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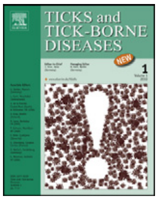

Original article

Reproducibility of local environmental factors for the abundance of questing *Ixodes ricinus* nymphs on pastures

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ABSTRACT

In ecology and epidemiology, exploratory field studies based on multivariate statistical models commonly are used to identify factors that are associated with a phenomenon. The challenge is to evaluate whether these factors are indeed correlated to the phenomenon or if the statistical significance results from fortuitous association or type 1 statistical error (probability of rejecting a null hypothesis when it is true). This is particularly the case when the phenomenon is linked to environmental factors that usually are more or less correlated to each other and when the phenomenon is itself highly variable. The abundance of *Ixodes ricinus* ticks, a major disease vector in Europe, is highly variable and depends on environmental conditions that define suitable habitat and host presence. Our objective was to identify reproducible factors in space and time that influenced the abundance of questing *I. ricinus* nymphs. We sampled questing nymphs in pastures in 4 settings, i.e. during 3 periods (2003, 2004, and 2006) in one region, and during one period (2006) in another region, both regions located in Central France. The same data collection, data selection, and model analysis using negative binomial distribution were applied independently in the 4 data sets to identify 'reproducible' factors, i.e. explanatory factors that were significant in different time periods and spaces. The 3 most reproducible factors suggested that woodland type vegetation and woodland vicinity constantly favoured nymph abundance on pastures. In addition, the presence of fruit trees was significantly associated with nymph abundance in one region. The other factors were not reproducible. The study confirmed the status of key factors for nymph abundance while avoiding having to redefine the statistical model to model the different sampling conditions. It also shows the difficulty to identify factors with general significance acting on a very variable phenomenon, based on a study made one year in one region. Relevant study design should consider appropriate repetitions based on the range of values of the studied factors. Furthermore, discussion of the results is essential to identify factors that have a general significance.

Introduction

In ecology and epidemiology, exploratory studies based on the collection of field data commonly are used to identify factors associated with a biological phenomenon. In such studies, the response variable (e.g., disease occurrence or species abundance) is recorded along with a set of potential explanatory factors collected from observations and/or measurements (e.g., Schwartz and Goldstein, 1990; Barnouin et al., 2005). Because such studies are labour intensive, they usually are performed only once. Associative statistical models are then run to identify the significant explanatory factors for the response variable. The challenge is to evaluate whether the significant factors are indeed associated to the biological phenomenon. The statistical significance of explanatory factors arises for 3 main reasons (Guisan and Thuiller, 2005; De Muth, 2009): (i) the factors are consistently associated with the phenomenon regardless of the study conditions, (ii) they are associated in a particular study through specific conditions, or (iii) they are part of a type 1 error of the model, i.e. the error of rejecting a null hypothesis when it actually is true. To assess the reproducibility of the identified factors under different conditions, several strategies can be used. The data set can be randomly divided into 2 parts; one part is used to explore the relationships between factors and to select a statistical model which is applied to the second part of the data set. However, due to the small size of the 2 data sets, this approach can lead to low statistical power. The data also can be difficult to model if several conditions are to be taken into account. Another strategy is to use the same methodology to analyze several data sets collected at various times and (or) in different spaces. The aim is to check whether independent results obtained with the same sampling design and on identical or very close site characteristics but under

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different conditions (different years, geographic conditions) are in agreement.

Ixodes ricinus ticks are major vectors of animal and human diseases in Europe (de la Fuente et al., 2008). Each of *I. ricinus* postembryonic life stages requires its own specific host type and minimal microclimatic conditions. The risk for a host to contract a tick-borne pathogen is associated with the density of infected questing ticks, in particular nymphs, which are the tick stage with the greatest epidemiological importance (Mather et al., 1996; Beytout et al., 2007). Identifying the most relevant environmental factors associated with questing *I. ricinus* nymphs' abundance will help to develop predictive risk models and target preventive and control measures against disease transmission.

As it is true for other parasites, the abundance of questing nymphs is highly variable and difficult to characterize (e.g., Vassallo et al., 2000; Jouda et al., 2004). It ultimately depends on the microclimate and the presence of hosts that together allow the tick to survive, to feed, and to reproduce (Randolph, 2004). Moreover, habitat and climate factors have an indirect influence on tick abundance because they determine vegetation and animal abundance and diversity (Sonenshine and Mather, 1994).

However, because these factors are complex to monitor precisely, studies commonly consider various vegetation and weather factors that characterize habitat in their stead (Estrada-Peña, 2002). As these factors generally are correlated closely to each other (Guisan and Thuiller, 2005), it is a challenge to distinguish which factors have a general significance from those that are linked to a specific setting.

Our objective was to assess which factors, collected during the sampling period, that influenced the abundance of questing *I. ricinus* nymphs in pastures located in Central France were reproducible.

