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Assessment of the risk of infestation of pastures by *Ixodes ricinus* due to their phyto-ecological characteristics

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Abstract – This study aims to assess the relationships between the phyto-ecological characteristics of grazing pastures and their risk of being infested by the hard tick *Ixodes ricinus*. To that purpose, 128 pastures belonging to 20 dairy farms were observed from April to July 1994. The farms were located in western France. Assessment of the tick population was made by four monthly, blanket-dragging sessions. The average infestation rate was 40.2 %. Six types of pastures were identified according to their basic phyto-ecological characteristics using a factorial analysis of correspondences followed by a hierarchical ascending classification. These identified types were significantly related to the infestation rates, thereby making it possible to provide a predictive value in risk assessment. The infestation rates were high (96 % on average) in two types of pastures characterized by their proximity to woods, and low (13 %) in two other types characterized by seeded grass species located at some distance from woods, and intermediate (39 % on average) in the last two types. © Inra/Elsevier, Paris

Ixodes ricinus / **pasture / France**

Résumé – Évaluation du risque d'infestation de parcelles de pâturage par *Ixodes ricinus* à partir de leurs caractéristiques phytoécologiques. L'étude a pour objectif d'évaluer les relations entre les caractéristiques phytoécologiques de parcelles de pâturage et leur infestation par *Ixodes ricinus*. À cette fin, 128 parcelles ont été observées dans 20 exploitations bovines de l'ouest de la France. 40,2 % des parcelles ont été identifiées comme infestées par quatre passages de détection à l'aide de la méthode du drapeau, réalisés d'avril à juillet 1994. Les caractéristiques phytoécologiques ont permis de définir six types de parcelles par une analyse factorielle des correspondances suivie d'une classification hiérachique ascendante sur les six premiers facteurs. Ces

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types de parcelles sont associés à trois niveaux de taux d'infestation très différents : 96 % en moyenne pour les deux types les plus infestés qui correspondent aux parcelles proches de bois de feuillus ou de conifères, 13 % en moyenne pour les deux types les moins infestés qui correspondent à des parcelles de prairie artificielle, éloignées de tout bois, et 39 % en moyenne pour les deux types intermédiaires, qui correspondent principalement à des parcelles bordées par des haies et à des prairies naturelles.

***Ixodes ricinus* / pâturage / France**

1. INTRODUCTION

Ixodes ricinus (Acari, Ixodidae) is known in Europe to be a vector of human disease, such as viral encephalitis [4] or Lyme disease due to *Borrelia burgdorferi* [23]. *I. ricinus* is also responsible for the transmission of animal disease agents in north-western Europe. The tick is particularly implicated in the epidemiology of bovine babesiosis due to *Babesia divergens* [29]. As a result of its role in human health and, more frequently, its economic impact as a vector of animal diseases, the biology and activity cycle of *Ixodes ricinus* have been previously studied in the north-western part of Europe: in Ireland [15], in Wales [2, 7, 9], in England [2, 16, 26, 27], in Switzerland [1, 24, 25], and in the Netherlands [28].

In France, studies have been carried out in forested environments, in Alpine areas in the south-east [10–13] and in areas where a more continental climate is prevalent, in the north-east [30].

These studies suggested the existence of significant differences in seasonal tick activity patterns which are linked to the phyto-ecological conditions of the area. Recently, a survey carried out over 2 years in the area of Sarthe (western France) provided the first information about the seasonal activity of *I. ricinus* in the pastures of western France where there is an oceanic climate [18]. Nevertheless, these observations needed to be validated with a larger sample under the same conditions.

The control of disease transmission to grazing cattle can depend on either chemoprophylaxis or on preventing exposure of susceptible grazing animals to tick infestation. In the second type of control method, the ability to make a cursory assessment of the probability that a given pasture, may be infested by *I. ricinus* would be very practical.

