

# Identification of environmental risk factors for the presence of *Echinococcus multilocularis*.

Florian TOLLE<sup>a</sup>, David PLEYDELL<sup>b</sup>, Benoit COMBES<sup>c</sup>, Florence CLIQUET<sup>d</sup>, Martine PIARROUX<sup>e</sup>,  
Patrick GIRAUDOUX<sup>b</sup> and François-Pierre TOURNEUX<sup>a</sup>

<sup>a</sup> ThéMA UMR 6049 CNRS - Université de Franche-Comté, 32 Rue Mégevand, 25030 Besançon Cedex.

<sup>b</sup> Laboratoire de Biologie environnementale EA3184 usc INRA, Université de Franche-Comté, Besançon.

<sup>c</sup> Entente Rage et Zoonoses – Domaine de Pixérécourt – BP 43, 54220 Malzéville, France.

<sup>d</sup> Agence Française de Sécurité Sanitaire des Aliments – BP 9, 54220 Malzéville, France.

<sup>e</sup> ThéMA UMR 6049 CNRS – Réseau FrancEchino - Université de Franche-Comté, 32 Rue Mégevand, 25030 Besançon.

The tapeworm *Echinococcus multilocularis* is dependent upon foxes and microtine rodents to complete its life cycle and is a parasite of public health importance causing the fatal zoonotic disease alveolar echinococcosis (AE). Concerns that AE could be in emergence in Europe have arisen from recent studies. In France, a monitoring survey led to the collection of 2813 georeferenced faecal samples of which 82 have been diagnosed positive for the presence of the parasite.

A geographically weighted logistic regression was used to assess potential spatial variation in the effect of putative environmental risk variables. Landscape and climatic variables were expected to play a role in epidemiological factors. The results showed no significant spatial heterogeneity. This suggests a constant prevalence across the study area. The parasite is now identified in regions where it was formerly unknown, outside of traditional endemic areas.

## KEYWORDS

Landscape composition, eco-epidemiology, *Echinococcus multilocularis*, geographically weighted regression, GIS

## INTRODUCTION

The cestode tapeworm *Echinococcus multilocularis* is a parasite responsible for the fatal zoonotic disease alveolar echinococcosis in humans [19]. The life cycle of *E. multilocularis* is dependent on two types of host: Microtine rodents (usually voles) are intermediate hosts and carry the larval form of the parasite; definitive hosts become infected following predation of infected rodents. Foxes (*Vulpes vulpes*) are the most common carriers of the adult worm, but other mammals like dogs or cats can also become infected [25]. Adult worms locate in the intestine of definitive hosts and their eggs are dispersed in the environment through the faeces. The larval form of the worm locates and develops in the liver of the intermediate host and accidental infections in humans lead to debilitating, chronic and often fatal disease that can be extremely difficult to cure once symptomatic [19]. Historically, the highest incidence rates for human alveolar echinococcosis (AE) within France have been recorded in the Doubs department of Franche-Comté (eastern France)(figure 1).

