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Tick ecology and Lyme borreliosis prevention: a regional survey of pharmacists' knowledge in Auvergne-Rhône-Alpes, France

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1 Ticks and tick-born Disease (TTBD)

2

3 Tick ecology and Lyme borreliosis prevention: a regional survey of pharmacists'
4 knowledge in Auvergne-Rhône-Alpes, France

5

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22 Highlights:

- 23 • Ticks are the vectors that **transmit the pathogen responsible for** Lyme borreliosis,
24 considered to be one of the most prevalent vector-borne diseases in the
25 temperate zone of the Northern hemisphere; in France, pharmacists are often the
26 first medical source of information in the case of a tick bite.
- 27 • This study questioned the knowledge of 364 pharmacists and assistants, mainly in
28 Auvergne-Rhône-Alpes region (France), on ticks, Lyme biology and Lyme
29 borreliosis prevention.
- 30 • The study showed solid knowledge of preventive measures for tick bites but
31 sometimes approximate knowledge about the main characteristics of ticks (host,
32 living area, etc.), potentially detrimental to the accuracy of prevention.

33

34 Abstract:

35 The most **prevalent** vector-borne diseases in **Europe** are **caused by tick-borne pathogens,**
36 **such as bacteria of the genus *Borrelia*** that cause Lyme borreliosis.

37 In this context, retail pharmacists are frequently the first medical source of information in
38 the event of a tick bite. The objective of this study was to assess pharmacy professionals'
39 knowledge about both tick ecology and the appropriate measures for tick bites and Lyme
40 borreliosis prevention. It was based on an online survey of 364 pharmacists and pharmacy
41 assistants located in the Auvergne-Rhône-Alpes region of France. The results showed solid
42 knowledge about preventive measures for tick bite and Lyme borreliosis, but weaker
43 knowledge about tick biology (hosts, suitable habitats, favorable conditions for tick activity,
44 etc.). In particular, several stereotypes were observed in the responses of the pharmacy
45 professionals. These appear to result from a social construction of the knowledge on ticks
46 and tick-borne diseases previously shown to the general population in the region. The
47 results highlight the need for continuous training about ticks and tick-borne diseases for
48 healthcare professionals serving local populations that live in endemic areas.

49

50 Key-words:

51 tick-borne disease, pharmacists, survey, prevention measure.

52 Introduction

53 In a context of global change, especially in terms of climate, land use, and increased
54 mobility, the risk of vector-borne diseases evolves with the modification of vector distribution,
55 host distribution, and ecosystem properties (Aenishaenslin et al., 2017; Gallana et al., 2013).
56 This is the case for tick-borne diseases in areas where climate change has altered the
57 distribution of ticks and the temporal pattern of their activity: in Europe ticks are found at
58 higher altitudes and further north (Akl et al., 2019; Ragagli et al., 2016), the result of
59 increasingly milder winters (Dautel et al., 2008). Similarly, changes to the landscape, such
60 as fragmented forests and fewer predators, also modify the risk of these diseases
61 (Hofmeester et al., 2017; Levi et al., 2012; Ostfeld and Keesing, 2012).

62 **As a specific example**, the emerging presence of *Hyalomma marginatum* ticks has
63 increased the zoonotic risk of Crimean-Congo hemorrhagic fever (CCHF) **in Europe**
64 (Fontenille et al., 2020; Stachurski and Vial, 2018; Vial et al., 2016). **But the accumulation of**
65 **changes has also impacted the risk of** more common **diseases** such as Lyme borreliosis,
66 which is the most **widespread** tick-borne disease **in** humans (Van Hout, 2018). Lyme
67 borreliosis **is caused by pathogens transmitted** by ticks of the *Ixodes* **genus, and the** *I.*
68 *ricinus* **is the most common** *Ixodes* **species** found in metropolitan France. This tick is found
69 in ecological environments where suitable land cover and climate conditions are present
70 (Beugnet et al., 2009; Hönig et al., 2011; Swart et al., 2014; Tack et al., 2012) and hosts are
71 abundant.

72 The incidence of Lyme borreliosis has increased in European countries (Medlock et al.,
73 2013) including metropolitan France. The annual estimated incidence of Lyme borreliosis in
74 France was stable between 2009 and 2015 (55 cases per 100 000 inhabitants in 2013), but
75 increased to 76 cases per 100 000 inhabitants in 2019 with large disparities between regions
76 (Sentinelles, 2020). In the Auvergne sub-region, annual incidence per 100 000 inhabitants
77 was estimated at 92 [IC95% 39;145] and in the Rhône-Alpes sub-region at 137 [IC95%: 92;
78 176] (Sentinelles, 2020). The risk of Lyme borreliosis depends on **the** density of infected
79 ticks and human exposure (De Keukeleire et al., 2015; Zeimes et al., 2014).

80 Thus, it becomes essential to sensitize populations by promoting the adoption of preventive
81 behaviors to avoid tick bites (Aenishaenslin et al., 2015). It should be noted that these ticks
82 can also be present in urban and suburban areas (Mathews-Martin et al., 2020).

