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Environmental determinants of the spatial distribution of *Echinococcus multilocularis* in Hungary

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ABSTRACT

Human alveolar echinococcosis, caused by the metacestode stage of *Echinococcus multilocularis*, is one of the most pathogenic zoonoses in the temperate and arctic region of the Northern Hemisphere. To investigate the spatial distribution of *E. multilocularis* and the factors influencing this distribution in the recently identified endemic area of Hungary, 1612 red fox (*Vulpes vulpes*) carcasses were randomly collected from the whole Hungarian territory from November 2008 to February 2009 and from November 2012 to February 2013. The topographic positions of foxes were recorded in geographic information system database. The digitized home ranges and the vector data were used to calculate the altitude, mean annual temperature, annual precipitation, soil water retention, soil permeability, areas of land cover types and the presence and buffer zone of permanent water bodies within the fox territories. The intestinal mucosa from all the foxes was tested by sedimentation and counting technique. Multiple regression analysis was performed with environmental parameter values and *E. multilocularis* counts. The spatial distribution of the parasite was clumped. Based on statistical analysis, mean annual temperature and annual precipitation were the major determinants of the spatial distribution of *E. multilocularis* in Hungary. It can be attributed to the sensitivity of *E. multilocularis* eggs to high temperatures and desiccation. Although spreading and emergence of the parasite was observed in Hungary before 2009, the prevalence and intensity of infection did not change significantly between the two collection periods. It can be explained by the considerably lower annual precipitation before the second collection period.

1. Introduction

Alveolar echinococcosis (AE) caused by the metacestode stage of *Echinococcus multilocularis*, is a lethal human disease occurring in the temperate and arctic regions of the Northern Hemisphere (Eckert et al., 2000). Until the end of the 1980s, the *E. multilocularis* endemic areas in Europe were known to exist only in eastern France, Switzerland, southern Germany and western Austria, and the number of human cases was limited (Deplazes and Eckert, 2001; Kern et al., 2003). In the last 20 years, the population size of the red fox (*Vulpes vulpes*) and the infection rate of *E. multilocularis* in this host species increased (Chautan et al., 2000; Berke et al., 2008; Staubach et al., 2011), and new endemic foci were detected in these countries. These epidemiological changes have resulted in an emerging epidemic of AE 10–15 years later (Schweiger et al., 2007; Schneider et al., 2013). The parasite was also reported from 14 countries surrounding the historical endemic area, and the parasite is currently known to be endemic in 18 countries of the European Union (Casulli et al., 2010; Combes et al., 2012). Although the number of human cases of AE might be still underreported, several autochthonous cases were described in humans in the newly endemic countries in

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the recent years, and this number is likely to increase in the future (Romig, 2009; Nahorski et al., 2013). Similar changes of the parasite distribution and prevalence in carnivores were also observed in North America (Storandt and Kazacos, 2012), and AE is one of the most significant zoonotic disease in China and other countries of central Asia (Vuitton et al., 2003, 2011; Usubalieva et al., 2013; Torgerson, in press). To assess the risk areas for AE, it is important to know the spatial distribution of the parasite and factors influencing this distribution. There might be a range of factors which affect the pattern of *E. multilocularis* transmission in wildlife, and these environmental factors operate at a range of spatial scale (Otero-Abad and Torgerson, 2013). Geographical information systems and remote sensing represent new tools for studying these factors (Rinaldi et al., 2006). These tools extended our knowledge in the field of the distribution of *E. multilocularis* in man and animals and allow us to know the underlying factors. Nevertheless, the majority of studies dealing with the spatial distribution of *E. multilocularis* were carried out in China (Craig et al., 2000; Giradoux et al., 2003; Danson et al., 2003, 2004; Graham et al., 2004; Pleydell et al., 2008; Giradoux et al., 2013), and the number of European studies is limited. Moreover, European investigations were carried out in the historically known endemic region (Viel et al., 1999; Raoul et al., 2001; Staubach et al., 2001; Stieger et al., 2002; Denzin et al., 2005; Immelt et al., 2009; Burlet et al., 2011), and no data are available from the recently identified endemic areas. To investigate the spatial distribution of the parasite and factors influencing this distribution in a new endemic area (Sréter et al., 2003, 2004; Casulli et al., 2010), red foxes were sampled and examined for infection with *E. multilocularis* in Hungary. The spatial distribution of *E. multilocularis* and relationship of this distribution with the landscape and the climate were analyzed by geographic information system.