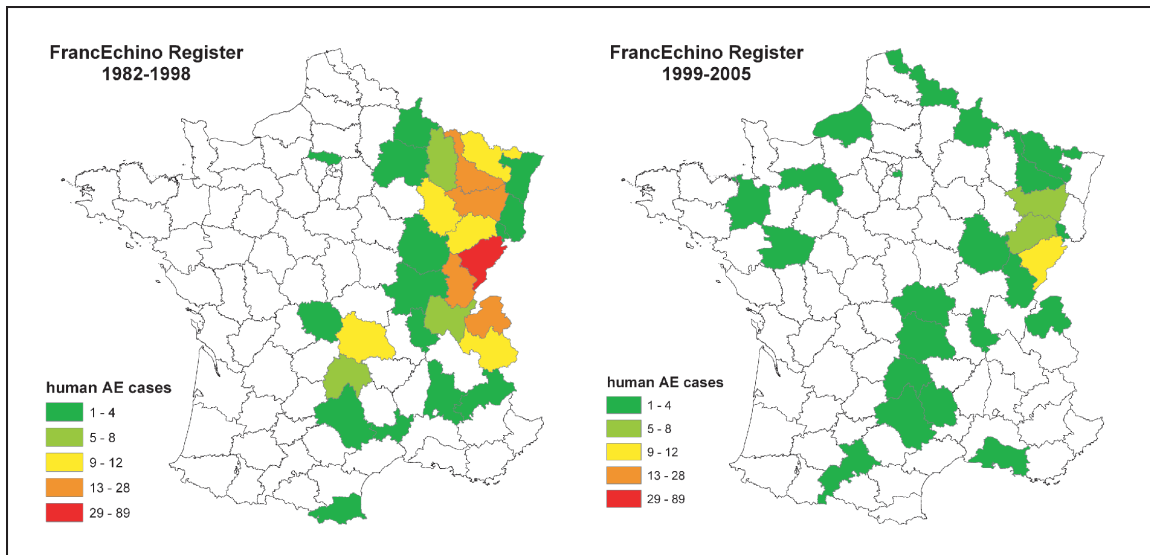


Figure 1: Spatial distribution of alveolar echinococcosis cases in France (Doubs is in red on the first map)

Geographical variation in prevalence and infection rates of *E. multilocularis* has previously been described using the Ratio of Optimal to Marginal Patch Area (ROMPA) hypothesis of Lidicker [13, 14]. This approach attempts to explain observed levels of parasitic infection in terms of the availability of habitat patches within which the population densities of intermediate hosts may become cyclic with regular multi-annual outbreaks of very high density. The hypothesis attempts to combine bottom-up forces imposed by the limiting factor of habitat availability with the top-down forces imposed by predator-prey interactions: in areas of low ROMPA microtine dynamics are stabilised by predators and the landscape acts as a large sink; in areas with abundant optimal habitat (high ROMPA) the landscape may sustain high densities and population dynamics stabilise; at intermediate ROMPA resource and predation effects interact in complex ways and multi-annual cycles are often observed. Observational support for the ROMPA hypothesis was found in northern and central France with densities of the common vole *Microtus arvalis* low in areas of <5% grassland, multiannually cyclic where grassland percentage was 5-50% and annually cyclic in areas with >50% grassland [6]. Moreover, in the Doubs department both population outbreaks of *A. terrestris* and incidence rates of AE among agricultural workers have been related to the proportion of permanent grassland there [9]. Similarly, the analyses of [20] indicated that trends in fox infection across Franche-Comté were correlated to grassland percentage while [4, 5] provide empirical support for the relation between human AE and land use in central China.

While the ROMPA hypothesis attempts to explain regional trends in *E. multilocularis* epidemics more localised clustering is also known to occur. Specifically the parasite is known to be over-dispersed in the host populations with up to 70% of the biomass of adult worms typically occurring in just 10% of foxes [12, 21]. As such the pattern of environmental contamination at any one point in time will become aggregated within the home ranges of the most heavily infected individuals. More over faeces within a home range are not randomly distributed but are used to mark territorial boundaries or favourite hunting grounds. Further egg mortality rates are highly dependant upon microclimatic conditions and in favourable cool and humid conditions eggs may survive for periods of up to eight months [26], however eggs dessicate readily in hot dry conditions imposing climatic restrictions on endemic areas. There is some evidence that prevalence rates in rodents living in open fields can be lower than in rodents living close to field borders where both fox defecation rates may be higher and microclimatic conditions more favourable to egg survival [10].

It is evident that a variety of environmental conditions are required such that the pathogenic agent may remain infective in the environment and that the parasite may complete its life cycle. First, the joint presence of intermediate and definitive hosts is mandatory [10] and the absence of one of the hosts may lead to either a temporary or even complete interruption of the parasite's life cycle. With this first condition satisfied, other environmental factors play a role on the eggs' survival rates. The nature of land use at defecation sites in part determines the protection that eggs will receive from heat and dessication.

Current concerns that alveolar echinococcosis could be in emergence in Europe have arisen from recent studies which have shown increases in: the population densities of foxes; the prevalence of *E. multilocularis* in foxes in endemic areas [22, 23, 8]; and range extensions of endemic areas [7, 15]. This potential emergence justifies the monitoring of the endemic status in foxes across large areas. Recently, a monitoring survey has been undertaken covering a large area of eastern and northern France (figure 2). This program was the result of a collaboration between the ERZ (Interdepartmental Office for Rabies and other Zoonoses Control), the AFSSA (French Food Sanitary and Safety Agency) and the University of Franche-Comté (departments of Environmental biology and ThéMA). The current paper presents this data set and attempts to look for potential environmental risk factors which may relate to spatial variations in faecal prevalence. The identification of eco-epidemiological risk factors for the presence of *E. multilocularis* is central to understanding the transmission of the parasite.

