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# Unravelling the phenology of *Ixodes frontalis*, a common but understudied tick species in Europe

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ABSTRACT

Tick-borne diseases have a complex epidemiology that depends on different ecological communities, associating several species of vertebrate hosts, vectors and pathogens. While most studies in Europe are focused on *Ixodes ricinus*, the common sheep tick, other *Ixodes* species may also be involved in the transmission or maintenance of different pathogens. In this study, we describe for the first time the activity pattern of *I. frontalis*, an understudied but widespread tick species associated with several common bird species in Europe. Questing ixodid tick stages (larvae, nymphs and adults) of both *I. frontalis* and *I. ricinus* were monitored by the drag sampling method over three years at the same locations in Western France. Differential activities were observed depending on *I. frontalis* life stages: nymphs and adults were present sporadically on the ground throughout the year, while larvae exhibited a marked peak of activity around October-November with tens or even hundreds of individuals per m$^2$, followed by a slow decrease in winter. Larvae were completely absent in summer, which contrasts with the high numbers of *I. ricinus* larvae at this time of the year. The vegetation and the litter where the two tick species were found also exhibited marked differences, with *I. frontalis* mostly collected under bamboo bushes.

## 1. Introduction

Vector-borne diseases constitute a major concern for both human and animal health. In temperate zones, hard ticks are considered to be the most important vectors in terms of diversity of pathogens, including zoonotic agents (such as *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, tick-borne encephalitis virus, *Babesia* spp.). They are also the vectors with the highest economical and medical impact on animals and Man (McCoy and Boulanger, 2015; Sonenshine, 1993). The transmission cycles of tick-borne pathogens are complex, involving generally several host species and sometimes more than one tick species (Estrada-Peña and de la Fuente, 2014; Kurtenbach et al., 2006; Mannelli et al., 2012; McCoy and Boulanger, 2015). Therefore, the study of a particular tick-borne pathogen requires an integrated community approach, in a diversity of host and tick species.

European research has only recently started to thoroughly investigate the role of tick species other than *I. ricinus* in the transmission of *I. ricinus*-associated pathogens (Heylen et al., 2017b, 2014b, 2013; Jahfari et al., 2014; Obsomer et al., 2013). In Europe, the generalist *I. ricinus* is the most important vector to which humans are exposed, but some of the pathogens carried by this species can originate from other tick species, where they may exhibit a higher prevalence (Heylen et al., 2017b; Literak et al., 2015). When *I. ricinus* shares the hosts on which other tick species feed, pathogens can be bridged towards humans (Bown et al., 2008; Heylen et al., 2017b). Many of the other tick species within the tick community have been neglected, either because (i) they are rare or difficult to collect (*e.g.,* endophilic ticks), (ii) they are easily misidentified (Estrada-Peña et al., 2017a) or (iii) because they are not known to affect Man or animals of interest. This lack of interest leads to probable underestimation of their presence and of their real implication in pathosystems. The knowledge of basic ecological life history parameters of those understudied species, including their seasonal activity patterns, is crucial to understand the role of such tick species in tick-borne disease epidemiology. Ticks associated with birds, such as *I. frontalis* or *I. arboricola*, are of particular interest in this context because (i) they are known to carry several zoonotic pathogens such as *Anaplasma phagocytophilum*, *Borrelia*, or *Rickettsia* spp. (Heylen et al., 2017a), (ii) birds participate in the dissemination of ticks and their associated pathogens - at long distance but also in urbanised regions that are less accessible to migrating mammals (de la Fuente et al., 2015;

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Heylen, 2016) - and (iii) immature stages of *I. ricinus* - the most frequently reported species on humans - commonly feed on birds (Hornok et al., 2013), acting as a potential bridge species with possible transfer of pathogens between birds and mammals.

In this article, we investigate seasonal activity patterns of the ornithophilic tick *Ixodes frontalis*, an abundant species from Europe (Estrada-Peña et al., 2017b), however with a poorly-investigated phenology of its free stages and with a largely unknown ecology (Heylen, 2016). *I. frontalis* is commonly found feeding on a great diversity of bird species that are shared with *I. ricinus* (Cringoli et al., 2005; Doby, 1998; Martyn, 1988; Monks et al., 2006; Norte et al., 2012). It has also been reported to exceptionally bite humans (Cull et al., 2018; Gilot et al., 1997). However, it has rarely been found questing on the ground (but see Barandika et al., 2006; Bona and Stanko, 2013; Ceballos et al., 2014; Doby, 1998; Gilot et al., 1997, 1995; Schorn et al., 2011), possibly because it is easily overlooked, especially for the larval stages that are difficult to identify due to their small size (Heylen et al., 2014a). *I. frontalis* is also known to be implicated in direct (*i.e.,* without infectious agents) paralytic and mortality phenomena in certain birds (Mans et al., 2004; Monks et al., 2006), in the transmission of Chizé virus, fatal for birds (Chastel et al., 1999) and on the possible contribution to the maintenance of 'Candidatus *Neoehrlichia mikurensis*' (Movila et al., 2013), *Anaplasma phagocytophilum* (Jahfari et al., 2014) or *Borrelia burgdorferi* s.l. (Heylen et al., 2017a, 2013; Norte et al., 2013) in bird populations. As *I. ricinus* may bridge *I. frontalis*-related pathogens towards humans, the investigation of co-occurrences of both tick species in time and space, which will be described in this study, is of prime importance.