Therefore, our study aimed at assessing the relationships between the infestation intensity of grazing pastures for cattle by *I. ricinus* in western France and the phyto-ecological characteristics of these pastures. Special interest was given to identifying the predictable characteristics that could easily be used in routine assessments of the infestation risk for a given pasture.

2. MATERIALS AND METHODS

2.1. Sample and study period

The study was carried out in the area of Sarthe (western France) because of the previously reported high prevalence of bovine babesiosis due to *Babesia divergens* [17] in that area. Data were collected from April to July 1994. This 4-month period was chosen because it would give us the best chance to observe the peak activity of *I. ricinus* in the pastures, under the geoclimatic conditions of this area [18].

The unit of observation was the pasture. All the grazing pastures of 20 farms were included. To ensure a sufficient variability in the assumed prevalence of infestation of the pastures by *I. ricinus*, the selection of farms included ten farms which had experienced at least one case of clinical bovine babesiosis within the last 5 years and ten farms without any documented case in the last 5 years. However, to minimize the differences in weather conditions, all the farms were located in the same area (maximum distance: 40 km).

2.2. Data collection

There are several methods for measuring the intensity of tick infestation in pastures [14]. In their studies, L'Hostis et al. [18–20] assessed three methods (counting the ticks on cattle and micromammals, blanket dragging over pastures) simultaneously. These three methods, given the methods used, resulted in similar conclusions, even when there was little infestation.

Assessment of *I. ricinus* density in pastures was performed by the blanket-dragging method described by Macleod [21] and following the protocol defined by L'Hostis et al. [18]. Every month, a white flannel blanket (230 x 125 cm) was dragged across the pasture over a distance of 90 m, choosing the side of the pasture with the most dense vegetation. After collection, the ticks were put in vials containing alcohol to allow for further identification and more precise counting. Dragging was carried out once a month from April to July after 11 a.m., to avoid the dew effects [34]. Parallel samples were carried out the same week in all the farms.

To describe the pastures, nine variables were used (*table I*). Except for the geological type of soil, these characteristics were visually assessed directly by the investigator in June. The geological type of soil was determined using geological maps (nos 322, 358, 393) published by the Bureau de Recherches Géologiques et Minières, Service Géologique National (Orléans, France). Three types were defined: 1) clay and calcareous soils with a pH of over 7.2 and containing a large amount of water; 2) colluvial deposits with a pH ranging from 5.7 to 6.0 and having an intermediate level of water reserve; 3) all other types, mostly sandy soils, with low clay content, various levels of pH, and with low water reserves.

2.3. Data analysis

2.3.1. *Tick infestation of pastures*

Results of tick counting were calculated for each dragging. The cumulative results of counting for a given pasture were then used first to define a qualitative yes/no infestation variable: a pasture was declared infested if at least one dragging was positive (i.e. at least one *I. ricinus* was found during one counting). For the sample, this variable made the calculation of the overall infestation rate possible for the pastures. A quantitative variable assessing the intensity of the infestation for a given pasture was also calculated. This intensity was obtained by totalling the counts of adults and nymphs of *I. ricinus* that were found during the four dragging evaluations.

2.3.2. *Characterization and definition of phyto-ecological type of pastures*

Univariate analysis of pasture characterization variables was performed first. Bivariate analyses were then run for these variables to identify redundant variables. After elimination of one of the variables found bilaterally redundant [6] a factorial analysis of correspondences (FAC) was run. FAC provides a new set of quantitative variables (coordinates on factorial axes) which summarize the information from the previous categorical variables included as active variables [8]. These new variables were then submitted to the hierarchic ascending classification (HAC) algorithm to determine the phyto-ecological types present in our sample. HAC is a method that defines groups of observations, based on the maximization of the inter-group variance and the minimization of the intra-group variance [32]. The number of groups finally obtained depends on the number of nodes kept by considering the dendogram of distances between these nodes.

Table I. Description of grazing pastures.

