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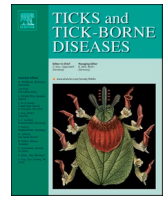
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## Where to find questing *Ixodes frontalis* ticks? Under bamboo bushes!

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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*, other *Ixodes* species may also be involved in the transmission or maintenance of pathogens. This is the case of *Ixodes frontalis*, a poorly known species associated with different bird species such as blackbirds, thrushes and robins, with a wide distribution covering most European countries.

In a previous study, high densities of questing *I. frontalis* larvae were found during autumn-winter at a site close to Nantes (western France) where a long-term survey focused on *I. ricinus* was conducted. These *I. frontalis* were mostly observed under bamboo bushes. In the present study, we investigated the presence of *I. frontalis* under bamboo bushes at various locations. With that aim in mind, a systematic search for questing *I. frontalis* was undertaken by the flagging method in public urban parks and private gardens presenting bamboo bushes (32 sites). This survey was carried out during autumn-winter to maximize the probability of finding the most abundant stage, *i.e.* larvae. We searched for *I. frontalis* first in the area of Nantes (10 sites), then in other regions of France (21 sites) and at one site in northern Italy. A single visit to each site revealed the presence of *I. frontalis* at 29 out of 32 sites: larvae were always present, nymphs were frequent (59 % of the positive sites), while adults were found at only 14 % of the sites. Questing stages of this understudied species are thus easy to find, by dragging or flagging under bamboo bushes in autumn or winter. We make the assumption that bamboo offers a favourable place for birds to roost overnight outside their breeding period (*i.e.* spring), sheltered from both predators and wind. This would explain higher densities of *I. frontalis* under bamboo, relative to other biotopes.

As *I. frontalis* is known to harbour zoonotic pathogens, the consequences of this discovery on the epidemiology of tick-borne diseases are discussed.

### 1. Introduction

While most studies of tick-borne diseases including field sampling usually focus on widespread tick species known to have a strong impact

on animal and public health, it is crucial to keep in mind that the eco-epidemiology of those diseases is also related to the occurrence of other tick species, even if they are considered to be rare. The most common tick species in Europe, *Ixodes ricinus*, is a generalist tick with a

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prominent role in the transmission of pathogens (responsible for Lyme borreliosis or tick-borne encephalitis as major examples) to humans. Moreover, *I. ricinus*, by its broad host spectrum, can behave as a bridge vector species able to transmit pathogens, notably to humans, that would otherwise stay confined to a more restricted association between a particular host and a more specialised tick species that would not bite humans (Heylen et al., 2017). For example, particular strains of *Anaplasma phagocytophilum* have been reported to be associated with *Ixodes ventraloi*, a nidicolous specialist tick parasitizing rabbits (Jaarsma et al., 2019), or to *Ixodes trianguliceps*, a nidicolous specialist tick infesting small rodents (Bown et al., 2006), while those hosts are also used by *I. ricinus* for its blood meals. Another example involves birds, notably blackbirds and other species of the *Turdus* genus, that are frequently parasitized by the tick *Ixodes frontalis*, an exclusively ornithophilic species (Drehmann et al., 2019; Martyn, 1988; Remesar et al., 2019a, b). As birds have often been quoted as potential reservoirs of *Borrelia* that can be transmitted to humans by *I. ricinus* bites (Coipan and Sprong, 2016; Hanincová et al., 2003), the implication of specialist ticks associated with birds, such as *I. frontalis*, in the maintenance of zoonotic pathogens within bird populations can be suspected. Besides human health, some bird diseases have also been reported as associated with *I. frontalis*, including the Chizé Virus (Chastel et al., 1999) and avian tick-related syndrome (TRS) (Monks et al., 2006).

