Peer Community In

An extensive review of Ixodes ricinus in European France

Ana Sofia Santos based on peer reviews by **Ana Palomar** and 1 anonymous reviewer

Grégoire Perez, Laure Bournez, Nathalie Boulanger, Johanna Fite, Barbara Livoreil, Karen D. McCoy, Elsa Quillery, Magalie René-Martellet, and Sarah I. Bonnet (2023) The distribution, phenology, host range and pathogen prevalence of *lxodes ricinus* in France: a systematic map and narrative review. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Infections. https://doi.org/10.1101/2023.04.18.537315

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Santos, A. (2023) An extensive review of Ixodes ricinus in European France. *Peer Community in Infections*, 100076. 10.24072/pci.infections.100076

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Ticks are obligate, bloodsucking, nonpermanent ectoparasitic arthropods. Among them, *Ixodes ricinus* is a classic example of an extreme generalist tick, presenting a highly permissive feeding behavior using different groups of vertebrates as hosts, such as mammalian (including humans), avian and reptilian species (Hoogstraal & Aeschlimann, 1982; Dantas-Torresa & Otranto, 2013). This ecological adaptation can account for the broad geographical distribution of *I. ricinus* populations, which extends from the western end of the European continent to the Ural Mountains in Russia, and from northern Norway to the Mediterranean basin, including the North African countries - Morocco, Algeria and Tunisia (https://ecdc.europa.eu/en/di sease-vectors/surveillance-and-disease-data/tick-maps). The contact with different hosts also promotes the exposure/acquisition and transmission of various pathogenic agents (viruses, bacteriae, protists and nematodes) of veterinary and medical relevance (Aeschlimann et al., 1979). As one of the prime ticks found on humans, this species is implicated in diseases such as Lyme borreliosis, Spotted Fever Group rickettsiosis, Human Anaplasmosis, Human Babesiosis and Tick-borne Encephalitis (Velez et al., 2023).

The climate change projections drawn for *I. ricinus*, in the scenario of global warming, point for the expansion/increase activity in both latitude and altitude (Medlock et al., 2013). The adequacy of vector modeling is relaying in the proper characterization of complex biological systems. Thus, it is essential to increase knowledge on *I. ricinus*, focusing on aspects such as genetic background, ecology and eco-epidemiology on a microscale but also at a country and region level, due to possible local adaptations of tick populations and genetic drift.

In the present systematic revision, Perez et al. (2023) combine old and recently published data (mostly up to 2020) regarding *I. ricinus* distribution, phenology, host range and pathogen association in continental France

and Corsica Island. Based on a keyword search of peer-reviewed papers on seven databases, as well as other sources of grey literature (mostly, thesis), the authors have synthesized information on: 1) Host parasitism to detect potential differences in host use comparing to other areas in Europe; 2) The spatiotemporal distribution of *I. ricinus*, to identify possible geographic trends in tick density, variation in activity patterns and the influence of environmental factors; 3) Tick-borne pathogens detected in this species, to better assess their spatial distribution and variation in exposure risk.

As pointed out by both reviewers, this work clearly summarizes the information regarding *I. ricinus* and associated microorganisms from European France. This review also identifies remaining knowledge gaps, providing a comparable basis to orient future research. This is why I chose to recommend Perez et al (2023)'s preprint for Peer Community Infections.

References:

Aeschlimann, A., Burgdorfer, W., Matile, H., Peter, O., Wyler, R. (1979) Aspects nouveaux du rôle de vecteur joué par Ixodes ricinus L. en Suisse. Acta Tropica, 36, 181-191.

Dantas-Torresa, F., Otranto, D. (2013) Seasonal dynamics of Ixodes ricinus on ground level and higher vegetation in a preserved wooded area in southern Europe. Veterinary Parasitology, 192, 253-258. https://doi.org/10.1016/j.vetpar.2012.09.034

Hoogstraal, H., Aeschlimann, A. (1982) Tick-host specificity. Mitteilungen der Schweizerischen Entomologischen Gesellschaft, 55, 5-32.