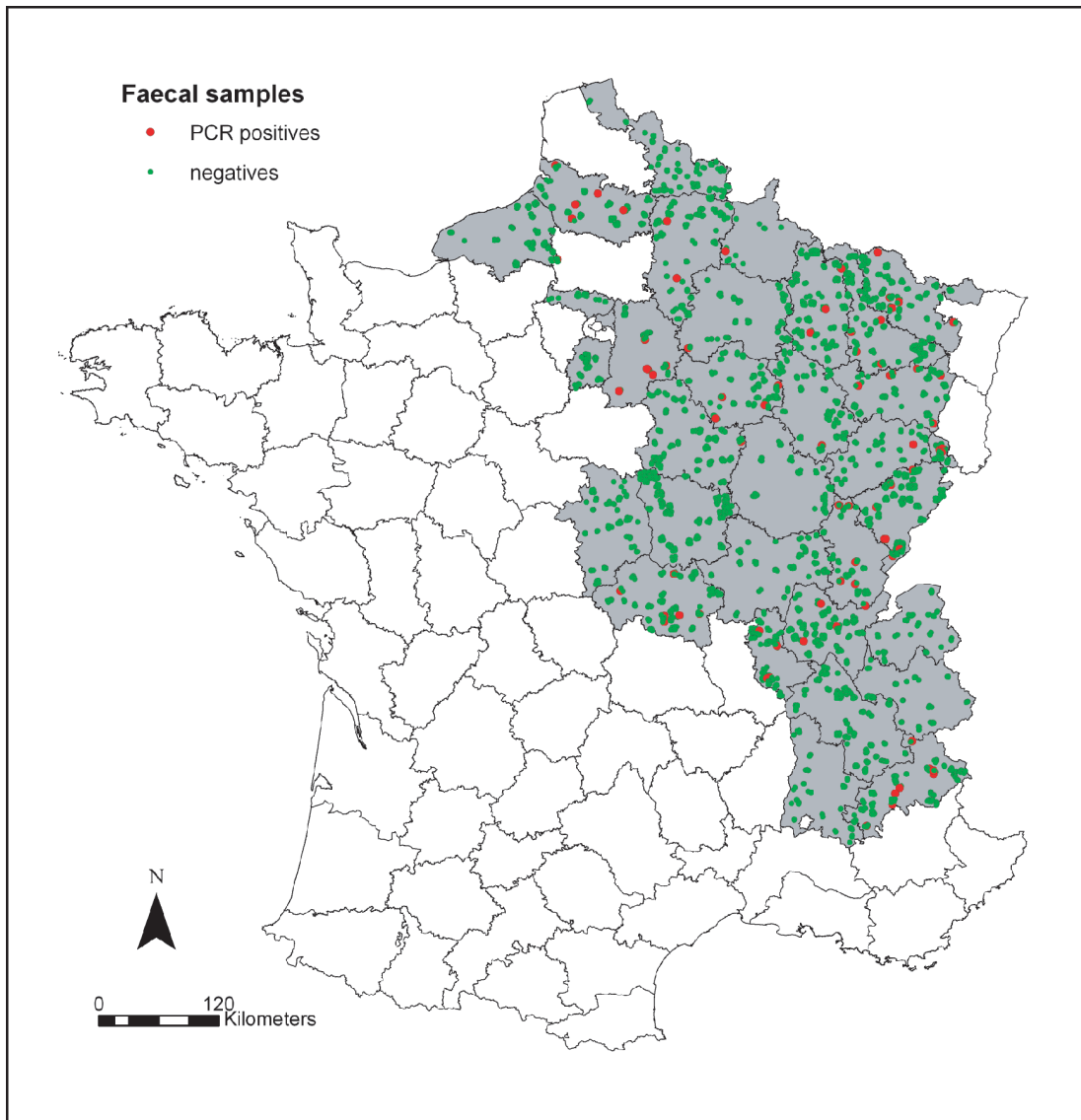


Figure 2: Study area and samples location

#### MATERIAL AND METHODS

In France, the sampling design consisted of randomly selecting 4km x 4km sampling units in which 5 faecal samples were collected whenever possible. Fox faeces collected in the field were sent to laboratories in Nancy where they were tested for the presence of *E. multilocularis* specific antibodies and DNA. A first

screening was conducted using an ELISA test which provided a quick and cheap method to remove negative samples. All ELISA positive samples were then PCR-tested in order to remove false-positive faeces. The exact locations of the collected faeces were marked on a cartographic map enabling geographical coordinates to be recorded in the ERZ database along with the biological test results. To date, the French monitoring survey has yielded 5299 georeferenced faecal samples of which 2813 samples have been analysed and 82 diagnosed positive for the presence of the parasite's eggs.