## 2. Material and methods

A study site was selected in spring 2014 near Nantes (47°19′N, 01°29′W) to survey exophilic hard tick densities on vegetation, estimated with the drag sampling method: a wooded private park was chosen for its high density of *I. ricinus* nymphs. Ticks were collected one day per month from June 2014 to May 2017 (Supplementary material I and II), during the second half of each month as regularly as possible (the collection could not occur on rainy days), by 1 to 4 operators, between 13:30 and 18:00. To collect questing ticks, ten marked transects of 10 m x 1 m were chosen along trails covered by short grass or a leaf litter, with a minimum distance of 20 m between them (Fig. 1). Tick collection was repeated 3 times consecutively on the same marked transects, in order to collect more individuals and to improve accuracy in the assessment of tick abundance (Bord et al., 2014). On each transect, a 1 m$^2$ white flannel cloth was slowly dragged (0.5 m/s) along the 10 m transect (Agoulon et al., 2012): ticks were counted, removed from the cloth with tweezers and stored alive (except larvae, which were stored in 70% ethanol for further identification from February 2015 onwards – see below) for further identification concerning stage and species according to the keys provided in Heylen et al. (2014a), Hillyard (1996) and Pérez-Eid (2007). Larvae were initially only counted and then discarded, without collection and identification. They were systematically collected and identified under an optical microscope (100x magnification) from February 2015 onwards.

The park was characterized by mainly deciduous trees (*Quercus* spp., *Betula* sp.), the presence of two ponds, a humid peaty soil (with *Carex* spp., *Salix* sp., *Myrica gale*) and several non-native plants along different parts of the trails, including bamboo (*Phyllostachys* spp.). The ten transects presented marked differences in terms of habitat: three transects (hereafter called "Transects with bamboo": n°8 to 10) were surrounded and overhung by bamboo and presented a litter covered with bamboo leaves (Fig. 1). The ground of the seven other transects (hereafter called "Transects without bamboo": n°1 to 7) was either a litter of deciduous (n°2, n°3, n°5) or coniferous needle leaves (n°5), or short (< 15 cm) grass (n°1, n°4, n°6, n°7) (Fig. 1).

A meteorological station was installed in January 2015 in a wooded part of the study site (Fig. 1), recording hourly macroclimatic data exploited at the level of the whole site: temperature, hygrometry and wind speed. Saturation deficit was calculated from temperature and hygrometry according to Hartmann (1994). Mean values of these four meteorological factors were considered as explanatory variables for analyses, at different time intervals: 0–30 days, 0–10 days and 0–5 days prior to the day of tick collection. Thus, twelve meteorological variables were considered, crossing mean values of four factors and three time intervals (Table 1).

The temporal dynamics of the different stages of *I. frontalis* was described at the level of the whole study site. For the larval stage, to evaluate the influence of meteorology, the relationship between tick counts and meteorological variables was considered from February 2015 to May 2017 by Spearman's rank correlation, and the best relationship was selected according to the rho and its p-value. After log transformation of larval counts (log(larval count + 1)), because of the overdispersion of the data, a general linear model was built to assess the influence of this selected meteorological variable, based on R$^2$. In