2. Materials and methods

2.1. Sample collection

Carcasses of red foxes sent by hunters 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 programme, were included in this study. The animals 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 as described by Conraths et al. (2003) (Fig. 1). 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/). This database contains the yearly estimated fox population size of each Hungarian county. Red fox carcasses, representing more than 1% of the total fox population of each county in both sampling periods (Table 1), were randomly selected out of the 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 for 10 days before examination. 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). Adult worms of *E. multilocularis* were identified by morphology as previously described (Sréter et al., 2003). The intensity of infection was determined by counting of worms in low level infections (<100 worms/fox) or by the estimation of the worm number by counting in a known percentage of the sediment following dilution in high level infections (≥100 worms/fox). For molecular studies, 1–25 worms were collected from each infected foxes and stored in 70% (v/v) ethanol. The taxonomic status of the worms was confirmed by a multi-locus microsatellite analysis (Casulli et al., 2010) or a polymerase chain reaction (PCR) assay of the mitochondrial 12S rRNA gene (Dinkel et al., 1998).

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, permanent water bodies, soil water retention and soil permeability 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 by Quantum GIS 1.8.0 on the basis of the georeferenced digital maps of the Hungarian Meteorological Service. The spatial resolutions of the vector layers varied between 10 and 100 m. The vector-based analysis was carried out by Quantum GIS 1.8.0. The radius around the 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 water bodies, a 100 m broad buffer zone was created, where the probability of the presence of semi-aquatic intermediate hosts is high (Széll et al., 2013). The digitized home ranges and the vector data were used to calculate the altitude, mean annual temperature, annual precipitation, areas of land cover types, soil water retention, soil permeability and the presence and buffer zone of permanent water bodies within the fox territories.

2.3. Statistical analysis

The relationship between the prevalence of the two collection periods and between the presence of permanent water bodies and *E. multilocularis* infection in foxes was analyzed by the Fisher's exact test. The 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 correct even if population