In order to include landscape and climatic factors in the analysis different spatial data sources have been used. The area covered by the French program was approximately 220 000 km<sup>2</sup>. Given the extent of the study, the European CORINE Land Cover map (CLC)[3] presented the most readily available means by which landscape composition around each sample could be quantified. The CLC was used in raster format at a resolution of 100 m. The composition ratio of each CORINE class was computed around each faecal sample using Fragstats [16]. This was conducted at radii ranging from 500 to 5000m (500m increments) and the results for each landscape class were expressed as a ratio of total area. Three landscape composition variables were extracted from CORINE in this way: first, grassland percentage (both natural grassland and pasture) was investigated since it was expected to relate to the ROMPA for key intermediate host species; complex agricultural zones (here called "mixed") were also tested since these CORINE classes usually incorporate semi-natural areas like abandoned agricultural land, scrub areas or shrub areas potentially favourable to intermediate host populations; finally forest percentage was included since, in France at least, foxes depend upon forest areas for making their dens (figure 3).

Climatic variables were retrieved from the Climatic Research Institute [17]. The CRU provides high-resolution datasets of mean monthly earth-surface climatic data obtained by interpolating 30-year means from a set of meteorological stations, altitude was taken into account in their interpolations. Considering the sensitivity of *E.multilocularis* eggs to dessication, mean temperature and average number of wet days (precipitation > 0.1 mm) in July were included as potential variables limiting the distribution of the parasite. The interpolated value corresponding to each faecal sample location was added to the database. The hypothesis underlying the choice of these two climatic variables was the assumed negative influence of high temperatures on the survival rate of *E. multilocularis* eggs and the positive influence of wet environments on the conservation of the pathogenic material.