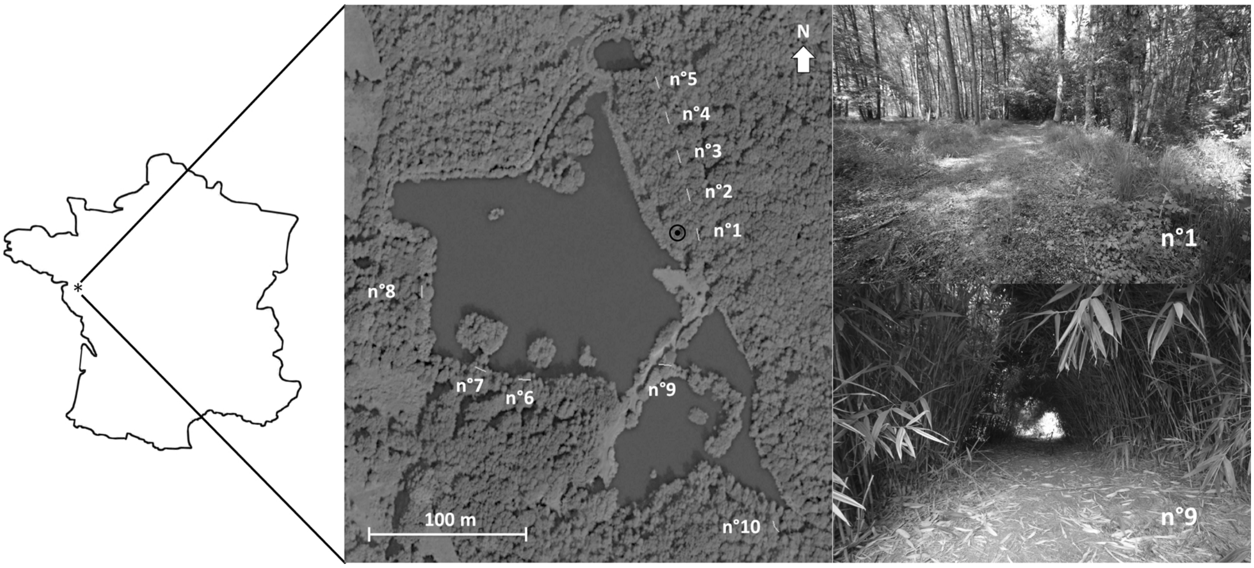

**Fig. 1.** Geographical position (*) of the study site in France (left). Aerial photograph of the observatory with position (⊙) of the meteorological station (center). Two transects, illustrating transects without (n°1) or with (n°9) bamboo (right).

**Table 1**

Relationship between *Ixodes frontalis* or *Ixodes ricinus* larval counts and meteorological variables or day length, attested by Spearman's rank correlation.

| | 0-30 days* | 0-10 days* | 0-5 days* |
|---|---|---|---|
| a) *Ixodes frontalis* | | | |
| Mean hygrometry | **p = 0.003**<br>**rho = 0.534** | p = 0.008<br>rho = 0.494 | p = 0.008<br>rho = 0.488 |
| Mean temperature | p = 0.002<br>rho = -0.565 | **p < 0.001**<br>**rho = -0.672** | p < 0.001<br>rho = -0.645 |
| Mean wind speed | p = 0.657<br>rho = -0.088 | p = 0.407<br>rho = -0.163 | p = 0.597<br>rho = -0.104 |
| Mean saturation deficit | p = 0.001<br>rho = -0.597 | **p < 0.001**<br>**rho = -0.623** | p < 0.001<br>rho = -0.616 |
| Day length (on the day of tick collection) | | p < 0.001<br>rho = -0.808 | |
| b) *Ixodes ricinus* | | | |
| Mean hygrometry | **p = 0.002**<br>**rho = -0.560** | p = 0.010<br>rho = -0.480 | p = 0.048<br>rho = -0.377 |
| Mean temperature | p < 0.001<br>rho = 0.668 | **p < 0.001**<br>**rho = 0.717** | p < 0.001<br>rho = 0.680 |
| Mean wind speed | p = 0.108<br>rho = -0.310 | p = 0.039<br>rho = -0.392 | **p = 0.030**<br>**rho = -0.412** |
| Mean saturation deficit | **p < 0.001**<br>**rho = 0.704** | p < 0.001<br>rho = 0.599 | p = 0.002<br>rho = 0.553 |
| Day length (on the day of tick collection) | | p = 0.004<br>rho = 0.527 | |

*prior to the day of tick collection.

For each meteorological factor and time interval prior to tick collection, and for day length on the day of tick collection, the relationship with larval counts was estimated by Spearman's rank correlation, with indication of p-value (p) and rho (rho). For each meteorological factor, the cell in bold indicates the most relevant time interval to be considered. The cell with the best correlation is underlined.

Meteorological variables, day length and larval counts were considered from February 2015 to May 2017 (28 dates) for the whole observatory.

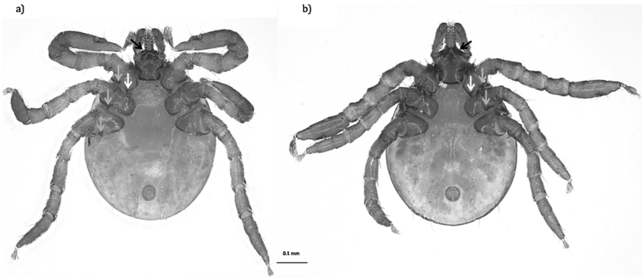

**Fig. 2.** Distinction of *Ixodes frontalis* (a) and *Ixodes ricinus* (b) at the larval stage (light microscopy).

*Ixodes frontalis* larvae (a) have a marked protuberance on the front margin of the basis of the capitulum, between palp and hypostome (black arrow), external spurs on coxa I, II and III (grey arrows), and an internal spur on coxa I (white arrow).

*Ixodes ricinus* larvae (b) have a straight front margin of the basis of the capitulum, between palp and hypostome (black arrow), small external spurs on coxa I and II (grey arrows), and an internal spur on coxa I (white arrow).

parallel, to evaluate the influence of regular seasonal phenomena, the relationship between *I. frontalis* larval counts and day length on the day of tick collection was estimated in the same manner, leading to a similar model, hereafter called "seasonal model". Finally, 4 models were compared, the best one being selected according to the lowest Akaike information criterion (AIC): 1) "meteorological model" (1 selected meteorological variable); 2) "seasonal model" (1 variable: day length); 3) "combined model" (1 meteorological variable and day length); 4) "combined model with interaction" (1 meteorological variable, day length and interaction between them). The same statistical process was applied to the larval stage of *I. ricinus* for comparison.