Variable	Levels and definition	Absolute frequency
Dominant grass species in pastures		
	1) rye-grass	31
	2) seeded Graminae and white clover	50
	3) native grass species	47
Willow or poplar trees in pastures		
	1) absence	89
	2) at least one willow or poplar tree	39
Oak or chestnut trees in pastures		
	1) absence	103
	2) at least one oak or chestnut tree	25
Small vegetation in pastures		
	1) absence	52
	2) presence of thorns and/or ferns	76
Average thickness of the pasture hedges		
	1) none or discontinuous hedges	42
	2) hedges (less than 5 m wide)	50
	3) hedges (more than 5 m wide)	36
Nearness of a wood with deciduous trees		
	1) absence (or distance > 250 m)	93
	2) presence (less than or to 250 m)	35
Nearness of a wood with conifers		
	1) absence (or distance > 250 m)	116
	2) presence (less than or to 250 m)	12
Water in pastures		
	1) absence	63
	2) presence of a pond, brook or river	65
Geological type of soil		
	1) clay and calcareous, pH >7.2	31
	2) colluvial deposits, pH of 5.7 to 6.0	49
	3) other soils*	48

2.3.3. Relationships between pasture phyto-ecological type and infestation by I. ricinus

Differences in the proportion of infested pastures for the phyto-ecological types were assessed by a Chi-square test.

2.3.4. Statistical software used

Factorial analysis of correspondences, hierarchical ascending classification and Chi-square tests were run on the Statlab package [33].

3. RESULTS

A total of 128 pastures were grazed by at least one part of the herd during one cycle of grass growth on the 20 farms, and were included in the study. However, seven pastures were only investigated three times by dragging and, of the seven, only one had a positive tick cumulative count, the six others were negative during the three evaluations. These six pastures were excluded from steps of the study dealing with infestation description and analysis.

3.1. Infestation of pastures by *Ixodes ricinus*

Numbers of positive tick counts obtained by the dragging technique were quite similar from May to July: 25 in May, 26 in June and 29 in July. Conversely, in April only four pastures had a positive count. The distribution of the total number of *I. ricinus*, collected during the 4 months whatever the stage is in *table II*.

The overall infestation rate of the pastures was 40.2 % (calculated from 122 pastures). The intensity of infestation varied from 0 to 22 units between different months. Mean and standard deviation were 1.0 and 2.7, respectively. The distribution was strongly skewed (*figure 1*).

Only a small proportion of pastures had more than two positive counts: two infested pastures had four positive counts, and four had three positive counts (*figure 2*).

3.2. Characterization and definition of the phyto-ecological pasture types

Table I displays the absolute frequency of the several levels for the characterization variables. The samples were gathered from quite different pastures with each variable, except for respect to their nearness to a conifer wood and the presence of oak or chestnut trees in the pasture, for which one level was clearly more prevalent.

Because of its redundancy with other variables, the variable accounting for the edge thickness was deleted. FAC was run on the eight remaining variables. Six principal axes were retained to perform the HAC. This number was chosen to account for 80.3 % of the total inertia. Finally, six classes of phyto-ecological type were identified. Their frequency varied from 8.2 to 27.9 % of the total number of pastures (*table III*).

3.3. Relationships between pasture phyto-ecological type and infestation by *I. ricinus*

Given the results in frequency of detecting *I. ricinus* in an infested pasture, no analysis of the relationships between

Table II. Total number of harvested *Ixodes ricinus*.