*Ixodes frontalis*, sometimes called the passerine tick (Hillyard, 1996), has been detected on birds in most European countries (Estrada-Peña et al., 2017), even if reports of this species on birds are still less frequent than those of *I. ricinus* (Norte et al., 2020b). Surprisingly, questing *I. frontalis* have been rarely reported on vegetation, despite a recent increase in the number of reports (Agoulon et al., 2019; Bona and Stanko, 2013; Gillingham et al., 2020; Kahl et al., 2019; Schorn et al., 2011). The finding of free stages of *I. frontalis* remains infrequent and no

description of a particular habitat, that could help to find this tick species elsewhere than on its host, has yet been provided, except by Agoulon et al. (2019). These authors discovered thousands of *I. frontalis* larvae under bamboo bushes, collected by the dragging method in autumn and winter, even revealing a higher frequency of the larval stage of this species relative to *I. ricinus* during those seasons.

In this article, to investigate if this finding can be generalised, tick samplings were carried out under bamboo bushes at several locations in France and Italy to search for *I. frontalis* questing stages.

## 2. Material and methods

We targeted sites presenting bamboo bushes with a minimum height of 4 m and a minimum surface of 5 m<sup>2</sup>. The search for new sites was performed first around the initial site in western France, in the vicinity of Nantes (Agoulon et al., 2019), then at a larger scale in France, thus covering different climatic conditions, and in the vicinity of Pavia in northern Italy. Thirty-two sites were found (Fig. 1), either in public urban parks or in private gardens. A typical example of a collection site is illustrated in Fig. 2. Table 1 summarizes study site characteristics (site number and name, GPS coordinates, surface of prospected bamboo patches, sampling date, time spent on flagging) and tick counts (by species and stages).

Tick collections were performed from the beginning of November to the end of April, according to previous results from Agoulon et al. (2019), who showed that this period was the most favourable to collect questing larvae of *I. frontalis*. This stage was particularly targeted because it was the most abundant one, maximizing the probability of finding the tick species. Tick samplings were carried out during two periods: late 2017-early 2018 and late 2018-early 2019.