The distribution of the different stages of *I. frontalis* was described at the study site for each transect to evaluate spatial distribution. It was compared to *I. ricinus* on two types of transects: with bamboo *versus* without bamboo. Comparisons were performed by bilateral Fisher's exact test. All analyses were carried out in R version 3.4.1 (R Core Team, 2017).

## 3. Results

### 3.1. *Temporal dynamics of* I. frontalis

From September to November 2014, an increase in the density of questing larvae was observed on transect n°9 (*i.e.,* 14 larvae in September, 1230 in October, more than 3000 (rough count) in November and 466 in December 2014). As at that time of the study larvae were assumed to belong to *I. ricinus*, they were just counted and discarded without prior identification. In January 2015, larvae of transect n°9 (n = 490) were kept and all were identified as *I. frontalis* (Fig. 2). Since that date, larvae from the whole study site were then stored and identified for the remaining 28 months of the study (from February 2015 to May 2017). Larval questing seasonality of *I. frontalis* could therefore be assessed for that period: a marked peak of activity of *I. frontalis* larvae was observed around October-November, followed by a slow decrease in winter and by the absence of larvae in summer (Fig. 3a and Supplementary material I). Assuming that most of the larvae collected earlier than January 2015 on transect n°9 belonged to *I. frontalis* emphasizes this observed seasonality.

All meteorological variables were correlated to *I. frontalis* larval counts, except mean wind speed. The best correlation concerned mean temperature over 10 days prior to tick collection (Table 1: Spearman's rank correlation rho = -0.6721; p-value = $8.955 \times 10^{-5}$): larvae could be abundant (n > 50) only when the mean temperature did not rise above 12 °C over the previous 10 days ($R^2$ of the corresponding meteorological model = 0.4693) (Fig. 4a). Day length was more correlated to larval counts (Spearman's rank correlation rho = -0.8078; p-value = $2.027 \times 10^{-7}$): larvae could be abundant (n > 50) only when day length was below 12 h ($R^2$ of the corresponding seasonal model = 0.7531) (Fig. 4b). Meteorological and combined models (with or without interaction) exhibited a higher AIC than the seasonal model: the seasonal model was therefore selected for *I. frontalis* larvae, day length explaining 75.3% of the variance (Table 2). In comparison, the same process led to the selection of the meteorological model for *I. ricinus* larvae, mean temperature over 10 days prior to tick collection explaining 53.3% of the variance (Table 1, Fig. 4c and d, Table 2).

Besides *I. frontalis* larvae, for which thousands of individuals were found, only a few individuals of *I. frontalis* were identified at the nymphal (n = 28) and adult (n = 5, all males) stages, with no clear seasonality (Fig. 3b, c and Supplementary material I). The relative rarity of those nymphal and adult stages of *I. frontalis* contrasts with the abundant collection of those stages for *I. ricinus* ticks (Supplementary material II).

### 3.2. *Spatial distribution of* I. frontalis

*I. frontalis* stages were clustered in 3 main transects (n°8, 9, 10), with occasional presence (in decreasing order of importance) on transects n°5, n°2, n°3, n°6 or n°7 (Supplementary material I). The distribution of *I. frontalis* was significantly different from that of *I. ricinus* when considering on the one hand transects with bamboo (n°8 to 10) and on the other hand other transects (n°1 to 7) (Fisher's exact tests: $p < 2.2 \times 10^{-16}$ for larvae, $p = 6.9 \times 10^{-6}$ for nymphs and p = 0.0161 for adults) (Table 3). Considering the ratio of *I. frontalis versus I. ricinus*, places with bamboo appeared to be more favourable to *I. frontalis*, with the highest contrast for larvae (O.R. = 842 (C.I.95% =

