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Environmental determinants of the spatial distribution of *Alaria alata* in Hungary

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ABSTRACT

Alaria alata is a potential zoonotic parasite, which is widely distributed in Eurasia. To assess the risk of human infection, it is important to know the spatial distribution pattern of the parasite and factors influencing this pattern. To investigate these relationships, 1612 red fox (*Vulpes vulpes*) carcasses were randomly collected from the whole Hungarian territory, and the intestines were examined by sedimentation and counting technique. The spatial distribution of the parasite was highly clumped. The topographic positions where the foxes had been shot and the intensity of infections were recorded in geographic information system database. Digitized home ranges of infected and uninfected foxes were analysed on the background of geographic vector data of altitude, land cover types, permanent waters, mean annual temperature, annual precipitation and soil permeability. Multiple regression analysis was performed with environmental parameter values and *A. alata* scores. Based on the statistical analysis, lack of permanent waters, mean annual temperature, annual precipitation and soil permeability were the major determinants of the spatial distribution of *A. alata*. It can be explained by the use of biotopes by the intermediate hosts. The lack of permanent waters results in the use of temporary waters by the second intermediate hosts, frogs. The higher temperature, the lower precipitation and the higher soil permeability lead to earlier desiccation of temporary waters, and tadpoles and frogs infected with mesocercariae can be more easily predated by the final hosts (e.g., red foxes). Moreover, temporary waters are more easily contaminated with the faeces of the final hosts containing eggs than permanent waters. Therefore, high infection rate with *A. alata* can be expected mainly in lowland areas, where the hydrogeography of permanent waters is less complex, the precipitation is lower, the mean temperature and the soil permeability are higher than in highland areas.

1. Introduction

The digenean trematode, *Alaria alata*, is a potential zoonotic parasite, which is widely distributed in Eurasia. *A. alata* adults reach their maturity in the intestine of the definitive hosts, wild and domesticated carnivores (e.g., red foxes, wolves, raccoon dogs, minks, dogs, cats). Unembryonated eggs are passed with the faeces of the definitive hosts. After embryonation and hatching of the eggs, miracidia penetrate the first intermediate host, freshwater snails. Following multiplication, large numbers of furcocercariae emerge from the snail, penetrate tadpoles and frogs and develop into mesocercariae (Möhl et al., 2009). A wide range of paratenic hosts (e.g., wild boars) can acquire infection by ingesting tadpoles and frogs, or infected paratenic hosts. In paratenic hosts including man, migrating mesocercariae of some *Alaria* spp. can cause

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serious disease leading to death in some cases (Möhl et al., 2009). As *A. alata* mesocercariae infects primates, it is most likely infectious for man (Odening, 1961). Carnivores become infected by feeding on tadpoles and frogs or infected paratenic hosts. The young flukes migrate through various organs of the definitive host, including the diaphragm and lungs, before reaching the small intestine (Möhl et al., 2009). Although the flukes are generally considered to be non-pathogenic for the definitive host, large numbers of mesocercariae may cause pulmonary haemorrhages during migration, and high intensity infection with adults can be responsible for enteritis and general intoxication (Möhl et al., 2009).