Medlock, J.M., Hansford, K.M., Bormane, A., Derdakova, M., Estrada-Peña, A., George, J.C., Golovljova, I., Jaenson, T.G.T., Jensen, J.K., Jensen, P.M., Kazimirova, M., Oteo, J.A., Papa, A., Pfister, K., Plantard, O., Randolph, S.E., Rizzoli, A., Santos-Silva, M.M., Sprong, H., Vial, L., Hendrickx, G., Zeller, H., Van Bortel, W. (2013) Driving forces for changes in geographical distribution of Ixodes ricinus ticks in Europe. Parasites and Vectors, 6. https://doi.org/10.1186/1756-3305-6-1

Perez, G., Bournez, L., Boulanger, N., Fite, J., Livoreil, B., McCoy, K., Quillery, E., René-Martellet, M., Bonnet, S. (2023) The distribution, phenology, host range and pathogen prevalence of lxodes ricinus in France: a systematic map and narrative review. bioRxiv, ver. 1 peer-reviewed and recommended by Peer Community in Infections. https://doi.org/10.1101/2023.04.18.537315

Velez, R., De Meeûs, T., Beati, L., Younsi, H., Zhioua, E., Antunes, S., Domingos, A., Ataíde Sampaio, D., Carpinteiro, D., Moerbeck, L., Estrada-Peña, A., Santos-Silva, M.M., Santos, A.S. (2023) Development and testing of microsatellite loci for the study of population genetics of Ixodes ricinus Linnaeus, 1758 and Ixodes inopinatus Estrada-Peña, Nava & Petney, 2014 (Acari: Ixodidae) in the western Mediterranean region. Acarologia, 63, 356-372. https://doi.org/10.24349/bvem-4h49

Reviews

Evaluation round #1

DOI or URL of the preprint: https://drive.google.com/drive/folders/1Uy-kVzEle9xg1DGxRUmsXe qVT7EjGZCM?usp=share_link

Version of the preprint: 1

Authors' reply, 02 April 2023

Dear Recommender,

Thank you for handling our manuscript.

We did all the requested corrections and additions asked by you and by the two reviewers.

Particularly, we added two figures: a presence map of Neoerhlichia mikurensis (Figure 7) and a presence map of Rickettsia monacensis (Figure 9c). We corrected the Table 1, the Figure 1 heading, and added references where it was necessary. To comply with the PCI rules and make data available, SI 04 (fields description) was replaced by the dataset table itself with the previous table as its metadata. Following the previous work, we noticed a mistake in the number of DMV theses (21 and not 23). The total number of references was corrected and related figures (Figure 1) and SI files (SI 06) were changed accordingly, without changing substantially the manuscript. Additionally, we corrected little mistakes in SI 09 and SI 11 and the related Figures 3 and 5 that do not imply any substantial changes.

Please find below a more detailed point to point reply to your and reviewers' comments.

We thank you and the two reviewers for your remarks that helped us to improve our manuscript. We stay available for any suggestion of further improvement, Sincerely,

In behalf of all co-authors.

1. Pag 2, Introduction, line 1 – "Among the forty or so tick species present in European France (...)" Please replace by an accurate sentence/number, "forty or so" sounds strange in a scientist article.

Response: The sentence was changed to: "Among the forty one tick species known to be present in European (metropolitan) France [...]"

2. Pag4, table 1, 2nd row –"context"- Please note that context description in tabl 1 is different from what is found in the text body, right below table 1 "The "context" corresponded to European France as the target area (...)"

Response: Context was corrected in the table: "European France, i.e. excluding overseas French territories"

3. Pag5, 2.1 Research approach, last line (regarding the Outcome) – "The assessment of tick-borne pathogens in ticks was also considered as an outcome variable"

and SI 03 "Exclusion criteria - No comparator was excluded"

If the objective was to provide a complete image of tick-borne occurrence it would be interesting to include studies that does not have temporal or spacial and ecological comparator.

