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Short Communication

Environmental determinants of the spatial distribution of *Angiostrongylus vasorum*, *Crenosoma vulpis* and *Eucoleus aerophilus* in Hungary

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ABSTRACT

Angiostrongylus vasorum, *Crenosoma vulpis* and *Eucoleus aerophilus* (syn. *Capillaria aerophila*) are the most important lungworm species infecting wild and domesticated canids in Europe. To investigate the spatial distribution of these parasites and the factors influencing their circulation in the fox populations, 937 red foxes (*Vulpes vulpes*) were tested for lungworm infection in Hungary. The prevalence of *A. vasorum*, *C. vulpis* and *E. aerophilus* infection was high (17.9, 24.6 and 61.7%). The distribution pattern of infection in foxes and the relationship of this pattern with landscape and climate was analyzed by geographic information system. Based on the analysis, the annual precipitation was the major determinant of the spatial distribution of *A. vasorum* and *C. vulpis* and *E. aerophilus*. Nevertheless, the mean annual temperature also influenced the distribution of *A. vasorum* and *E. aerophilus*. The positive relationship with annual precipitation and the negative relationship with mean annual temperature can be attributed to the sensitivity of larvae, eggs and intermediate hosts (snails and slugs) of lungworms for desiccation. Based on the highly clumped distribution of *A. vasorum* and *C. vulpis*, the indirect life cycle (larvae, slugs and snails) of these parasites seems to be particularly sensitive for environmental effects. The distribution of *E. aerophilus* was considerably less clumped indicating a lower sensitivity of the direct life cycle (eggs) of this parasite for environmental factors. Based on these results, lungworm infections in canids including dogs can be expected mainly in relatively wet and cool areas.

1. Introduction

Angiostrongylus vasorum, *Crenosoma vulpis* and *Eucoleus aerophilus* (syn. *Capillaria aerophila*) are the most important lungworm species infecting wild and domesticated canids in Europe (Morgan et al., 2005; Traversa et al., 2010). Recently, emergence of these parasites was observed in several European countries (Traversa et al., 2010). Red foxes (*Vulpes vulpes*) are the major reservoir hosts of these parasites in Europe. In the past decades, considerable population increase and urbanization of foxes could be observed on the continent (Deplazes et al., 2004). These changes increase the circulation of lungworms in human habitation and the risk for infection of dogs (Morgan et al., 2005). Moreover, as *E. aerophilus* is a zoonotic parasite (Lalosević et al., 2008), the contamination of human environment with this parasite is likely to increase opportunities for transmission from foxes to humans. To assess the risk for lungworm infections, it is important to know the spatial distribution pattern of these parasites

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and environmental factors (e.g., temperature, land cover) influencing this pattern. To investigate environmental determinants which can play a role in lungworm transmission in canids, red foxes were sampled and examined for lungworm infection in Hungary.

2. Materials and methods

2.1. Sample collection, parasite isolation and identification

From November 2013 to June 2014, carcasses of red foxes killed by hunters in connection with the *Trichinella* 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 labeled 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 as described by Conraths et al. (2003) (Fig. 1). Red fox carcasses ($n = 937$), representing more than 1% 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) (Széll et al., 2013). At necropsy, heart and lungs were removed. The heart, pulmonary arteries, the trachea, bronchi and larger bronchioles were cut open, and lungworms were washed to a large beaker containing warm water. Then, the lungs were sliced into small pieces, floated them in the beaker, squeezed them by hand, and the beakers were allowed to stand at room temperature overnight. Lung tissues were removed, supernatant was discarded, and the beaker was filled with water. Following sedimentation for 5 min, supernatant was discarded, and the beaker was filled with water. The last step was repeated twice. Finally, the sediment was transferred into Petri dishes, and worms were counted under a stereomicroscope using 10–63× magnification. The parasites were identified on the basis of the characteristic morphological features described by Soulsby (1965).

2.2. Geographic information system database, spatial and statistical analysis

The locality of origin of foxes and the number of worms were marked on a point layer by the Quantum GIS 2.2 software (Quantum GIS, 2014). The vector layers of altitude, land cover, permanent water bodies, protected areas, soil water retention and soil permeability, were obtained from VÁTI Hungarian Nonprofit Ltd. for Regional Development and Planning (Budapest, Hungary). The vector layers of the mean annual temperature and annual precipitation of 2013 were created and vector-based analysis was carried out by the Quantum GIS 2.2 software on the basis of the georeferenced digital map of the Hungarian Meteorological Service. The spatial resolution of the vector layers were 50–100 m. 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 (Staubach et al., 2001). Along permanent water bodies, a 100 m wide buffer zone was created, where the probability of the presence of final hosts was high. 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. Multiple linear regression analysis was performed with the log transformed environmental parameter values (see above) and the lungworm counts to identify the environmental conditions which affected the abundance of these parasites in the area. For each X variable, its best-fit coefficient with standard error and 95% confidence interval, and a P value testing whether the variable contributes significantly to the model was calculated. The variance inflation factor was also calculated to test for multicollinearity. The logistic regression analysis was also performed using non-transformed data. Statistical analyses were carried out with InStat 3.1 (GraphPad Inc., La Jolla, CA) and MedCalc 12.7 (Medcalc

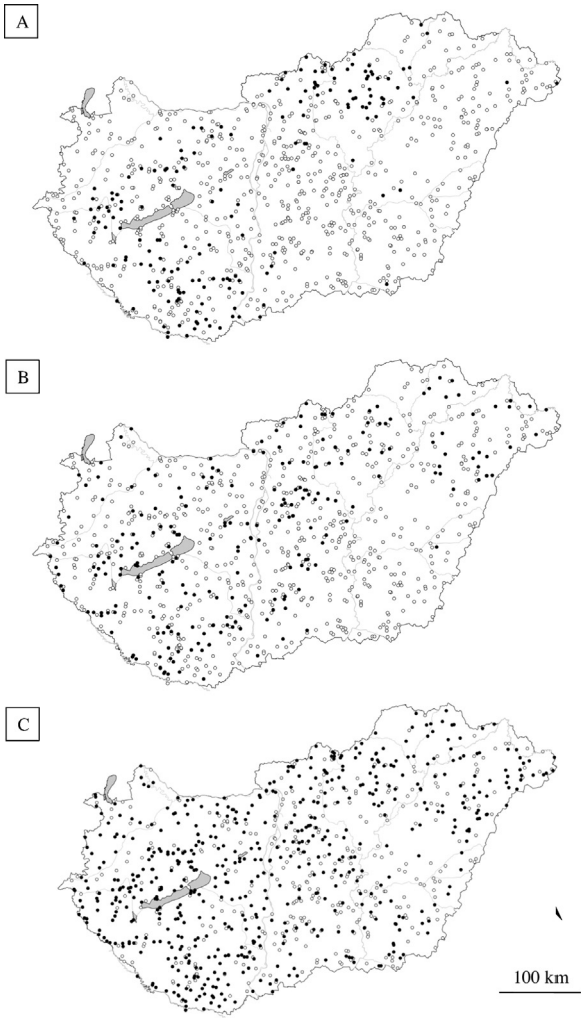

Fig. 1. Map of Hungary showing uninfected (white dots) and infected (black dots) red foxes (*Vulpes vulpes*) sampled from 2013 to 2014. Panel A. *Angiostrongylus vasorum*. Panel B. *Crenosoma vulpis*. Panel C. *Eucoleus aerophilus*.