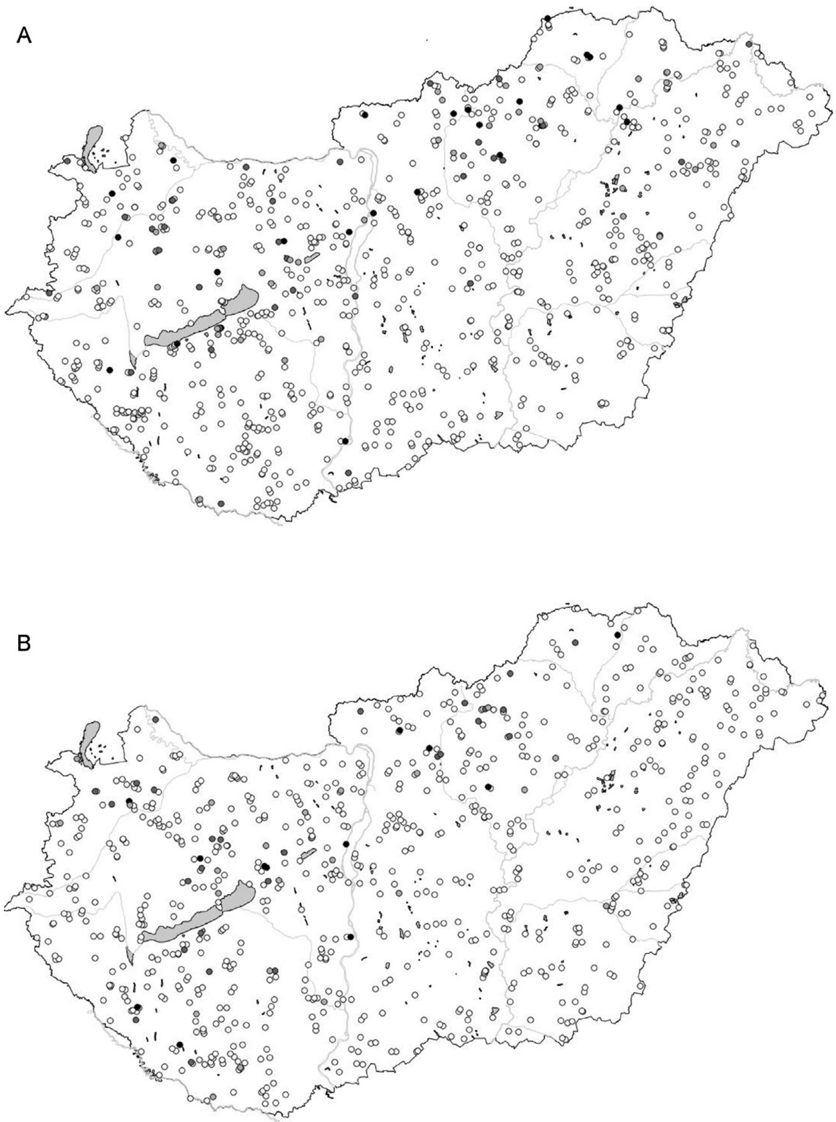

Fig. 1. Distribution of uninfected and *Echinococcus multilocularis* infected red foxes (*Vulpes vulpes*) collected in Hungary between November 2008 and February 2009 (Panel A) and between November 2012 and February 2013 (Panel B). The darkness of the dots is in line with the intensity of infection (white: uninfected; light grey: <10 worms; grey: 10–100 worms; black: >100 worms).

is fairly far from normal distribution (Motulsky, 1995, 1998), parameters were log transformed to provide a closer approximation to the normal distribution prior to the parametric analysis. The associations between environmental factors (altitude, land cover types, permanent water bodies, soil water retention, soil permeability, mean annual temperature and annual precipitation) and the intensity of *E. multilocularis* infection were initially analyzed by calculation of Pearson correlation coefficients. These relationships were also confirmed by the calculation of Spearman rank correlation coefficients using non-transformed data. Once the significant variables at an individual level were selected on the basis of the results of the preliminary statistical analysis, a multiple linear regression was performed with the log transformed environmental parameter values and the *E. multilocularis* counts to identify the environmental conditions which affected the abundance of *E. multilocularis* 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. As multicollinearity was a problem (variables provided redundant information), some variables were removed from the regression model. 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 Software, Ostend, Belgium). The prevalence, 95% confidence interval (95% CI) and freedom from

Table 1
Epidemiological features of the red fox (*Vulpes vulpes*) population tested for *Echinococcus multilocularis* in Hungary in 2008–2009 and 2012–2013.