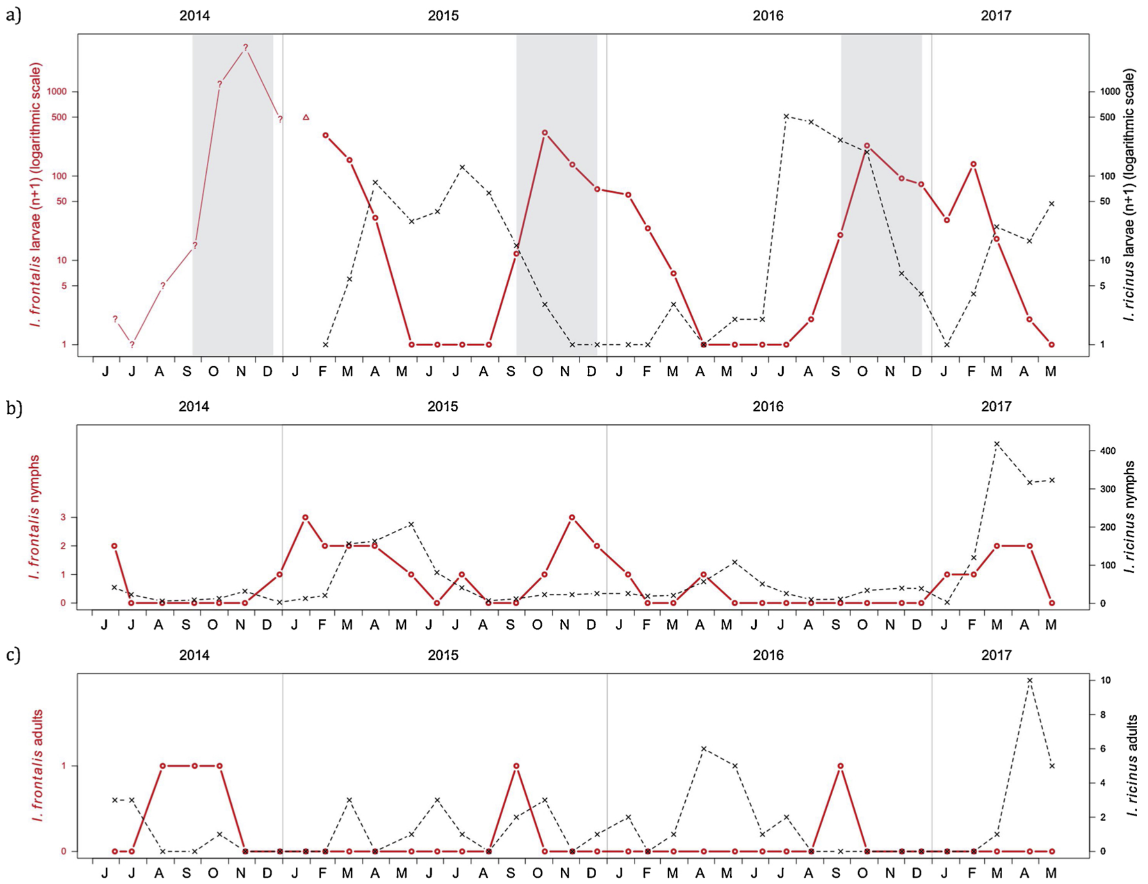

**Fig. 3.** Temporal distribution of questing stages of *Ixodes frontalis* and *Ixodes ricinus*.
a) larvae, b) nymphs, c) adults.
For larvae, the scale is logarithmic, and 1 was added to the counts (n): the baseline (« 1 » on the Y axes) corresponds to the absence of larva. Note that larvae collected before 27/01/2015 were not identified, but (1) those collected on transect n°9 until 29/12/2014 are represented by « ? » as they were presumed to belong to *I. frontalis* (see text and Supplementary Material I and II), (2) and those collected on transect n°9 on 27/01/2015 are represented by $^{\Delta}$ as they were confirmed for the first time as *I. frontalis*. From 18/02/2015 to 16/05/2017, all the transects were considered because larval identification was systematic. For larvae only, shaded grey areas highlight the seasonality of *I. frontalis* during autumn seasons. $^{\times}$ and a dashed black line represents *I. ricinus*. $^{\circ}$ and a continuous red line represents *I. frontalis*.

[427–1905])), followed by nymphs (O.R. = 8 (C.I.95% = [3–31])) and adults (O.R. incalculable because of the absence of *I. frontalis* adults on places without bamboo) (Table 3). On transects with bamboo, *I. frontalis* larvae were predominant compared to *I. ricinus* larvae (representing 82% of the captures), while *I. frontalis* nymphs and adults represented respectively 2% and 19% of the captures. On transects without bamboo, *I. frontalis* larvae and nymphs represented respectively 0.5% and 0.3% of the captures, and adults were absent (Table 3).

## 4. Discussion

Our study on the seasonal activity of exophilic hard ticks yielded unique information on *I. frontalis*, an ornithophilic tick with a largely unknown ecology. To the best of our knowledge, this is the first description of the phenology of the free stages of *I. frontalis*. The most remarkable feature consists in an abundant population of *I. frontalis* questing larvae during autumn-winter. Moreover, the distribution of *I. frontalis* appeared highly aggregated, with favourable sites consisting in transects surrounded and overhung by bamboo.

### 4.1. *Abundance and recurrence of questing stages of* I. frontalis

*I. frontalis* was firmly established at our study site, as evidenced by long-term presence (up to 36 months, from June 2014 to May 2017) of the three questing developmental stages, with 2218 confirmed *I. frontalis* larvae (from January 2015 to May 2017: Supplementary material I), 28 nymphs and 5 adults (males). To the best of our knowledge, this abundance has never been described before for *I. frontalis* questing ticks, especially for the larval stage. In other studies in Europe, *I. frontalis* has been collected by drag sampling, but only at the nymphal and adult stages (France: Doby, 1998; Gilot et al., 1997, 1995 - Spain: Barandika et al., 2006 - Germany: Schorn et al., 2011 - Slovakia: Bona and Stanko, 2013 - Italy: Ceballos et al., 2014). Therefore, *I. frontalis* questing larvae may be more abundant than one would assume, due to confusion with *I. ricinus* (Heylen et al., 2014a).