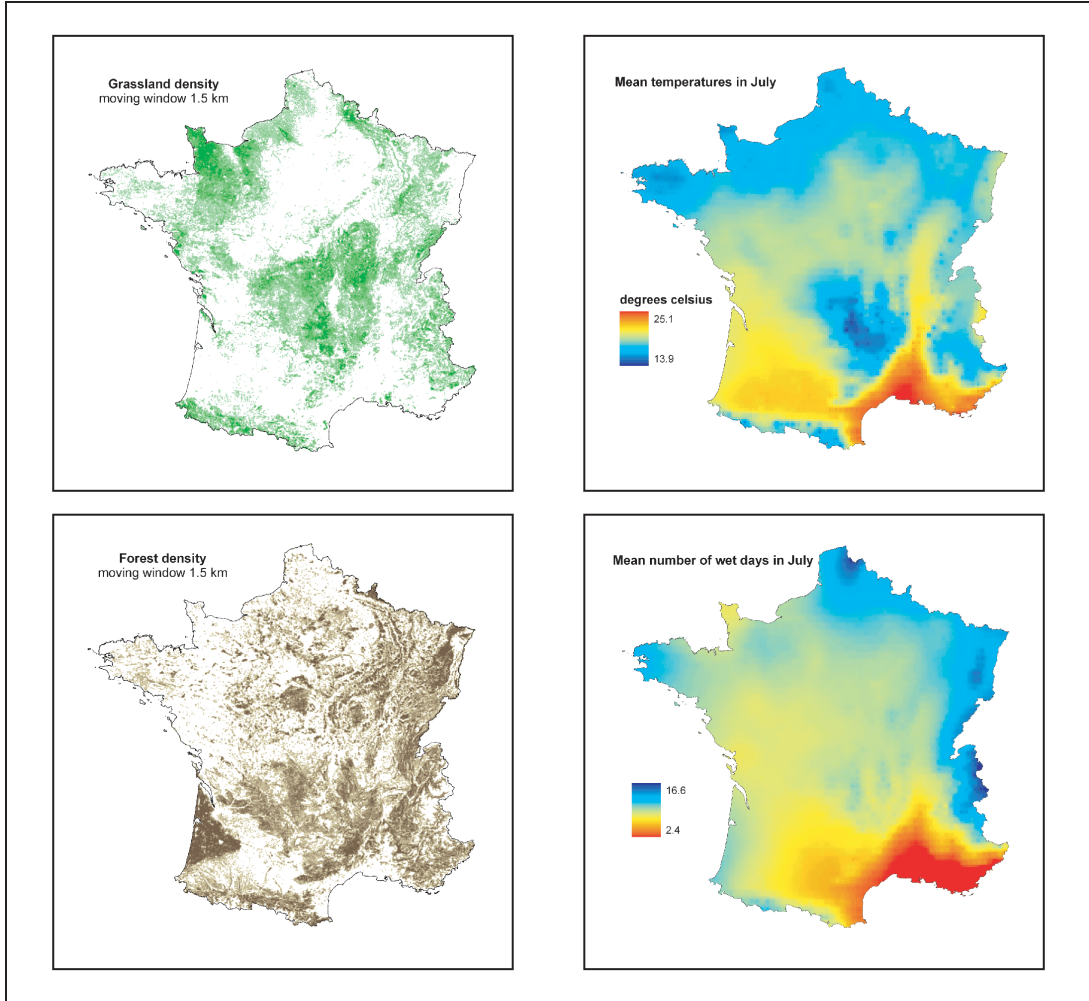


Figure 3: Grassland ratio (top left), Forest ratio (bottom left), mean temperature in July (top right) and mean number of wet days in July (bottom right) in France

Geographically weighted logistic regression (GWLR) was used to assess potential spatial variation in the effect of putative environmental risk variables. This is a useful approach in situations where the effect of environmental conditions may not be homogeneous over an entire study area. By extending logistic regression to allow the regression coefficient associated with a given environmental variable to vary smoothly across the study area the model can be written as

$$\log\left(\frac{p_i}{1-p_i}\right) = c + f_x(a_i)$$

where  $c$  is a constant,  $p_i$  is the expectation of a PCR positive result for faecal sample  $i$ ,  $a_i$  is the environmental variable of interest at or surrounding the  $i^{\text{th}}$  faecal sample and  $f_x$  is a function enabling the regression parameter associated with  $a$  to vary smoothly over the study area. Each faecal sample was then associated with its spatially corresponding grid square. The smooth function  $f_x$  was chosen to be a Markov random field where the realisation  $\beta$  at grid location  $x$  follows a normal distribution specified conditionally on the local neighbourhood  $x'$  consisting of the  $n$  adjoining grid squares such that

$$\beta_x | \beta_{x'} \sim \text{Normal}\left(\frac{\sum \beta_{x'}}{n}, \frac{\tau^2}{n}\right)$$

The grids used to model the random fields were generated to cover the entire sampling area plus a 100km buffer zone (to reduce edge effects of the random field). A 20km grid resolution was used. The joint

distribution of the parameters and random field given by this geographically weighted regression model was sampled conditionally on the faecal PCR data using Markov chain Monte Carlo (MCMC) simulation techniques as implemented in the software BayesX [1] using the default hyper-prior for the smoothing parameter  $\tau^2$ . Following a burn-in period of 20000 iterations the Markov chains were run for a further 100000 iterations and thinning was applied such that every 100<sup>th</sup> simulation was saved for posterior inference.

Each of the variables of interest was tested using this approach and these variables included: the composition ratios of both grassland, forest and mixed areas calculated at a range of radii (from 500 to 5000m, 500m increments); the mean temperature and number of wet days in July; and finally  $a_i$  was replaced with the unit constant such that the Markov random field could fit spatial trends without environmental constraints. For each variable under consideration the GWLR model outlined above was implemented and the models were compared using the deviance information criterion (DIC) [24] which is a suitable statistic for comparing hierarchical models of this nature fitted using MCMC and is similar in spirit to the popular Akaike information criterion (AIC) for maximum likelihood. The geographically weighted logistic regression was conducted using the independent variables outlined above.