Counties (n = 19)	No. of examined foxes (no. of positives)		Prevalence (95% CI)		Mean intensity (± SE)	
	2008–2009	2012–2013	2008–2009	2012–2013	2008–2009	2012–2013
Bács-Kiskun	60 (2)	45 (1)	3.3 (1.0–5.6)	2.2 (1.0–5.6)	69 (±52)	2 (–)
Baranya	56 (2)	44 (3)	3.6 (1.2–6.0)	6.8 (3.1–10.5)	26 (±24)	22 (±17)
Békés	38 (0)	36 (1)	0	2.8 (0.1–5.5)	0	1 (–)
Borsod-Abaúj-Zemlén	48 (9)	45 (4)	18.7 (13.2–24.2)	8.9 (4.7–13.1)	471 (±328)	53 (±28)
Csongrád	32 (0)	37 (0)	0	0	0	0
Fejér	61 (10)	41 (10)	16.4 (11.8–21.0)	24.4 (17.8–30.9)	352 (±331)	658 (±541)
Győr-Sopron	40 (6)	35 (9)	15.0 (9.5–20.5)	25.7 (18.5–32.9)	61 (±25)	61 (±35)
Jász-Nagykun-Szolnok	38 (0)	34 (0)	0	0	0	0
Hajdú-Bihar	51 (7)	49 (0)	13.7 (9.0–18.4)	0	110 (±98)	0
Heves	34 (13)	42 (8)	38.2 (30.0–46.4)	19.0 (13.1–24.9)	85 (±28)	129 (±96)
Komárom-Esztergom	22 (3)	22 (0)	13.6 (6.4–20.8)	0	29 (±11)	0
Nógrád	27 (7)	26 (4)	25.9 (17.6–34.2)	15.4 (8.5–22.3)	8792 (±7038)	131 (±47)
Pest including Budapest	58 (5)	54 (2)	8.6 (5.0–12.2)	3.7 (1.2–6.2)	169 (±75)	1 (±0)
Somogy	85 (7)	66 (7)	8.2 (5.3–11.1)	10.6 (6.9–14.3)	440 (±274)	602 (±534)
Szabolcs-Szatmár	51 (4)	48 (0)	7.8 (4.1–11.5)	0	105 (±98)	0
Tolna	34 (1)	30 (4)	2.9 (0.1–5.7)	13.3 (8.9–18.1)	4 (–)	85 (±72)
Vas	30 (3)	27 (1)	10.0 (4.6–15.4)	3.7 (0.1–7.3)	148 (±126)	5 (–)
Veszprém	39 (9)	52 (7)	23.1 (16.5–29.7)	13.5 (16.5–29.7)	44 (±27)	189 (±156)
Zala	36 (2)	39 (0)	5.5 (1.8–9.2)	0	175 (±125)	0
Average	840 (90)	772 (61)	10.7 (9.7–11.7)	7.9 (6.9–8.9)	746 (±556)	243 (±110)

infection, based on sample size, were calculated by Survey Toolbox 1.04 (Cameron, 1999; www.ausvet.com.au/).

3. Results and discussion

Although spreading and emergence of the parasite was observed in Hungary before 2009 (Sréter et al., 2003, 2004; Casulli et al., 2010), a non-significant ($P > 0.05$) decrease of the prevalence and intensity of infection was noted between the two collection periods (Table 1). It can be explained by the considerably different mean annual precipitation in 2007–2008 and 2011–2012 (541–572 mm vs. 382–440 mm) and the high sensitivity of *E. multilocularis* eggs to desiccation (Veit et al., 1995). The latter two years were ones of the driest since 1900. The mean annual temperature of the two periods did not differ significantly. These results indicate that precipitation is a significant determinant of the temporal distribution of the parasite in Hungary. A significant negative correlation with temperature and precipitation was also found in Slovakia (Miterpaková et al., 2006; Miterpaková and Dubinsky, 2011).

The spatial distribution of *E. multilocularis* was highly clumped in Hungary (Fig. 1). In northern counties, the prevalence was 0–38.2%, while in other counties the prevalence was 0–13.7% (Table 1). Based on the calculation of freedom from infection (Cameron, 1999), none of these counties can be considered free of the parasite with a prevalence of 5–7%. The intensity of infection was lower than that seen in the historically known endemic areas (Table 1).

There was no correlation between soil water retention or soil permeability in the home range of foxes and the intensity of *E. multilocularis* infection.

There was a positive correlation between the average altitude of the home range of foxes and the intensity of *E. multilocularis* infection ($P < 0.0001$). However, the multiple regression analysis revealed multicollinearity with temperature (redundant information). Therefore, this variable was removed from the regression models.

Permanent water bodies were significantly more frequently detected in the home range of *E. multilocularis* infected foxes than that of uninfected foxes ($P < 0.001$). However, there was no correlation between the total area of buffer zone of waters in the home range of foxes and the intensity of *E. multilocularis* infection, and multiple regression models did not reveal a significant relationship with the area of buffer zones of water. The relationship between waters and *E. multilocularis* infection might be explained by the longer survival of eggs in the humid environment of permanent water bodies and the occurrence of two important semi-aquatic intermediate hosts of the parasite, the water vole (*Arvicola terrestris*) and the muskrat (*Ondatra zibethicus*). The relationship with permanent water bodies was also observed by Staubach et al. (2001) in Germany and by Milner-Gulland et al. (2004) in Kazakhstan.