Saying in other words, the present review may not provide a complete view of the tick-borne agents present in France, as some studies might have been skipped for not having a temporal/special/ecological comparator, and this should be clearly stated in the text.

Response: No comparator was excluded. This is now stated more explicitly in the table.

4. Pag5, 2.2 Search strategy – No clear indication if the first search using the keyword combinations "Ixodes ricinus" AND (abundance OR activity OR [behaviour OR behavior] OR burden OR density OR dispersal OR distribution OR dynamics OR infestation OR presence OR prevalence OR questing).", and that retrieved 19,654 references, was just performed on titles. Please clarify that. The authors mention in pag 6 (2.3 Eligibility screening , 2.3.1 Eligibility criteria) that "Screening was conducted by GP, first on titles, then on abstracts, and finally on the full text when available." but is not clear if this was applied to all. Please clarify this in the text. What is GP?

It seems reasonable to think that the first round was not based just on title analysis, otherwise several paper having a more generalist title, such as "lxodes" or "ticks", may have been skipped from this revision.

Response: GP is the first author. We added a precision in the text. The text was changed: "Only references in French or English were considered. Screening was conducted by one of the authors (GP), first on titles, then on abstracts, and finally on the full text when available."

5. Pag10, Figure 1 heading – "Refer to SI 5 for the name of the departments." Should be SI 7 Response: This correction was done.

6. Pag10, fist 6 lines of the text – "In south-western France (...). Thus, I. ricinus ticks seem to be present in some Mediterranean departments, but only under specific environmental conditions and above a certain altitude (Stachurski & Vial 2018).

Please note that, Sevestre et al, 2021 has published a paper on tick-borne agents found on questing I. ricinus (but also, host-feeding ticks) above 400 m in the Alpes Maritimes region, questioning the general idea of considering Mediterranean rim unsuitable for this ticks species. Maybe this should be, as well commented, in the paper, as post-analysis reference.

Sevestre J, Diarra AZ, Oumarou AH, Durant J, Delaunay P, Parola P. Detection of emerging tick-borne disease agents in the Alpes-Maritimes region, southeastern France. Ticks Tick Borne Dis. (2021) 12:101800. doi: 10.1016/j.ttbdis.2021.101800

Response: Thank you for the remark and the reference. This section was rewritten accordingly and the references included.

7. Pag17, 3.3.1. The genus Anaplasma, "These ecotypes also differ by their vectors. Ecotypes I and II are mainly transmitted by I. ricinus, but ecotype III seems mainly transmitted by I. trianguliceps; vectors of ecotype IV are still uncertain, but are suspected to be among the hard ticks that specifically exploit birds."

Please note that in Europe I. ventalloi was found associated with an A. phagocytophilum ecotype IV-like. This A. phagocytophilum variant was found in I. ventalloi from Portugal (Santos et al, 2004, 2017) and is sharing only 95% homology to those sequences of the agent derived from birds, and included in ecotype IV (Jaarsma et al, 2019). Beside I. triangulicepts and I. ventalloi other species may as well be associated with A. phagocytophilum.

Jaarsma et al, 2019 doi.org/10.1186/s13071-019-3583-8

Santos AS, Santos-Silva MM, Almeida VC, Bacellar F, Dumler JS. Detection of Anaplasma phagocytophilum DNA in Ixodes ticks (Acari: Ixodidae) from Madeira Island and Setúbal District, mainland Portugal. Emerging Infectious Diseases. 2004;10(9):1643-1648

Santos AS, de Bruin A, Veloso AR, Marques C, Pereira da Fonseca I, de Sousa I, et al. Detection of Anaplasma phagocytophilum, Candidatus Neoehrlichia sp., Coxiella burnetii, and Rickettsia spp. in questing ticks from a recreational park, Portugal. Tick and Tick-Borne Diseases. 2018;9(6):1555-1564. DOI: 10.1016/j.ttbdis.2018.07.010

Response: Thank your for the information and references, it was accounted for in the text: "; and an ecotype IV-like was associated with I. ventalloi, a tick species mostly feeding on European rabbits, Oryctolagus cuniculus, and present in France (Gilot, Rogers & Lachet 1985; Santos et al. 2018; Jaarsma et al. 2019)."