The flagging method was adopted, because it was more appropriate

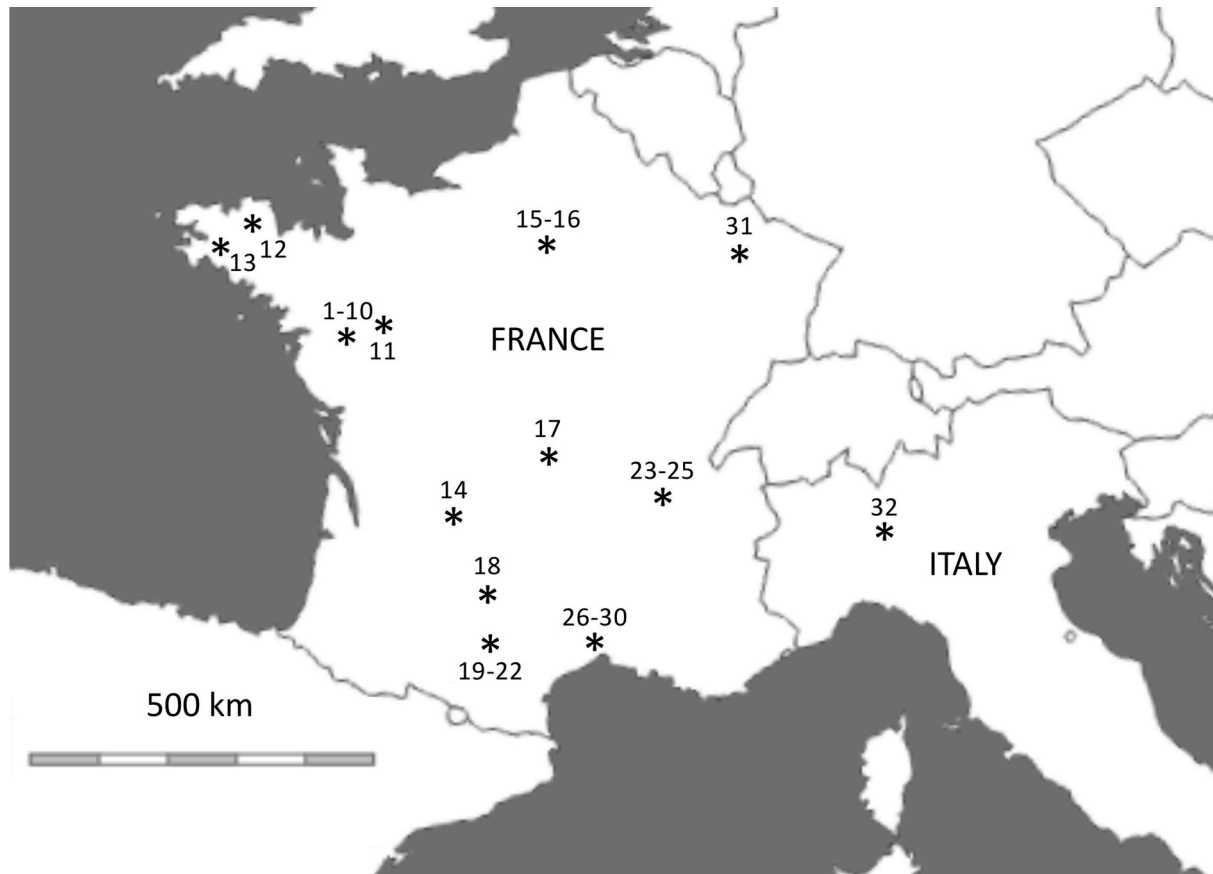


Fig. 1. Map of the 32 collection sites (\*). Some sites correspond to several locations (see Table 1: sites no 1-10, 15-16, 19-22, 23-25, 26-30).



Fig. 2. Illustration of a collection site (site no 9).

for tick sampling within dense vegetation areas (see Fig. 2) than the dragging method, which requires areas free of obstacles such as trees, bushes or bamboo stems. Sampling was carried out on the ground, between bamboo stems or along trails surrounded by bamboo bushes, with a flannel flag (dimensions around 40 × 40 cm) mounted on a handle with a 45° angle. The time spent on flagging ranged from 5–160 min, including time needed to extract ticks from the flag with tweezers to put them in 70 % ethanol. Ticks were identified under an optical microscope (100X magnification) according to the morphological keys provided in Heylen et al. (2014a), Pérez-Eid (2007) and Estrada-Peña et al. (2017). A site was considered positive as soon as a single individual from any stage of *I. frontalis* was found.

### 3. Results

During the first period (late 2017-early 2018), we found eight locations with bamboo patches conforming to the required criteria of surface area and height in the region of Nantes, France, and all sites revealed the presence of *I. frontalis* (Table 1). During the second period (late 2018-early 2019), we extended investigations to 23 additional sites in France (2 locations near Nantes and 21 sites elsewhere in France) and one site in Italy (Table 1). All the sites, except three, were again found positive for the presence of *I. frontalis*.

A total of 2676 larvae, 247 nymphs and 7 adults (all females) of *I. frontalis* were collected during this sampling. In addition, *I. ricinus* was also occasionally found (2 larvae, 43 nymphs and 7 adults [4 females

and 3 males]). Although only a limited time was spent sampling at most sites (usually less than one hour), a high number of *I. frontalis* was found, mostly at the larval stage (91 % of collected individuals were larvae). Among the 29 positive sites, larvae were always present, nymphs were frequent (59 % of the sites) and adults were only occasionally found (14 % of the sites).

### 4. Discussion

To the best of our knowledge, our study is the first one that clearly identifies a suitable biotope, i.e. below bamboo bushes, in which *I. frontalis* can be found with a very high likelihood (90 % of the 32 prospected sites). *Ixodes frontalis* ticks were not detected at only three sites. Two of them (no 14 and no 20) were probably too small (5 and 12 m<sup>2</sup> respectively, corresponding to the two smallest bamboo bushes of our sampling) to detect the passerine tick, perhaps because small bamboo patches may attract too few birds. At the third site (no 15), located in central Paris, the area of bamboo can be considered as atypical in comparison to the other sampled sites as it was not surrounded by any other kind of vegetation and a woven mat (geotextile) was covering the humus layer below the bushes. Thus, our results show that *I. frontalis* is widespread, at least in western Europe, and it can probably be found in most bamboo patches of a sufficient area and height.