### 4.2. *Phenology of* I. frontalis

In our study, questing adults and nymphs were present sporadically throughout the year. The larval stage, however, showed a marked

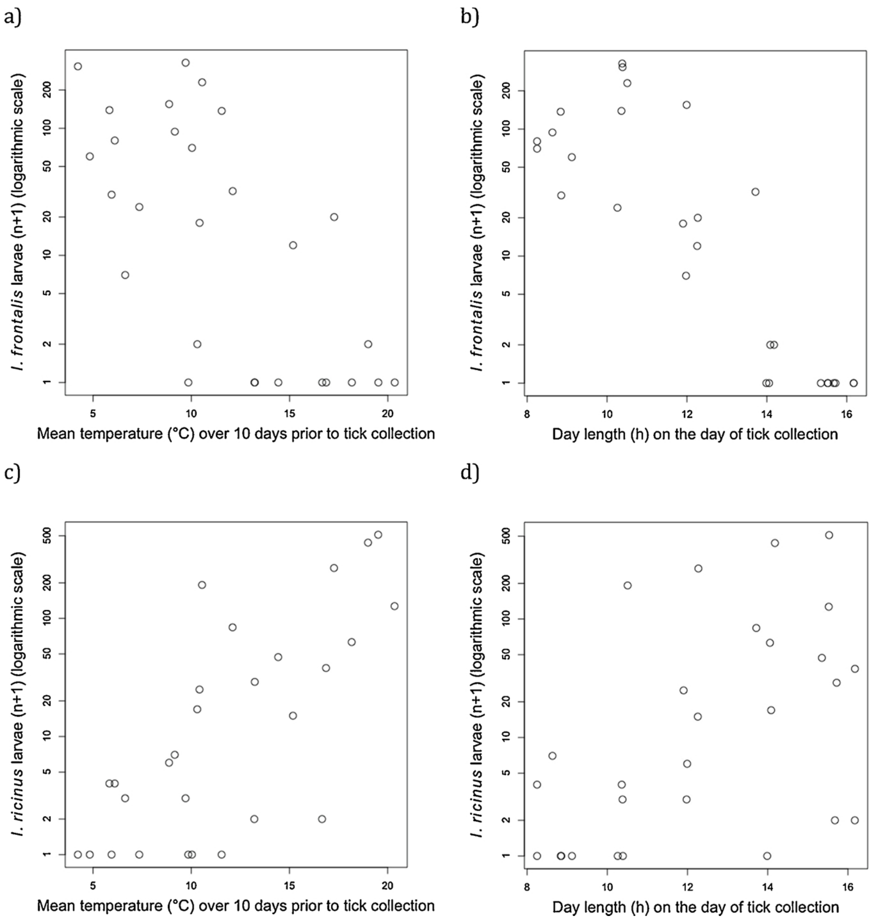

**Fig. 4.** Relationship between mean temperature over 10 days prior to tick collection (a, c) or day length (b, d) and *Ixodes frontalis* (a, b) or *I. ricinus* (c, d) larval counts (n + 1). The relationship between mean temperature (over 10 days prior to tick collection) and *Ixodes frontalis* or *I. ricinus* larval counts (on the day of tick collection) was considered from February 2015 to May 2017 (28 dates) for the whole observatory. The same set of data was used for the influence of day length. To represent larval counts on a logarithmic scale, 1 had to be added to the counts (n): the baseline (« 1 » on the Y axes) corresponds to the absence of larva.

seasonality, with an onset of questing activity in September, followed by a peak in October-November, a slow decrease in winter and an absence of activity in summer. Current literature reports only on the phenology of host-feeding individuals in the wild (Doby, 1998; Norte et al., 2015, 2012). Doby (1998) described the feeding activity of *I. frontalis* (by the time synonymized with « *Ixodes pari* ») on blackbirds (*Turdus merula*) in France. The three parasitic life stages were found constantly between late autumn and early winter, but not during summer. The studies in Portugal by Norte et al. (2015, 2012) found that feeding activity takes place between October and March. Larval activity peaks in November and secondary peaks are observed in February or August, whereas nymphal peak activity is found in December. The activity pattern of those bird-feeding ticks is consistent with the autumnal peak of questing larvae found at our site (Fig. 3).