8. Pag 18-19, "Only five studies have attempted to detect the DNA of other Anaplasma species in questing I. ricinus ticks in France (Bonnet et al. 2013; Michelet et al. 2014; Lejal et al. 2019a; Lejal et al. 2019b; Grech-Angelini et al. 2020a)."

Which Anaplasma spp were detected in questing I. ricinus? Not stated in the text. Please be clearer if the studies in questing ticks ever found any other Anaplasma sp..

Response: The Anaplasma species searched for are those cited in the paragraph. The paragraph was reorganised to make it more clear. The details are in SI 09.

9. Pag 28, 3.3.7 The genus Rickettsia, Fig 8 – why not presenting a map of R. monacensis distribution too? Response: A map of R. monacensis was created and added with the other maps of Rickettsia (Figure 9c in the revised version)

10. SI 4 – Description of the dataset encoding fields

Please correct the number of this Supplementary information – is 4 not 3

First row – Study type – "Data were obtained from field sampling or in laboratory or it was a review: field/lab/review (...)"

Laboratory reared colonies were not included, right? What does it means to have data obtained from laboratory?

Response: We acknowledge that this statement was confusing. We meant, and reformulated to "Data were obtained when sampling or from field samples analysed in laboratory (field), from lab experiments (lab) or it was a review (review)". Also, we will provide the full dataset instead and this file as metadata for it.

Reply to Ana Palomar

The manuscript summarize clearly the information regarding lxodes ricinus and associated microorganisms from France. I have only minor comments.

Line 71: Please correct 552,000 or omit the gap.

Response: This correction was done.

Line 208: research articles and reviews are 153, while in the legend of the SI 6 the number indicate is 1. The latter should be corrected

Response: This correction was done.

Line 229: In the title of other sections Ixodes ricinus is complete e.g. line 271 Response: Titles have been homogenised with the full name. Line 243: SI 5 are the final references, the names of the departments are in SI 07. Response: This correction was done.

Line 247: The Figure 2 demonstrated the occurrence of I. ricinus in the Mediterranean climatic region, at least on host, so the species should not be considered absent in this area, or where all of the migratory species? Response: The section was rewritten.

Line 430, 434, 433...: Why Carnivores whit capital letter? It is not in line 428 Response: This correction was done. The "Carnivores" are the phylogenetic mammalian clade.

Line 448: Italics for orders? Response: This correction was done.

Line 485: Anaplasma capra should be mentioned Response: From the analysed literature, Anaplasma capra was not searched for in France.

Line 491: Which others species? Why (A. marginale) at the end of the sentence? Response: The paragraph was rewritten to be more clear.

Line 492: Delete the plot from SI. 9 Response: This correction was done.

Line 528: Please correct B. spielmanii Response: This correction was done.

Line 575: sensu stricto could be deleted, it is is the first sentence of the legend Response: This correction was done.

Line 586: Borrelia theileri and Borrelia lonestari are also transmitted by hard ticks. B. miyamotoi is the only in Europe.

Response: This correction was done.

Line 615: The vector competence of ticks should be referenced. Response: We added the reference: Duron et al. 2015. Line 720: There are pathogens without a map, such as Neoehrlichia mikurensis, and neither R. monacensis is included in these maps while I. ricinus is vector of it and is not recognized as vector of R. felis.

Response: A map of N. mikurensis was created (Figure 7 in the revised version), and a map of R. monacensis and a map of Rickettsia spp. were created and added with the other maps of Rickettsia (Figure 9c in the revised version).

Line 734: Correct R. slovaca. In my opinion this study is not relevant, because the detection of Rickettsia in other studies also include these and other Rcikettsia species, because most of the times are not PCR specie-specific, are genus-dpecific.

Response: You are right that this study, like others, include a genus-specific PCR for Rickettsia SFG, so we revised the text and the Figure 9 (formerly Figure 8) accordingly.