Because *I. frontalis* is ornithophilic (Martyn, 1988), we hypothesize that bamboo bushes constitute high quality roost sites for birds, especially during the night and outside the breeding period (i.e. spring) of the birds used by this tick species as hosts. In particular, bamboos (Poaceae) are grasses and not trees and thus do not bear robust lateral branches that could allow terrestrial predators (including mustelids, or cats in more urban areas) to climb in the foliage and thus reach birds during their sleep. Moreover, the density of small lateral side branches in bamboo is so high that it also probably hampers predation by raptors (including both nocturnal and diurnal birds of prey such as goshawks or buzzards, and even some species that can be found in urban areas such as sparrowhawks or owls). Finally, bamboo also has persistent foliage and this characteristic allows roosting birds to save energy due to heat loss during colder and windier winter times. Because of the scarcity of biological data available for *I. frontalis*, the peak activity season of *I. frontalis* females is still debated (Agoulon et al., 2019; Doby, 1998; Heylen et al., 2017, 2014a, b). However, some observations on the passerine ticks collected on birds suggest that it may be in late summer-early autumn (Monks et al., 2006). Bamboo bushes could thus concentrate bird roosting in this period and increase the likelihood that engorged *I. frontalis* females fall to the ground beneath the bamboo, hence producing larvae in this particular habitat in autumn-winter. The bird species using bamboo as roosting sites remain to be determined, but passerines such as blackbirds, thrushes, robins, house sparrows, finches or tits are good candidates. These species are also known to be used for their blood meals by *I. frontalis* (Martyn et al., 1988). In the USA, the use of bamboo as “blackbird lure roost habitat” has been proposed to avoid local urban and agricultural blackbird problems, illustrating the attractivity of these plant species for bird roosting (Flynt and Glahn, 1993; Glahn et al., 1991). We do not mean that this tick species is exclusively associated with this particular plant (as it is also found in other places where no bamboo is present; AA, OP, personal observation), but we emphasize that it can be more easily found underneath bamboo, due to the concentration effect of this plant on birds, probably in relation to its quality as a nocturnal perching site.

Our sampling thus confirmed and generalised the results of the finding described in the Agoulon et al. (2019) paper, revealing a dramatic concentration of *I. frontalis* larvae underneath bamboo. Compared to the site of western France studied in the first paper, a higher proportion of nymphs was found at most of the different sites investigated in the present study. At their site, Agoulon et al. (2019) found a total of 1728 larvae, 28 nymphs and 5 adults (all males) of *I. frontalis* with the dragging method along trails under bamboo bushes. The generally

**Table 1**

Collection data of questing ticks across the 32 study sites (L = larva, N = nymph, F = female, M = male, for each tick species).