### 4.3. *Life cycle duration of* I. frontalis

The understanding of the entire life cycle duration of *I. frontalis* is still debated. Doby (1998) suggested a one-year life cycle, based on the developmental durations of ticks obtained from experimental bird infestations. Also Heylen (unpublished results) suggests that the life cycle may take only one year, if ticks have the opportunity to feed soon after moulting. He monitored ticks in a breeding colony that was initiated for vector-competence experiments (Heylen et al., 2017b, 2014b) and morphological analysis (Heylen et al., 2014a). Still, if ticks have to wait for the host or enter diapause, the life cycle will probably take much longer. A one-year life cycle is compatible with our field data, where a clear seasonality is observed for the questing larvae (Fig. 3a), which could correspond to the interval between two generations. However, as Doby (1998) found the three stages on hosts at the same time (between late autumn and early winter), it could suggest a longer life cycle. Further controlled monitoring studies under quasi-natural conditions are clearly needed to deduce the real life cycle duration of *I. frontalis*, by measuring the tick development duration of each stage and their longevity between blood meals.

### 4.4. *Determinants of the phenology of questing stages of* I. frontalis

We found that day length, rather than meteorological conditions, showed the strongest correlation with larval counts of *I. frontalis*: a higher abundance of larvae was observed on short days, suggesting a main influence of regular annual determinants. On the contrary, we found that the questing activity of the larval stage of *I. ricinus* was mainly driven by temperature, with an increase in the population in warm conditions. Indeed, questing behaviour in this species is known to be influenced by meteorological conditions - at least for the nymphal stage (Cat et al., 2017; Jensen, 2000; Kiewra et al., 2014; Perret et al., 2003, 2000; Schulz et al., 2014; Walker, 2001). Because *I. frontalis* larvae are active during autumn and winter, we assume that the determinant for the phenology of this tick species may be different from *I. ricinus*. We hypothesize that the autumn-winter peak of *I. frontalis* larvae may correspond to the feeding of female ticks on birds after bird reproduction (*i.e.,* in summer) during a short period. This would induce a synchronization of egg development, with a fast increase in the larval

**Table 2**
Summary of general linear models explaining log-transformed *Ixodes frontalis* and *Ixodes ricinus* larval counts (log(larval count + 1)).

| | | *Ixodes frontalis* | | | | | | | *Ixodes ricinus* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Estimate | SE | $t$ | $p$ | | $R^2$ | AIC | Estimate | SE | $t$ | $p$ | | $R^2$ | AIC |
| **Meteorological model** | Intercept | 6.18125 | 0.81965 | 7.541 | < 0.001 | *** | 0.4693 | 110.1175 | **−1.36635** | **0.73859** | **−1.850** | **0.0757** | . | **0.5328** | **104.2857** |
| | Mean temperature (0-10 days)° | −0.31205 | 0.06507 | −4.795 | < 0.001 | *** | | | **0.31930** | **0.05864** | **5.445** | **< 0.001** | *** | | |
| **Seasonal model** | Intercept | **11.11442** | **0.98589** | **11.274** | **< 0.001** | *** | **0.7531** | **88.69496** | −2.5494 | 1.6302 | −1.564 | 0.1300 | | 0.268 | 116.8585 |
| | Day length# | **−0.69772** | **0.07835** | **−8.905** | **< 0.001** | *** | | | 0.3997 | 0.1296 | 3.085 | < 0.01 | ** | | |
| **Combined model** | Intercept | 10.91245 | 1.03358 | 10.558 | < 0.001 | *** | 0.7388 | 90.11617 | −1.14317 | 1.37854 | −0.829 | 0.4148 | | 0.4962 | 106.2439 |
| | Mean temperature (0-10 days)° | −0.04765 | 0.06595 | −0.723 | 0.477 | | | | 0.33178 | 0.08796 | 3.772 | < 0.001 | *** | | |
| | Day length# | −0.63600 | 0.11640 | −5.464 | < 0.001 | *** | | | −0.03000 | 0.15525 | −0.193 | 0.8483 | | | |
| **Combined model with interaction** | Intercept | 7.29429 | 3.36400 | 2.168 | < 0.05 | * | 0.7416 | 90.66578 | −2.81454 | 4.59050 | −0.613 | 0.5460 | | 0.4784 | 108.0738 |
| | Mean temperature (0-10 days)° | 0.29308 | 0.30869 | 0.949 | 0.3519 | | | | 0.48917 | 0.42124 | 1.161 | 0.2570 | | | |
| | Day length# | −0.33442 | 0.29100 | −1.149 | 0.2618 | | | | 0.10931 | 0.39709 | 0.275 | 0.7850 | | | |
| | Interaction | −0.02665 | 0.02359 | −1.130 | 0.2698 | | | | −0.01231 | 0.03219 | −0.382 | 0.7060 | | | |

°prior to the day of tick collection.

#on the day of tick collection.

For each explanatory variable, the parameter estimate, the standard error (SE), the *t* value (test statistics) and the *p* value (significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '1) are given.

For the whole model, $R^2$ and AIC are given.

For *Ixodes frontalis*, the seasonal model was selected as the best model according to AIC (in bold).