## RESULTS

Results from the numerous simulations consistently indicated an absence of significant spatial heterogeneity in the influence of the various estimated parameters. Moreover none of the tested variables were found to represent spatial trends in the data. The Markov random field fitted without an environmental variable interaction term similarly indicated a lack of spatial trend in the faecal prevalence, a result confirmed using a generalised linear model with second-order polynomial trend terms based on geographical coordinates alone (not shown). The lack of significant trend in the Markov random field (without environmental interaction) was observed with one exception, a small cluster of PCR positive faeces from the Doubs department led to more than 95% of the posterior samples for  $\beta$  being greater than zero in that one grid square. Further, more than 95% of the posterior samples for the MRFs specified with a grassland interaction were also greater than zero at the same grid square for grassland ratios calculated at radii between 2000 and 4500 meters, for those radii a single square in the north west of Vosges department also displayed a regression parameter that was significantly different to zero.

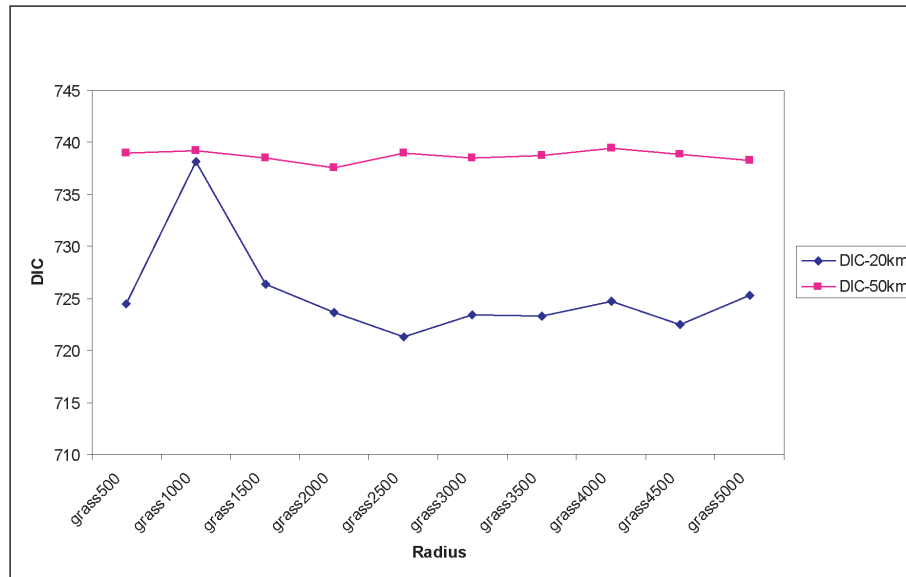


Figure 4: Model DIC scores using 20 and 50 km grids

In order to assess the relative significance of these results, the relation between the radii used to calculate grassland ratio and the DIC associated with each GWLR was compared (figure 4). Figure 4 also shows the DIC scores of each model fitted using both 20km and 50km resolution grid squares. Like the AIC the model which minimises the DIC can be selected as optimal among those models tried and tested, the results therefore indicate that the use of a 50 km grid was too coarse. A 15km grid was also tried with results mirroring those achieved with a 20km grid. Computational problems were experienced with finer grids.

Figure 4 indicates that the DIC was minimised at a 2500m radius (DIC=721.3) with a second minimum occurring at a radius of 4500m (DIC=722.5). Similarly the DIC associated with the aggregated mixed class was minimised at a radius of 1000m (DIC=722.4), the DIC for the forest models was minimised at a radius of 4000m (DIC=722.6) and the DICs associated with the models testing July wet days, mean July temperatures and no environmental interaction were 733.7, 726.6 and 720.7 respectively. This final result indicated that the predictive performance of the model was not improved by inclusion of the chosen environmental variables. The posterior mean of the grassland (radius 2500m) regression coefficient is presented in figure 5; as stated above the apparent trends were not significant:

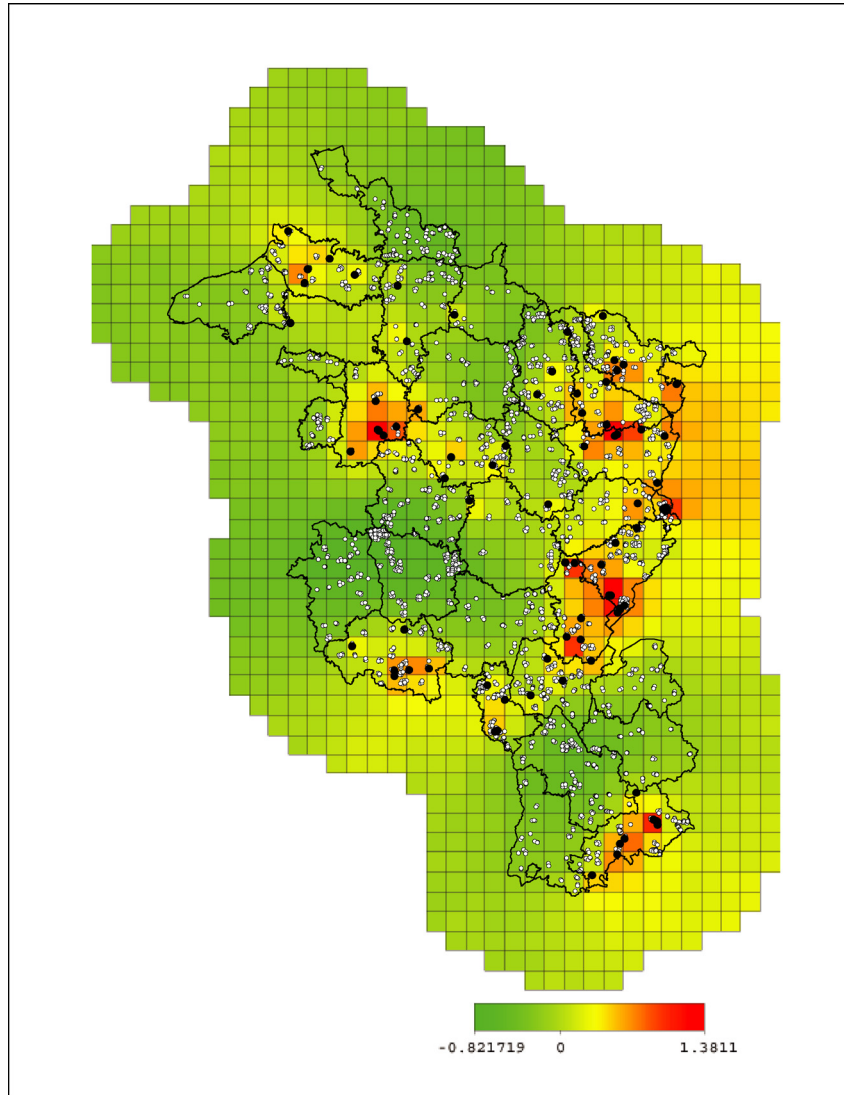


Figure 5: Spatial variation in the posterior mean of the regression parameter for grassland (radius of 2500 m) from the geographically weighted regression. (black and white dots are positive and negative faecal samples respectively)

## DISCUSSION

From the analyses presented here it was apparent that the PCR positive samples were homogeneously distributed over the entire study area and that no clear trends or link to landscape or climatic variables were apparent. This lack of spatial heterogeneity was confirmed by plotting an indicator variogram (not shown) which was flat. The absence of spatial heterogeneity indicates a constant prevalence across the study area and the global faecal prevalence was estimated as 2.9% (2.3 – 3.5 95% CI). Previous studies have shown



that fox prevalence rates of the order of 40% or even 60% are not uncommon in endemic areas [number of Hofer], it is highly likely therefore that the copro-ELISA / copro-PCR combination used here has seriously under-detected the true number of positive faeces, perhaps even to the order of just one in ten positives being detected accurately. The relationship between faecal prevalence and true prevalence has not previously been studied, but the results here suggest that very low faecal prevalences render it difficult to extract meaningful information relating to ecological conditions which give rise to heightened transmission rates of *E. multilocularis*. Despite the suspected under-detection in known established foci and main endemic areas, the important result arising from this study has been the identification of *E. multilocularis* across the whole study area. Although the parasite presence was clearly expected in identified endemic areas of north-east France and Massif-Central, it is striking that new regions like Ile-de-France (Paris), Picardie or Haute-Normandie (north-west of Paris) also seem to be hosting active *E. multilocularis* transmission. This is the first time *E. multilocularis* presence has been confirmed for such a wide continuous area of France. It is clear that the sampled area has not yet reached the range limit of *E. multilocularis* and as a result the limits of the French endemic area currently remain unknown.