Line 739: I think that sensu stricto is not correct here

Response: Babesia sensu stricto are true Babesia, excluding B. microti and B. microti-like which are phylogenetically closer to Theilaria genus, according to Schnittger et al. 2012, see also Jalovecka et al. 2019.

Schnittger, L., Rodriguez, A. E., Florin-Christensen, M., & Morrison, D. A. (2012). Babesia: a world emerging. Infection, Genetics and Evolution, 12(8), 1788-1809.

Jalovecka, M., Sojka, D., Ascencio, M., Schnittger, L., 2019. Babesia life cycle-when phylogeny meets biology. Trends Parasitol. 35, 356-368. https://doi.org/10.1016/j.pt.2019.01.007

Line 907: I think that I. ricinus is recognized vector of R. helvetica and R. monacensis Response: Although very likely, to our knowledge, there is no experimental evidence.

Reply to anonymous reviewer

The authors have reviewed published data for the Distribution, phenology, host range and pathogen prevalence of lxodes ricinus in FRance. The manuscript is very well written and is very clear

Only minor revisions are needed to improve the manuscript. Difficult to give the exact position of my comments since there is no line number....

In the Entire manuscript : European France is not really clear for me ? Why not metropolitan France ? Or just say France in the introduction and mentionning the fact you mean metropolitan one and Corsica ?

Response: European France is a more politically and inclusive way to say metropolitan France.

Abstract

Page 1 : " and the circulation of tick-borne human pathogens". You mention zoonotic pathogens in this review, not only human pathogens. So better say tick-borne zoonotic pathogens.

Response: This change was done.

Introduction

Page 2 : "conditions and the length of diapause periods." Add a reference Response: A reference was added (Gray et al. 2021) Page 2 : "between 5 and 30°C and a relative humidity above 80%." For me 30°C is quite high for Ixodes ricinus. 80% only ? Not a higher range ? Add a reference for both information.

Response: The text was changed to "and to a minimum relative humidity of 80%" and a reference was added (Gray et al. 2021).

Page 3 : "host parasitism". Not "Parasitized host by I. ricinus" ? Response: The sentence was changed to: "Host infestation by I. ricinus, [...]"

3.2.3. Host Range

Page 16 : "the role of black rates". to be modified by "black rats" ; "ungulates contribute to an increase I. ricinus population" one of is missing "to an increase of I. ricinus population".

Response: This corrections were done.

3.3 Tick-borne pathogens in I. ricinus ticks in FrancePage 18. First line add a comma : "Different tick-borne pathogens, that have..."Response: This correction was done.

Page 20 : line 5. a word is missing : ...is not considered "to" be the vector of Response: This correction was done.

Page 23 : in the list of Borrelia species. For Borrelia bavariensis. Better say Borrelia garinii bavariensis as you say later in the same paragraph. Or decide to call B. garinii and B. bavariensis ? Response: We rewrote "Borrelia garinii subsp. bavariensis".

Page 23 : B. bissettii (Line 6 from the bottom) Response: This correction was done.

Page 24 : "compared to other regions with more open landscapes and less forested areas." Add a reference that explain the different type of vegetation in France

Response: Information on forest cover in France are freely available from the IGN ("Institut national de l'information géographique et forestière"). See for instance the Mémento 2022 of the Inventaire Forestier National (https://inventaire-forestier.ign.fr/IMG/pdf/memento_2022.pdf), but we are not sure that this general description of need a reference.

Page 29 : For Coxiella burnetii, please mention the fact that this bacteria is often mistaken with Coxiella-like symbionts. So confirmation by sequencing is really important to avoid a false identification of Coxiella burnetii in ticks.

Response: The following sentence was added: "For this bacteria, sequencing is essential to avoid confusion with Coxiella-like symbionts (Jourdain et al. 2015)."

Page 31 : Same problem, Francisella tularensis could be mistaken with Francisella-like endosymbionts, so need confirmation by sequencing.

Response: The following sentence was added: "For this bacteria too, sequencing or specific PCR are necessary to discriminate between Francisella-like symbionts and F. tularensis (Michelet et al. 2013)."