Month	Larvae	Nymphs	Adults	Total
April	0	8	0	8
May	6	44	8	58
June	46	37	1	84
July	458	29	0	487
Total	510	118	9	637

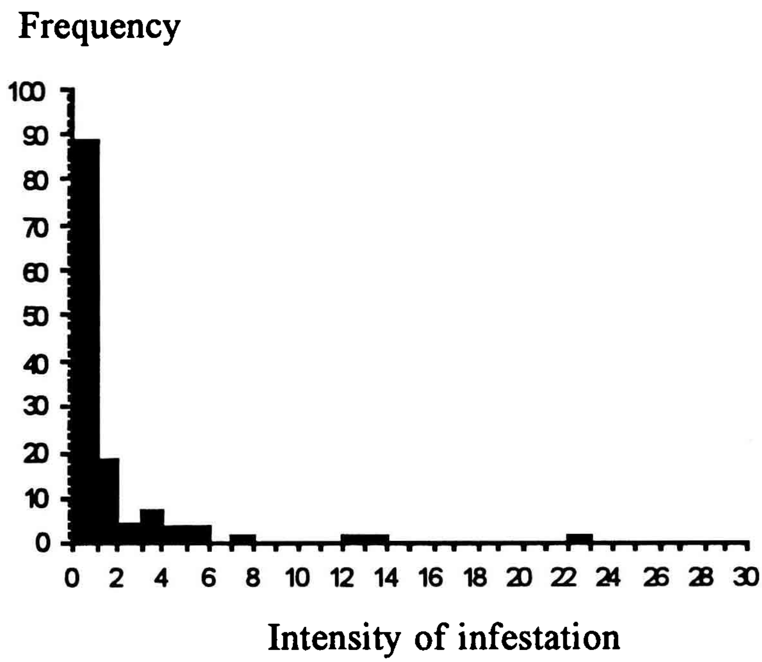

Figure 1. Distribution of the pasture infestation intensity (sum of the counts of nymph and adult) by *Ixodes ricinus*.

the infestation intensity parameter and the phyto-ecological type was undertaken and only the yes/no infestation parameter was investigated.

Table III displays the infestation rates for each phyto-ecological pasture type. Proportions of infested pastures differed significantly ($P < 0.05$) between types. Very high rates were observed for types B and E. Conversely, the rates were very low in types A and C. Types D and F showed intermediate infestation rates. The analysis of differences in groups of two finally made it possible to identify three groups with different infestation rates:

1) high infestation rate: types B and E (96 % on average);

2) intermediate infestation rate: types D and F (39 % on average);

3) low infestation rate: types A and C: (13 % on average).

4. DISCUSSION

This study provided a description and a classification of the different grazing pastures in an area of western France, based on their phyto-ecological characteristics. Types obtained from the classification differed significantly in the infestation rate by *I. ricinus* as determined by the blanket-dragging technique.

In the pastures, and given our methods used [18, 26], the blanket-dragging technique allowed us to detect mainly active

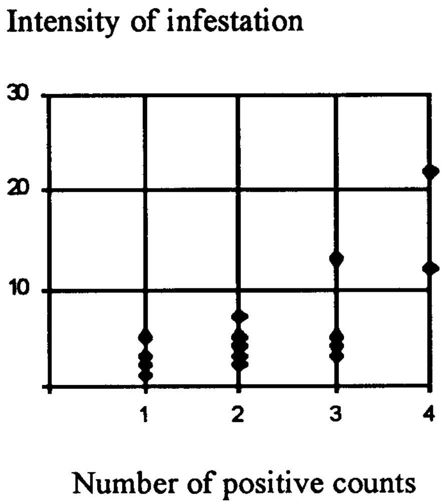

Figure 2. Intensity of *Ixodes ricinus* pasture infestation (sum of the counts of nymph and adult) by the number of positive counts from four blanket-dragging detection processes.

nymphs and larvae, the latter of which were often found in cluster activity [7, 15, 26].

A comparison with the results of L'Hostis et al. [18] can be made as our protocol is based on their study.

To limit the consequences of the non-dispersion of larvae from clusters, some authors have chosen to exclude larvae from the counts [30] and others preferred to increase the number of detections to improve the accuracy [31]. We have adopted here the first solution and decided to use four monthly detections on the same approximate location within the pasture (for the 90 m dragging distance). A relevant quantification of infestation density taking the larvae into account was not deemed feasible.

