were randomly selected out of all the foxes from 19 counties and from the Budapest municipality (covering 100% of the Hungarian territory, 93,029 km^2) (Tolnai et al., 2015). The intestinal tract was removed and stored at −20 °C. For safety reasons, the intestinal tract was frozen at −80 °C for 10 days before examination. After freezing, the gut was thawed at room temperature; the intestinal mucosa and content was collected and tested by sedimentation and counting technique according to a previously published protocol (Deplazes and Eckert, 1996). As the primary goal of the study was to assess the prevalence and intensity of *E. multilocularis* infection, the intensity of *Mesocestoides* infection was measured only by scoring (score 1: 1–10 scolicis; score 2: 11–20 scolicis; score 3: >20 scolicis). The parasites were identified on the basis of the characteristic morphological features described by Soulsby (1965). The sensitivity of the routine faecal flotation method for the detection of *Mesocestoides* infection was tested with the faecal samples of 180 foxes infected with *Mesocestoides* spp. Three grams of faeces from each fox were well mixed with modified Breza solution (one part of saturated $MgSO_4$ solution and one part of saturated $Na_2S_2O_3$ solution; specific gravity = 1.4) and strained through a sieve (0.6 mm mesh) by continuous and thorough mixing with a glass rod. The suspension was transferred to a 16-ml centrifuge tube and centrifuged at 2000 g for 3 min. The eggs were removed from the tube by touching the surface of the flotation fluid with the end of a glass rod, transferred to a microscopic slide and covered with a coverslip. The drops on the slide were scanned under 100 × magnification.

2.2. Geographic information system database, spatial and statistical analysis

The locality of origin of foxes and the infection status were marked on a point layer by the Quantum GIS 2.2 software (QGIS Team, 2012) as described by Tolnai et al. (2015). The radius around the locality of animal origin was restricted to 2.5 km, which was assumed to represent the average home range of foxes. The vector layers of altitude, mean annual temperature, annual precipitation, land cover and permanent water bodies were obtained from VÁTI Hungarian Nonprofit Ltd. (Budapest, Hungary) or created by Quantum GIS 2.2 on the basis of the georeferenced digital maps of the Hungarian Meteorological Service. The digitized home range and the vector data were used to calculate the altitude, mean annual temperature, annual precipitation, soil water retention and permeability, areas of land cover types, and the buffer zones of permanent water bodies as described by Tolnai et al. (2015). Linear and logistic regression analysis was performed with environmental parameter values and *Mesocestoides* infection to identify the environmental conditions which affected the prevalence of these pathogens in the area as described by Tolnai et al. (2015). The association between altitude and intensity of *Mesocestoides* infection was also analysed by the calculation of Spearman rank correlation coefficients. The difference between the prevalence of infection in foxes coming from the midland regions (150–750 m above sea level) and lowland regions (<150 m above sea level) was also compared by Fisher's exact test. Statistical analyses were carried out with MedCalc 12.7 (MedCalc Software, Ostend, Belgium).

Table 1
Prevalence (95% confidence interval) and intensity (±standard error) of *Mesocestoides* spp. infection in red foxes (*Vulpes vulpes*) in Hungary.

Counties	Prevalence (95% CI)	Intensity (±SE)[a]
Bács-Kiskun ($n = 26$)	4.0 (1.0–19.5)	1.5 (±0.3)
Baranya ($n = 23$)	8.7 (2.4–26.8)	1.7 (±0.2)
Békés ($n = 19$)	5.3 (0.1–26.0)	1.3 (±0.8)
Borsod-Abaúj-Zemlén ($n = 22$)	12.5 (4.3–31.0)	1.9 (±0.3)
Csongrád ($n = 19$)	5.3 (0.1–26.0)	1.3 (±0.1)
Fejér ($n = 22$)	18.2 (7.3–38.5)	1.7 (±0.2)
Győr-Sopron ($n = 18$)	27.8 (12.5–50.9)	1.3 (±0.2)
Hajdú-Bihar ($n = 23$)	26.1 (12.5–46.5)	1.2 (±0.2)
Heves ($n = 19$)	47.4 (27.3–68.3)	1.8 (±0.3)
Jász-Nagykun-Szolnok ($n = 21$)	28.6 (13.8–50.0)	1.5 (±0.2)
Komárom-Esztergom ($n = 11$)	63.6 (35.4–84.8)	2.3 (±0.3)
Nógrád ($n = 14$)	28.6 (11.7–54.6)	1.2 (±0.2)
Pest including Budapest ($n = 31$)	48.4 (32.0–65.2)	1.8 (±0.2)
Somogy ($n = 36$)	63.9 (47.6–77.5)	1.2 (±0.1)
Szabolcs-Szatmár-Bereg ($n = 25$)	0.0[b]	0.0 (±0.0)
Tolna ($n = 19$)	68.4 (46.0–84.6)	1.5 (±0.2)
Vas ($n = 15$)	66.7 (41.7–84.8)	1.4 (±0.2)
Veszprém ($n = 25$)	56.0 (37.1–73.3)	1.8 (±0.2)
-Zala ($n = 25$)	56.0 (37.1–73.3)	1.8 (±0.2)
Average ($n = 413$)	45.8 (41.0–50.6)	1.5 (±0.1)

[a] The intensity of *Mesocestoides* infection was measured only by scoring (score 1: 1–10 scolicis; score 2: 10–20 scolicis; score 3: >20 scolicis).
[b] Based on the calculation of freedom from infection, the county cannot be considered free of the parasite with a prevalence of 5–7% (95% CI).

3. Results and discussion

The prevalence and intensity of *Mesocestoides* infection was high in foxes (Table 1), nevertheless, the spatial distribution of the parasite was highly clumped (Fig. 1). The majority of infected foxes came from Transdanubia and the Northern Mountain Range, and the prevalence of infection was considerably lower in the lowland regions (Fig. 1).

Although the prevalence of *Mesocestoides* infection in foxes is highly variable in various European countries (4.7–89%) (Al-Sabi et al., 2014), knowledge of climatic and landscape influences on transmission of *Mesocestoides* spp. is very limited at present. In our study, multiple regression analyses revealed a positive relationship only with altitude ($P < 0.0001$). There was a positive correlation between altitude and intensity of infection ($r = 0.22$; $P < 0.0001$; $n = 413$). The difference between the prevalence of infection in foxes coming from the midland regions (150–750 m above sea level) and lowland regions (<150 m above sea level) was also significant (53% vs. 34%, $P < 0.0005$; $n = 155$ vs. 258). This is in line with the observation of other authors, i.e., these parasites are rare in

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