The prevalence of *A. alata* varies between 0.1% and 96.5% in wild carnivores in Eurasia (Möhl et al., 2009; Bružinskaitė-Schmidhalter et al., 2012; Li et al., in press). Wild boar is the most common paratenic host of *A. alata* in Europe. *A. alata* mesocecariae have been detected in muscle, glandular and fatty tissues of this species in various European countries with a prevalence of 0.2–19.4% and an intensity of 0.02–1.2 mesocercariae per gram (Jaksic et al., 2002; Große and Wüste, 2006; Portier et al., 2011; Esite et al., 2012; Paulsen et al., 2012, in press; Riehn et al., 2012, 2013). Humans could be infected by consumption of undercooked meat or meat products where food processing technologies did not guarantee an inactivation of mesocercariae. The Federal Office for the Environment of Switzerland categorized *A. alata* as a stage 2 risk for parasites with zoonotic potential as pertaining to occupational health risks (Anonymous, 2003). The Federal Institute of Risk Assessment of Germany concluded in its risk assessment that meat which contains *A. alata* mesocercariae should be regarded as unfit for human consumption (BfR, 2007). To assess the consumer exposition risk to *A. alata* mesocercariae, it is important to know the spatial distribution pattern of the parasite and factors influencing this pattern. There might be a range of factors which affect the pattern of *A. alata* transmission in wildlife, and these environmental factors operate at a range of spatial scale. Geographical information systems and remote sensing represent new tools for studying of these factors (Rinaldi et al., 2006). To investigate these environmental factors, red foxes (*Vulpes vulpes*), the most important final hosts of the parasite were sampled and examined for infection with *A. alata* in Hungary. The patterns of infection in foxes and relationship of these patterns with landscape and climate were analysed by geographic information system.

2. Materials and methods

2.1. Sample collection

Carcasses of red foxes sent to the National Food Chain Safety Office, Budapest, from November 2008 to February 2009 and from November 2012 to February 2013, in connection with the rabies immunization and control program, were included in this study. The animals were individually labelled with an identification number reporting the information on the nearest place to kill on the topographic map and the date of collection. Carcasses were forwarded in individual plastic bags at +4 °C. The fox sample size used in this study was previously established on the basis of the National Game Management Database (www.vvt.gau.hu/adattar/). Red fox carcasses, representing more than 1% of the total fox population of each county in both sampling period, were randomly selected out of these animals coming from 19 counties and from the Budapest municipality (covering 100% of the Hungarian territory, 93,029 km^2). The intestinal tract was removed and stored at −20 °C. For safety reasons, the intestinal tract was frozen at −80 °C before examination for 10 days. After freezing, the gut was thawed at room temperature; the intestinal mucosa was collected and tested by sedimentation and counting technique (SCT) 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 *Echinococcus multilocularis* infection (Casulli et al., 2010; Tolnai et al., submitted for publication), the intensity of *A. alata* infection was measured only by scoring (score 1: 1–20 worms; score 2: 21–40 worms; score 3: >40 worms).

2.2. Geographic information system database and spatial analysis

The topographic positions where the foxes had been shot and the intensity of infection were marked on a point layer in Quantum GIS 1.8.0 software (http://www.qgis.org/). The vector layers of altitude, land cover, soil permeability and permanent waters were obtained from VÁTI Hungarian Nonprofit Ltd. for Regional Development and Planning (Budapest, Hungary). The vector layers of mean annual temperature and annual precipitation of 2008 and 2012 were created on the basis of the maps of the Hungarian Meteorological Service. The spatial resolutions of vector layers varied between 10 and 100 m. Vector-based analyses were carried out by Quantum GIS 1.8.0. The search radius around topographic position of foxes was restricted to 2.5 km, which was assumed to represent the average home range of foxes (Staubach et al., 2001). Along permanent waters, a 100 m broad buffer zone was created, where the probability of the presence of water-related intermediate hosts is high. The digitized home ranges and the vector data were used to calculate the altitude, mean annual temperature, annual precipitation, soil permeability, areas of land cover types and the presence and buffer zone of permanent waters within the fox territories.

2.3. Statistical analysis

The relationship between the prevalence of the two collection periods and between the presence of permanent waters and *A. alata* infection was analysed by Fisher's exact test. Intensity of infection in the two collection periods was compared by the Mann–Whitney U-test. Although the central limit theorem ensures that parametric tests are robust to deviations from Gaussian distributions, so long as the sample size is large ($n > 100$) and P value will be nearly correct even if population is fairly far from normal distribution (Motulsky, 1995, 1998), data were log transformed to provide a closer approximation to normal