Although there was a negative correlation between non-forested area in the home range of foxes and the intensity of infection, multiple regression analysis revealed multicollinearity with altitude. Therefore, non-forested area was removed from the regression models. No other relationship with other land cover types could be observed in Hungary. In France and Germany, significant correlation was found between *E. multilocularis* infection of foxes and grassland (pasture) or agricultural areas (Viel et al., 1999; Staubach et al., 2001; Immelt et al., 2009). In these countries two terrestrial intermediate hosts exist, the common vole (*Microtus arvalis*) and the fossorial population of the water vole (*A. terrestris*) (Wust Saucy, 1998). The occurrence of fossorial population of the water vole is strongly related to grasslands (Viel et al., 1999). The lack of stronger relationship with land cover types might be attributed to the lack of fossorial population of the water vole in Hungary (Wust Saucy, 1998) and the different climate of these regions (see below). In China, significant correlation was found between landscape composition and AE (Craig et al., 2000; Giraudoux et al., 2003, 2013; Danson et al., 2003, 2004; Graham et al., 2004; Pleydell et al., 2008). Nevertheless, the intermediate host range of *E. multilocularis* is different and much wider, and the climate is considerably different in this country.

There was a negative correlation between the mean temperature of the home range of foxes and the intensity of *E. multilocularis* infection ($P < 0.0001$) and a positive correlation between the annual precipitation in the home range of foxes and the intensity of *E. multilocularis* infection ($P < 0.001$). The multiple regression analysis and the logistic regression analysis also confirmed these relationships ($P < 0.0001$; $P < 0.005$ and $P < 0.0005$; $P < 0.01$). Although the best fit model was the logistic regression model (classified correctly with $P = 0.5$: 90.6%; Hosmer–Lemeshow test: $P = 0.12$; area under ROC curve: 0.62 ± 0.02), the overall fit of both models was satisfactory ($P < 0.0001$). Based on the statistical analysis, the mean annual temperature and the annual precipitation (95% CI of odds ratios: 0.492–0.818 and 1.001–1.005) were the major determinants of the spatial distribution of *E. multilocularis* in Hungary. Comparison of the odds ratios indicates that mean annual temperature plays the most important role in the distribution of the parasite. It can be attributed to the sensitivity of *E. multilocularis* eggs to high temperature and desiccation. A temperature of 43 °C (e.g., direct exposition to sun in summer) kills the eggs within 2 h irrespective of the degree of humidity (Veit et al., 1995). *E. multilocularis* eggs are also highly sensitive to desiccation independently of the temperature; the exposure to low relative humidity ($\leq$27%) at 25 °C killed the eggs within 2 days (Veit et al., 1995). Similar relationships with temperature or precipitation and *E. multilocularis* infection of foxes or water voles were also observed in some regions of Germany and Switzerland (Denzin et al., 2005; Immelt et al., 2009; Burlet et al., 2011).

The partly different environmental determinants in the historically known endemic region (land cover types, permanent water bodies, temperature or precipitation) (Viel et al., 1999; Staubach et al., 2001; Denzin et al., 2005; Immelt et al., 2009; Burlet et al., 2011) and the new endemic region of Hungary (temperature and precipitation) can be explained by the different climate of these regions. The mean annual temperature and annual precipitation of Hungary is considerably different and less favourable for *E. multilocularis* in the historically known endemic areas of Europe. It might explain that only these two parameters play a role in the distribution of the parasite in Hungary. In some countries (e.g., Kazakhstan), where the climate is even more extreme in terms of temperature and rainfall, infected rodents and final hosts were only found in patches with humid microclimate (e.g., mountains and river valleys) (Milner-Gulland et al., 2004). However, it remains conceivable that there might be other predictor variables, not included in this analysis, that have a statistically significant impact on the spatial distribution of *E. multilocularis* in Hungary.

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