For *Ixodes ricinus*, the meteorological model was selected as the best model according to AIC (in bold).

population, as we observe. It would be consistent with the observation in the UK of a peak in August-September of tick-related syndrome cases on birds due to *I. frontalis* adult females (Monks et al., 2006), even if the situation may be different in other parts of the distribution area of *I. frontalis* (such as *I. ricinus*, showing a different phenology in western *versus* central Europe: Kurtenbach et al., 2006). For *I. ricinus*, the feeding of females is known to occur during a long spring period (Randolph, 2004), leading to a less synchronized egg development and hence perhaps a greater influence of temperature on larval emergence in summer. However, for *I. frontalis*, alternative hypotheses such as egg or larval diapause or delayed egg hatching cannot be excluded before conducting additional investigations.

### 4.5. *Spatial distribution of questing stages of* I. frontalis

Some transects exhibited frequent high densities of *I. frontalis*, especially for the larval stage. Despite being distant from each other, the transects shared the same physical characteristics with bamboo surrounding and overhanging the ground. The literature reports that the physical characteristics of the collection sites for questing *I. frontalis* are very similar to biotopes where *I. ricinus* can be found (Ceballos et al., 2014; Cringoli et al., 2005; Gilot et al., 1997, 1995; Schorn et al., 2011). To our knowledge, the presence of *I. frontalis* in bamboo bushes has never been described before. At our study site, we suspect that the abundance of *I. frontalis* under bamboo is a consequence of bird attendance. Bamboo may be a favourable place for birds to roost overnight, difficult to reach for predators. However, bamboo does not seem favourable for bird nesting, because of the lack of solid forked branches, hindering the attachment of nests. Thus, we hypothesize that the timing of detachment of engorged female ticks under bamboo is distinct from the reproduction period of birds, even if we have no data on the bird host species involved for the maintenance of the population of *I. frontalis* at our study site. *I. frontalis* is known to infest a wide range of birds, because virtually any bird species is submitted to parasitism when it walks on the ground (Doby, 1998; Hornok et al., 2016). A good candidate species could be the blackbird (*Turdus merula*), which has been observed at the site. It presents both the highest infestation prevalence and the highest tick burden in the literature (Doby, 1998; Norte et al., 2015). This point could be studied in the future, along with potential pathogen transmission consequences.

## 5. Conclusion

We have demonstrated that *I. frontalis* and *I. ricinus* have different phenologies and occupy distinct habitat types at our study site. Nevertheless, we have also shown that they can co-occur both in time and space (even at a very limited spatial scale of 10 m$^2$), indicating a possibility for those two tick species to parasitize the same host individuals and thus to share pathogens. We thus strongly encourage researchers to systematically identify the larval stage of hard ticks found on the ground. Even if *I. ricinus* is the most common species collected in Europe with the drag sampling method, *I. frontalis* may have been underestimated, especially during autumn and winter. A pathogen such as *Borrelia turdi*, belonging to the *Borrelia burgdorferi* s.l. group, can have a prevalence of 14.8% in some *I. frontalis* populations (Literak et al., 2015) and the vector competence of *I. ricinus* for this *Borrelia* species has been recently demonstrated experimentally (Heylen et al., 2017b). The generalist tick *I. ricinus* could thus function as a bridging species from *I. frontalis* hosts, allowing some pathogens to spill over and reach hosts other than birds, including humans or domestic animals. The spatio-temporal overlap between *I. ricinus* and *I. frontalis* may also be influenced by the currently observed climate change and thus influence the epidemiology of the tick-borne diseases associated with those ticks, an issue that should be investigated through dedicated studies.

**Table 3**
Habitat preference of *Ixodes frontalis* compared to *Ixodes ricinus* on two types of transects: with bamboo *versus* without bamboo.

| a) cumulative larval counts* | Transects with bamboo | Transects without bamboo |
|---|---|---|
| (last 28 dates: 18/02/2015 to 16/05/2017) | (n°8, n°9, n°10) | (n°1, n°2, n°3, n°4, n°5, n°6, n°7) |
| *Ixodes frontalis* | 1720 | 8 |
| *Ixodes ricinus* | 376 | 1487 |
| b) cumulative nymphal counts | Transects with bamboo | Transects without bamboo |
| (36 dates: 26/06/2014 to 16/05/2017) | (n°8, n°9, n°10) | (n°1, n°2, n°3, n°4, n°5, n°6, n°7) |
| *Ixodes frontalis* | 24 | 4 |
| *Ixodes ricinus* | 1088 | 1436 |
| c) cumulative adult counts | Transects with bamboo | Transects without bamboo |
| (36 dates: 26/06/2014 to 16/05/2017) | (n°8, n°9, n°10) | (n°1, n°2, n°3, n°4, n°5, n°6, n°7) |
| *Ixodes frontalis* | 5 | 0 |
| *Ixodes ricinus* | 22 | 32 |

* Larvae were systematically identified on each transect from 18/02/2015 to 16/05/2017 (last 28 dates): only these dates were considered.

## Declarations of interest

None.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ttbdis.2018.12.009.

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