83

84 For people spending time in an environment favorable to infected ticks, there are several
85 recommendations for reducing the risk of contact during and after an outing: tucking pants
86 into socks, walking in the center of trails, wearing light colored clothing, and using repellent
87 during an outing; showering/bathing, inspecting people and pets for ticks, and washing
88 clothes at 60° Celsius after an outing (Connally et al., 2009; Niesobecki et al., 2019; Bron et
89 al., 2020; Eisen, 2021).

90 Indeed, the risk of Lyme borreliosis is a **product** of the hazard, exposure, and human
91 vulnerability to the pathogen (De Keukeleire et al., 2015; Rousseau et al., 2017). The hazard
92 is defined as the presence or abundance of infected questing *I. ricinus* ticks in the
93 environment. It is influenced **by the prevalence of infection in the reservoir host community,**
94 **as well as environmental and meteorological factors.** Human exposure is a function of the
95 potential contact with an infected tick. The level of exposure often depends on the type of
96 human activity (e.g., sitting on logs, gathering wood, brushing against trees, hiking) (Dantas-
97 Torres, 2015; De Keukeleire et al., 2015, 2016). **Finally, vulnerability depends on individual**
98 **behavior and the actions taken to avoid infection (e.g., as taking account of prevention**
99 **messages and following recommendations on clothing, repellent, etc.). Therefore, controlling**
100 **the risk of this type of disease means evaluating the hazard, reducing exposure, and limiting**
101 **vulnerability when exposed.** These measures are affected by public prevention messages,
102 the population's understanding of the risk, and the preventive behaviors they undertake.
103 Unfortunately, public recommendations do not always achieve their objectives (Dernat and
104 Johany, 2019a; Puppo and Préau, 2018) and can be anxiety-provoking for some individuals
105 (Dernat and Johany, 2019b). Consequently, as shown by many studies, Lyme borreliosis
106 preventive behaviors are often unevenly adopted by the population (Aenishaenslin et al.,
107 2017, 2016; Butler et al., 2016; Hook et al., 2015). There is a need to move towards better

108 management of prevention methods and public health messages. Specific procedures
109 regarding the management of patients should be improved with respect to the organization
110 and infrastructure of local healthcare systems and for the implementation of preventive
111 healthcare measures. Several professionals are involved in this public health mission,
112 including pharmacists (Jen et al., 2016). In fact, pharmacists are often the first health care
113 professionals to be contacted by patients, particularly in medically underserved areas that
114 have convenient accessibility to pharmacies in France, due to their expansive distribution
115 throughout the country.

116 In France, retail pharmacists can provide information to patients on ticks and associated
117 risks of disease, guidance in selecting a repellent, advice on appropriate clothing before
118 exposure, and recommendations of what to do in case of a tick bite or after exposure. Thus,
119 pharmacists and their co-workers are on the front lines in the dissemination of knowledge
120 and prevention measures on ticks and tick-borne disease. They could also actively
121 participate in the surveillance of tick distribution.

122 One question concerns the level of pharmacists' knowledge in the field of ticks and Lyme
123 borreliosis and in the appropriate practices in terms of prevention and advice. Effective
124 prevention requires knowledge on key steps of the transmission: tick ecology, to prevent or
125 be aware of activities at risk according to the spatial and temporal context of tick activity, tick
126 bites, and *Borrelia* transmission.

127

128 Indeed, knowing how to identify ticks and the way they transmit *Borrelia burgdorferi* sensu
129 lato is an essential component of prevention and surveillance of ticks and tick-borne
130 diseases. This study addressed this issue by means of a large survey involving pharmacists
131 and their assistants in a geographic area that has a high incidence of Lyme borreliosis.

132

133 Materials and methods

134 Settings

135 An online survey was conducted among pharmacists and pharmacy assistants (PPA),
136 mainly in the Auvergne Rhône-Alpes (AURA) region of France. The online survey was
137 developed using LimeSurvey software (LimeSurvey Project Team / Carsten Schmitz, 2012).
138 Data were collected from February 1–27, 2019, with reminder e-mails sent two weeks before
139 the due date.

140 PPA were contacted via an e-mail sent by the regional pharmacist union and the regional
141 Order of Pharmacists. The annual incidence of Lyme borreliosis is estimated at 156 cases
142 per 100 000 inhabitants in 2016, compared to the national estimated rate (mainland France)
143 of 104 cases (Vaissière, E., Thabuis, A., Couturier, 2018). For every 100,000 inhabitants, the
144 AURA region has 31 pharmacies compared to an average of 32 in France.