Site number	Site name (department - city - locality)	GPS coordinates	Bamboo patch surface (m <sup>2</sup> )	Sampling date	Sampling duration (min)	<i>Ixodes frontalis</i>				<i>Ixodes ricinus</i>				
						L	N	F	M	L	N	F	M	
1	44 - Nantes - Parc de la Chantrerie	47°17'18"N, 1°31'31"W	960	06/11/ 2017	160	552	2	-	-	-	-	-	-	-
				09/02/ 2018	30	128	5	-	-	-	-	-	-	-
				19/03/ 2018	30	104	2	-	-	-	-	-	-	-
				10/12/ 2018	60	346	1	-	-	-	-	-	-	-
				20/03/ 2019	60	262	73	1	-	-	-	-	-	-
2	44 - Nantes - Oniris Chantrerie	47°17'23"N, 1°31'29"W	20	01/12/ 2017	10	1	-	-	-	-	-	-	-	-
3	44 - Sucé sur Erdre - La Héronnière	47°22'29"N, 1°32'02"W	20	08/11/ 2017	10	32	-	-	-	-	-	-	-	-
4	44 - Nantes - Parc du Grand Blottereau	47°13'46"N, 1°30'24"W	540	06/12/ 2017	45	202	2	-	-	-	-	-	-	-
5	44 - Nantes - Parc de la Godinière	47°14'38"N, 1°34'47"W	900	16/12/ 2017	30	56	3	-	-	-	1	-	-	-
6	44 - Nantes - Parc de la Beaujoire	47°15'40"N, 1°31'55"W	80	09/02/ 2018	45	69	-	-	-	-	-	-	-	-
7	44 - Nantes - Jardin des plantes	47°13'08"N, 1°32'37"W	135	21/02/ 2018	45	50	1	-	-	-	-	-	-	-
8	44 - Bouguenais - La Trocardière	47°10'54"N, 1°34'27"W	610	21/02/ 2018	30	97	2	-	-	-	-	-	-	-
9	44 - Saint Herblain - Bagatelle	47°14'37"N, 1°36'59"W	800	28/02/ 2019	35	64	14	1	-	-	-	-	-	-
10	44 - Orvault - Provotière	47°15'19"N, 1°36'44"W	195	10/04/ 2019	50	8	16	-	-	-	2	-	-	-
11	49 - Angers - Terra Botanica	47°30'00"N, 0°34'10"W	475	14/03/ 2019	40	70	8	-	-	-	-	-	-	1
12	22 - Treglasmus - Rumin	48°34'01"N, 3°15'12"W	50	22/04/ 2019	20	5	1	-	-	1	9	-	-	-
13	29 - Quimper - Men Foues	48°03'18"N, 4°06'03"W	100	27/02/ 2019	60	20	23	2	-	-	17	2	2	-
				28/02/ 2019	30	46	18	1	-	1	6	-	-	-
14	24 - Saint Paul de Serre - La Bouyerie	45°04'31"N, 0°37'54"E	12	16/03/ 2019	5	-	-	-	-	-	2	-	-	-
15	75 - Paris - Paris Expo Porte de Versailles	48°49'51"N, 2°17'19"E	500	03/03/ 2019	20	-	-	-	-	-	-	-	-	-
16	94 - Vincennes - Parc Floral & Tropical	48°50'15"N, 2°26'37"E	970	15/04/ 2019	60	9	56	2	-	-	1	-	-	-
17	63 - Biollet - Bamboueraie	46°00'04"N, 2°42'41"E	2180	22/02/ 2019	70	142	6	-	-	-	1	-	-	-
18	82 - Caussade - Rue de Lavaur	44°09'16"N, 1°32'32"E	30	29/12/ 2018	8	7	-	-	-	-	-	-	-	-
19	31 - Vieilleville - Roumingou	43°23'49"N, 1°39'17"E	24	22/02/ 2019	5	2	-	-	-	-	-	-	-	-
				24/02/ 2019	5	3	-	-	-	-	-	-	-	-
20	31 - Vieilleville - Chemin du Cammas	43°24'03"N, 1°39'22"E	5	23/02/ 2019	5	-	-	-	-	-	-	-	-	-
21	31 - Saint Léon - Bois de la Plano	43°25'20"N, 1°33'19"E	20	23/02/ 2019	15	37	-	-	-	-	-	-	-	-
22	31 - Montesquieu - Barrelis	43°25'47"N, 1°36'45"E	16	06/03/ 2019	15	3	-	-	-	-	-	-	-	-
23	69 - Tour de Salvagny - Rue de Sutin	45°47'52"N, 4°43'10"E	10	05/03/ 2019	30	2	-	-	-	-	2	1	-	-
24	69 - Fleurieux sur l'Arbresle - Servy	45°50'32"N, 4°39'22"E	500	05/03/ 2019	15	112	3	-	-	-	1	1	-	-
25	01 - Misérieux - Centre horticole	45°59'14"N, 4°47'45"E	1000	05/03/ 2019	90	12	2	-	-	-	-	-	-	-
26	34 - Montpellier - Vailhauquès	43°39'50"N, 3°42'38"E	130	13/02/ 2019	60	49	-	-	-	-	1	-	-	-
27	34 - Montpellier - Prades-le-Lez	43°41'29"N, 3°52'39"E	150	27/02/ 2019	60	12	3	-	-	-	-	-	-	-
28	34 - Montpellier - Plaine de Roques	43°40'52"N, 3°51'29"E	430	04/03/ 2019	75	3	-	-	-	-	-	-	-	-
29	34 - Montpellier - Lavallette	43°38'51"N, 3°52'29"E	900	05/03/ 2019	20	1	1	-	-	-	-	-	-	-
30	34 - Montpellier - Gigean	43°29'57"N, 3°42'30"E	50	05/03/ 2019	45	1	-	-	-	-	-	-	-	-
31			4370		90	152	5	-	-	-	-	-	-	-