We applied the same data collection, factors selection, and model analysis methodologies in 4 settings that varied in time (3 periods) and space (2 regions). We defined a 'reproducible factor' as being one that was significant and had the same effect across the 4 data sets. We hypothesized that reproducible factors would be more likely to have a 'generic' (verified in any situation or context) direct or indirect relationship with tick abundance than factors that only were significant in a specific situation.

Materials and methods

Data sets

The study was performed in 2 regions located in Central France, the Combrailles (45.8–46.2°N; 2.5–3.1°E) and the Cantal (44.9–45.3°N; 2.1–2.5°E). The 2 regions are characterized by a patchwork landscape of woodlands and pastures for ruminants, a continental climate, and an elevation that ranges between 400 and 850 m.

The Combrailles and Cantal pastures studied were selected randomly from a list of cattle farms possessing over 30 animals in the exhaustive farm database provided by the 'Etablissement Départemental de l'Elevage'. At each selected farm, we selected a permanent pasture on which at least 5 heifers grazed for at least 3 months over the 2 years preceding the study. The initial sampling (CO-P1) was run during the period P1 from April 23 to June 11, 2003, on 61 grazed permanent pastures located in the Combrailles (CO) (Boyard et al., 2007). The second sampling (CO-P2) concerned the same pastures and took place during P2 from April 26 to June 8, 2004. The third sampling (CO-P3) took place during P3 from May 17 to June 16, 2006, and involved a subset of 30 pastures that were selected randomly from the P1 to P2 pastures. The final sampling (CA-P3) was implemented during P3 in 30 pastures located in Cantal (CA). Drag sampling was done along the inner perimeters of the pasture using a 1 m^2 cloth (MacLeod, 1932) on a 10-m^2 surface unit known as a sub-transect (ST) (Vassallo et al., 2000). Each ST was separated from the next one by 20 m to ensure statistical independence. Ticks were collected, sorted into nymphs and adults, counted, and placed in vials with 70% ethanol for later species identification. The CO-P1 data set included 1089 STs, the CO-P2 1050 STs, the CO-P3 503 STs, and the CA-P3 757 STs.

For each data set, explanatory factors involved meteorological data of the sampling day, pasture topography, presence of heifers on the pastures, and tree and bush species at the pasture perimeter, in addition to date and hour of sampling. These factors were mostly collected during the sampling period. We acknowledge that factors occurring outside of the sampling period might play a role in determining tick abundance. However, our choice was made (i) because of logistic reasons, (ii) because such process is easily repeatable, and (iii) because we supposed that we have taken them into account indirectly. Factors outside of the sampling period, such as climatic factors in the last years, influence factors that we collected such as vegetation characteristics or fruit production.

Meteorological data obtained from the 'Météo France' stations for the Combrailles (2.8°E; 46.0°N) and for the Cantal (2.3°E; 45.3°N) included local humidity and temperature measured on the ground during tick collection, percentage of cloud cover, and general meteorological data, including the total amount of rainfall and daily mean temperatures and humidity (HUM) over the 10 days before sampling. Topography included pasture altitude and size, presence of water within the pasture, percentage of pasture perimeter with a tree layer (PP-TREE) and with a bush layer (PP-BUSH), and the number, perimeter, and surface area of woodlands within a 5-km radius around the pasture. This information was obtained from the Corine Land Cover data-base (CLC2000, 100 m, European Environment Agency). We also recorded 3 ST factors: the characteristics of the STs' outside border (i.e. everything that lay within 20 m of the ST border: a combination of trees and bushes, only trees, only bushes, or other), the ST-nearest woodland distance (whether or not the woodland nearest to the ST lay less than 20 m away), and ST grass height. Host populations were evaluated through county-level roe deer (*Capreolus capreolus*) and wild boar (*Sus scrofa*) hunting tables for each hunting season preceding the tick sampling, through the number of cattle on the pasture during the preceding spring season, and through the presence of cattle during sampling. In addition, the abundance of *I. ricinus* nymphs in the woodland nearest to each pasture (NY-WOOD) was evaluated from the total number of nymphs collected on 10 randomly selected STs. As vegetation factors, we used the 2 main tree species and bush species growing along the pasture perimeter. The presence on the pasture perimeter of each tree species carrying fleshy fruits was considered separately and together in the factor 'fleshy fruit tree presence'.

Finally, 59 factors were analysed as potential factors for nymph distribution in the CO-P1 and CO-P2 data sets, and 54 factors were considered in the CO-P3 and CA-P3 data sets. The difference between data sets arises from the number of vegetation factors.

Statistical analysis

We applied the same analysis strategy independently in the 4 data sets. Quantitative factors were categorized into 2 or 3 categories of equal size. We used negative binomial models that are frequently used to model aggregated parasite distribution (Shaw and Dobson, 1995). Before running such models, a factor selection was done, as these models do not converge when too many factors are tested together or when factors are too much correlated (McCullagh and Nelder, 1989). The modelling process consists of 3 steps (Dohoo et al., 2009). First, the factors were tested using uni-