The dataset presented here provides new insights into the epidemiological situation in western Europe. New areas have been identified as supporting the parasite's hosts and the parasite's life-cycle. It is hard to know whether this new situation is due to a real range extension of the parasite or to the rising interest in detection. The low number of reported human AE cases in the newly identified regions and the lack of information on the disease may have led to the ignorance of *E. multilocularis* presence even though it was already established. The lack of a previous baseline for the prevalence in these regions is the main reason why it is impossible to infer about temporal change and potential emergence of *E. multilocularis* in France. However, although this was the first time that the parasite had been specifically looked for in most of these new areas, the fact that faecal prevalence in these areas appeared to be indifferent from faecal prevalence in established foci with known human cases is surely a cause for concern.

The failure of landscape and climatic factors to explain variation in prevalence over the study area might be interpreted at different levels. First this may reveal that patterns in host interactions are more homogeneous than previously suspected, clearly sufficient communities of both foxes and rodents are present across the entire study area to support the transmission cycle. The absence of a link with landscape features may suggest that the parasite cycle is not as strongly reliant on the composition ratio of optimal microtine habitat as previously suspected. Perhaps this may be interpreted as an insight that a cycle can be sustained in landscapes where total patch area of optimal microtine habitat is not large. Therefore, we may formulate a new hypothesis that landscapes with low expected rodent densities might still allow for the persistence of the parasite cycle. If this were the case then the results here may represent an artifact arising from the use of the CLC which, with a minimum patch size of 25 ha, doesn't provide precise land use information and could feasibly miss some landscape features favorable to the establishment of rodent communities and of significance to the parasite. For example, certain cultured areas are known to support microtine populations yet are classified as Arable Land in the CLC typology. The apparent absence of explanatory influence of climatic variables seems to indicate that the study area completely lies within a parasite-favorable climatic area: the northern part of France is blessed with an oceanic or continental type climate which seems to suit the parasite; the southern limits of the study area mainly follow topographic features and these altitude zones have a climate clearly different from the typical Mediterranean climate found further south. Only the completion of the sampling program in France might allow for the determination of a southern limit to the parasite's extent, if there is one.

The lack of precision in CLC and the failure of CLC to describe the parasites distribution not only in France but also across the European endemic region suggests that new eco-epidemiological approaches are needed before the transmission of *E. multilocularis* can be understood across France and Europe. GIS technologies have led to new techniques in ecology and in epidemiology. A common approach is to use remotely sensed data to determine ecologically or epidemiologically distinct areas [11]. [2] used an unsupervised classification procedure to identify areas of ecological similarity in Africa. Such methods could be used to identify areas with high or low expected success of the parasite's host populations, the parasite cycle and the parasite's eggs. A wide variety of variables might be included in such an analysis. Landscape variables are expected to have an influence on host interactions and communities and other biogeographic variables could also influence the parasite's spatial distribution. Together, these descriptive datasets may help define the potential bounds of the range of *E. multilocularis* and human AE across large areas such as countries and continents.



## CONCLUSION

Field comparisons in extensively investigated regions seem to indicate that here faecal prevalences have clearly under-estimated true prevalence. The expected real prevalence can reach values 10 times higher than the faecal prevalence reported here. We might therefore expect true prevalences in the study area to reach at least 30% which would paint a totally different picture of the parasitological and epidemiological situation. Furthermore, the low number of positive samples in the database didn't allow for a clear distinction between areas. An increased sampling effort coupled with different diagnostic techniques (the gold standard being autopsy with sedimentation and counting) could help differentiate between high and low endemicity areas. There is still a strong interest in identifying the extent of the range of the parasite in France which hasn't been systematically investigated to date. New insights of foxes behavior and their increasing presence in cities make the estimation of the real range of the parasite a clear epidemiological issue as it defines the potential cities where a parasite cycle might appear and be sustained.

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## **AUTHORS INFORMATION**

### **Florian TOLLE**

florian.tolle@univ-fcomte.fr

ThéMA UMR 6049 CNRS - Université de Franche-Comté,  
32 Rue Mégevand, 25030 Besançon Cedex.

### **David PLEYDELL**

dpleydel@univ-fcomte.fr

Laboratoire de Biologie environnementale EA3184 usc INRA,  
Université de Franche-Comté, Besançon.