(continued on next page)

Table 1 (continued)

Site number	Site name (department - city - locality)	GPS coordinates	Bamboo patch surface (m <sup>2</sup> )	Sampling date	Sampling duration (min)	<i>Ixodes frontalis</i>				<i>Ixodes ricinus</i>			
						L	N	F	M	L	N	F	M
32	54 - Nancy - Jardin Botanique	48°39'33"N, 6°09'20"E	600	27/02/ 2019	60	17	-	-	-	-	-	-	-
	27 - Pavia (Italy) - Parco Sora	45°11'40"N, 9°06'15"E		18/03/ 2019									
<b>Total</b>						<b>2676</b>	<b>247</b>	<b>7</b>	<b>0</b>	<b>2</b>	<b>43</b>	<b>4</b>	<b>3</b>

higher frequency of nymphs in the present study, and also to a lesser extent of adults at certain sites, may at least be partially related to the use of the flagging instead of the dragging method: dragging is already known to let adult ticks fall more easily from the blanket when it is dragged over a certain distance (Dantas-Torres et al., 2013; Milne, 1943).

Although ticks are strictly hematophagous and do not rely on any other feeding resource than the one provided by their vertebrate host, the analysis of vegetation type has often been investigated to explain tick presence/absence or density. In particular, vegetation type acts on the habitat's microclimate and soil, which may have an effect on tick abundance and questing activity (Dobson et al., 2011; Gilot et al., 1979, 1975; Lindström and Jaenson, 2003). However, we are aware of very few studies that have reported a direct link between a particular plant species and the presence/absence or density of a tick species. This is the case for example for the non-native tree species *Solanum mauritanum* and *Ixodes holocyclus* (Buettner et al., 2013). The authors reported an outbreak of tick paralysis in an Australian population of bats (*Pteropus conspicillatus*) and hypothesized that this outbreak was linked to the introduction of this invasive new bush species that led those frugivorous bats to forage in a lower vegetation height than previously, exposing them to the bite of the indigenous tick *I. holocyclus*. The association of *I. frontalis* with bamboo could correspond to a similar relationship between a particular plant species and a particular tick species, induced by a concentration of the hosts in this particular vegetation type, favouring a higher density of this tick species. There are several reports of *I. frontalis* in urban areas, such as parks and gardens (Drehmann et al., 2019; Schorn et al., 2011). Because *I. frontalis* is found with a high likelihood in any bamboo bush of a sufficient surface area and because such bamboo bushes are especially frequent in urban areas, this tick species is probably a very common member of the tick community in such habitats). As *I. ricinus* can act as a possible bridge vector, investigations on *I. frontalis* and harboured pathogens such as *Borrelia* spp. may be relevant for public health issues (Buczek et al., 2020; Norte et al., 2020a, b). In the context of the increasing interest of the influence of urbanisation on infectious diseases (Hassell et al., 2017), including ticks and tick-borne diseases (Grochowska et al., 2020; Hansford et al., 2017; Rizzoli et al., 2014), our findings highlight the potential role of *I. frontalis* in the eco-epidemiology of tick and tick-borne diseases in such areas (Hamer et al., 2012).

Our results, favouring the sampling of this species, pave the way to additional investigations aiming at elucidating unknown aspects of the biology of this tick species, such as its population dynamics and its relationships with bird behaviour.

#### CRedit authorship contribution statement

**Olivier Plantard:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Thierry Hoch:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration. **Romain Daveu:** Investigation, Resources, Writing - review & editing. **Claude Rispe:** Investigation, Resources, Writing - review & editing. **Frédéric Stachurski:** Investigation, Resources, Writing - review & editing. **Franck**

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#### Declaration of Competing Interest

None.

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