Comparison of our results with those reported for the same period (April to July) on a smaller pasture sample L'Hostis et al. [18] shows that the present study had lower values for the infestation rate and especially low detection rates in April. This phenomenon was correlated with the climatic conditions observed during the first 3 weeks of this month, when there were higher than average precipitation levels (40 % increase) and average temperatures below annual mean values (difference of 1.7 °C). Several other studies have observed such meteorological effects on tick activity [1, 3, 11, 25, 30, 34]. Nevertheless, despite the lower values in unitary counts, the overall shape of the distribution curve for the intensity of infestation (*figure 1*) was similar to that found by L'Hostis et al. [18].

Unfortunately, we failed to show a positive relationship between the number of counts where at least one tick was found and the total intensity of infestation parameter (*figure 2*). This discrepancy led us to restrict the investigation on relationships between the phyto-ecological characteristics of pastures and their infestation with *I. ricinus*. Only the results of the qualitative (yes/no) assessment of infestation of pastures were considered.

Defining the overall types of pastures from their basic phyto-ecological characteristics was chosen because of the colinearity between these characteristics. In such a case, a regression method could also have been used, but could have resulted in a division of effects among the dependent correlated variables [6]. We used a factorial analysis in the first step of type identification. This factorial analysis did not demonstrate the existence of strong axes. In order to keep 80 % of the total inertia in the new quantitative variables, we selected six axes. Usually, for eight initial variables, a lower number of axes explaining a higher percentage of inertia would have been expected [5]. However, this confirmed that our preliminary elimination of a redundant variable

Table III. Phyto-ecological types of pastures and tick infestation rates by type.

Type characterization by levels of variables more frequently prevalent	Frequency (%)	Infestation (% within type)
A) rye-grass; absence of willow, poplar, oak and chestnut trees in the pasture; absence of wood within 250 m	18.9	13.0
B) near conifer wood; presence of pond, brook or river; soil of type 3[1]	8.2	90.0
C) seeded Graminae and white clover; none or discontinuous hedges; thorns or ferns; absence of wood within 250 m; soils of types 2[1] or 3[1]	18.0	13.6
D) seeded Graminae and white clover or other grass species; hedges; thorns or ferns; presence of willow or poplar trees; absence of oak or chestnut trees; presence of a pond, brook or river; soils of type 2[1] or 3[1]	27.9	38.2
E) near a wood with deciduous trees; presence of oak or chestnut trees; absence of a willow or poplar tree; absence of a pond, brook or river; soils of type 2[1]	10.7	100
F) native grass species; absence of a conifer wood within 250 m; no tree in the pasture	16.4	40.0

[1] For definition, see text.

was efficient for preventing the consequences of too much colinearity.

Characteristics of pastures belonging to the phyto-ecological types with the lowest and the highest infestation rates were found to agree with the data about *I. ricinus* biology. A deep vegetal carpet makes such conditions possible [2, 27]. Therefore, forests, undergrowth and their vicinity seem to be the favourite environment for *I. ricinus* activity in European countries [1, 2, 11, 18, 21, 22, 29]. In our study, 96 % of the pastures located near a wood were infested.

Geological characteristics of soils may indirectly influence their infestation by ticks owing to their draining capacity and thereby the selection of vegetation [2]. However, in our study very different rates of infestation were found for soils of the same type. We were dealing with grazing pastures that deviated to varying extents from natural conditions due to modifications made by generations of farmers. The role of geological soil characteristics may thus be smaller [27].

In conclusion, it seems that the phyto-ecological characteristics of grazing pastures in western France would make it possible to assess their risk of being infested by *I. ricinus*. Pastures located in the close vicinity of a wood containing deciduous or conifer trees, whatever their soil type or vegetation are at a six- to seven-fold increased risk than pastures without a wood in the close vicinity and consisting of seeded grass species. An intermediate risk level is related to the presence of large hedges and of naturally growing grass species.

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