Page 35 : Problem with the references cited line 11 for southern France : Vayssier-Taussat et al 2013 in from North-East/East of France, Two references of Lejal et al are from Paris region (Senart forest, Essonne) Response: References have been moved to avoid possible confusions.

Page 39 : Line 4 and Line 9 : Far-Eastern subtype (not Far-East subtype). Line 10 : more than 2% of mortality with Far-eastern subtypes !

Response: This correction was done.

Conclusion Page 42. "Mediterranean climates remain"instead of "remains" Response: This correction was done.

Page 43. Line 6. "to appropriate" not "appropriately" Response: We rewrote: "This highlights the need to scale studies according to expected outcomes [...]"

Decision by Ana Sofia Santos, posted 27 February 2023, validated 28 February 2023

Dear authors,

Thank you for submitting your article in PCI infections.

This paper merits publication as it is important to increase knowledge on the ecology and eco-epidemiology of I. ricinus, aspects focused by the authors. Experts have evaluated the paper and I should first advice changes to the text according to the reviewer's comments. I am also adding some suggestions myself:

1. Pag 2, Introduction, line 1 – "Among the forty or so tick species present in European France (...)" Please replace by an accurate sentence/number, "forty or so" sounds strange in a scientist article.

2. Pag4, table 1, 2nd row –"context"- Please note that context description in tabl 1 is different from what is found in the text body, right below table 1 "The "context" corresponded to European France as the target area (...)"

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Laboratory reared colonies were not included, right? What does it means to have data obtained from laboratory?

Please address all these points and submit both a revised version of your manuscript and rebuttal letter.

I look forward to receiving your revised manuscript.

Sincerely,

Com os melhores cumprimentos, Ana Sofia Pereira dos Santos, PhD LNR de Doenças Infeciosas Transmitidas por Vetores Departamento de Doenças Infeciosas

Reviewed by Ana Palomar, 15 February 2023

The manuscript summarize clearly the information regarding *lxodes ricinus* and associated microorganisms from France. I have only minor comments.

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Page 2 : "conditions and the length of diapause periods." Add a reference

Page 2 : "between 5 and 30°C and a relative humidity above 80%." For me 30°C is quite high for Ixodes ricinus. 80% only ? Not a higher range ? Add a reference for both information.

Page 3 : "host parasitism". Not "Parasitized host by I. ricinus" ?

3.2.3. Host Range

Page 16 : "the role of black rates". to be modified by "black rats" ; "ungulates contribute to an increase I. ricinus population" one of is missing "to an increase of I. ricinus population".

3.3 Tick-borne pathogens in I. ricinus ticks in France

Page 18. First line add a comma : "Different tick-borne pathogens, that have..."

Page 20 : line 5. a word is missing : ... is not considered "to" be the vector of

Page 23 : in the list of Borrelia species. For Borrelia bavariensis. Better say Borrelia garinii bavariensis as you say later in the same paragraph. Or decide to call B. garinii and B. bavariensis ?

Page 23 : B. bissettii (Line 6 from the bottom)

Page 24 : "compared to other regions with more open landscapes and less forested areas." Add a reference that explain the different type of vegetation in France

Page 29 : For Coxiella burnetii, please mention the fact that this bacteria is often mistaken with Coxiella-like symbionts. So confirmation by sequencing is really important to avoid a false identification of Coxiella burnetii in ticks.

Page 31 : Same problem, Francisella tularensis could be mistaken with Francisella-like endosymbionts, so need confirmation by sequencing.

Page 35 : Problem with the references cited line 11 for southern France : Vayssier-Taussat et al 2013 in from North-East/East of France, Two references of Lejal et al are from Paris region (Senart forest, Essonne)

Page 39 : Line 4 and Line 9 : Far-Eastern subtype (not Far-East subtype). Line 10 : more than 2% of mortality with Far-eastern subtypes !

Conclusion

Page 42. "Mediterranean climates remain"instead of "remains"

Page 43. Line 6. "to appropriate" not "appropriately"