145

146 Questionnaire development

147 A questionnaire (Appendix A) containing 22 multiple-true/false questions was divided into
148 three sections. Participants responding to the questionnaire were asked to select all the
149 proposed answers which correctly respond to the question. The feasibility and content
150 validity of the questionnaire were checked by a group of scientists: a lecturer in
151 pharmaceutical practices, a lecturer in veterinary parasitology, and a lecturer in medicine.
152 The questionnaire was pilot tested with 15 people, including six pharmacists and five
153 pharmacy assistants. Respondents' feedback from the pilot study was used to make minor
154 adjustments to the final questionnaire. The first section, consisting of 8 questions, collected
155 knowledge about ticks in general, the ability to identify ticks, and specific knowledge about
156 the vector of Lyme borreliosis (*Ixodes* spp.) and its biology. The first two questions (Q1 and
157 Q2) were based on the same pictures showing several arthropods: one insect, two spiders
158 and ticks of different genera *Hyalomma*, *Dermacentor*, *Ixodes*, *Rhipicephalus* engorged or
159 unfed. In Q1, respondents were asked which images show a tick, and in Q2, which images

160 identified the *I. ricinus* tick, the vector of the bacterial pathogen responsible for Lyme
161 borreliosis that is the primary vector in France and/or Europe. The second section,
162 consisting of 8 questions, evaluated knowledge on tick prevention measures and the
163 mechanism of Lyme borreliosis transmission. The third section collected descriptive
164 information about the respondent: gender, age, function at the pharmacy (pharmacist or
165 pharmacy assistant), location of the pharmacy (rural or urban, and postal code), and
166 feedback on tick bites. After validation of the questionnaire by the respondent, a page
167 identifying all the correct answers to the questions was available.

168

169 Formulation of questions and proposed answers

170 For each question, several answers were proposed: some false, others true. The
171 respondent's choice, whether, or not, to select each proposed answer, was considered
172 correct if (i) the answer was selected by the respondent when it was true or if (ii) the answer
173 was not selected by the respondent when it was false. The respondent's choice was
174 considered incorrect whenever a true answer was not selected or whenever a false answer
175 was selected.

176

177 Statistical analysis

178 Statistical analyses were conducted in R (R Core Team, 2019). Figures were produced
179 using the package ggplot2 (Wickham, 2016) and the appendix B was generated using the
180 package rmarkdown (Allaire et al., 2020). Descriptive statistics (frequencies, percentages)
181 were calculated for data analysis. The quality of the answers that respondents selected for
182 each question were assessed by the proportion of correctly selected answers to (i) the set of
183 propositions for each question, (ii) the true propositions, and (iii) the false propositions. The
184 objective was to assess knowledge in a global manner by determining the respondents'
185 ability to correctly identify the true and false propositions.

186 The chi-square tests of independence were performed to assess whether the distribution
187 of response quality was significantly different according to the respondents' characteristics
188 (pharmacists or assistants, rural or urban). All the results are available in the Appendix B.

189

190 Ethical approval

191 The implementation of an anonymous questionnaire among French pharmacists and
192 assistants does not require a specific ethics review process. Participation in the survey was
193 voluntary. Participants were informed in advance about details of how the data would be
194 used, assuring anonymity in accordance with French regulations at the time of data
195 collection.

196 Results

197 Questionnaire completion and respondents' characteristics

198 **There were** 546 people who clicked on the link to the online questionnaire. **The 152 who**
199 **did not respond to all the questions and the 30 who did not complete the online profile were**
200 **excluded. The questionnaires of the remaining 364 respondents (a 66.6% response rate)**
201 **were retained for the analysis.**

202 Among the **analyzed** questionnaires, the majority of respondents were located in the
203 Auvergne Rhône-Alpes (AURA) region, mainly in the proximity of large cities (Fig. 1).
204 Pharmacists comprise 85.2% of the total respondents, which represents about 4.5 % of the
205 6,820 retail pharmacists present in 2016 in the region. Declarations of habitation were evenly
206 distributed between rural (49.7%) and urban (50.3%) areas. The participants were 67.6%
207 women (n=246) and 32.4% men (n=118). The proportion of respondents included 21.7% of
208 participants aged between 18 and 30 years old, 34.6% between 31 and 45, 34.3% between
209 46 and 60, and 9.3% over the age of sixty. Among the respondents, 54.7% had already been
210 bitten by a tick once or several times, 66.2% had already had a close relation (family,
211 friends) who had been bitten, 58.5% had had a pet bitten, and 91.2% reported having been
212 consulted about a tick or ticks during their work at the pharmacy. Only 1.4% reported never
213 having been confronted with ticks.

214 Among the 16 questions concerning tick knowledge and Lyme prevention, one question
215 (Q15) about the probability of Lyme borreliosis transmission by an infected tick was
216 discarded. This question was confusing because it did not specify the period of time between
217 the bite and the removal of the tick, nor the way the tick was removed. This time lapse and
218 the removal method are key factors in the probability of transmission (Cook, 2014).

219

220 Global results

221 **The response to almost every question was correct** more than 50% of the time (see Fig.2A)
222 **The one exception was** question 10 **which concerned** the part of the body that should be
223 checked after being exposed to the risk of ticks.

224 One particular feature of the questionnaire was the formulation of questions with a series
225 of answers that the participants were required to identify as true or false. Figure 2B
226 highlights how participants responded.

227 On average, participants recognized false information, but they had more difficulty
228 confirming true information. Indeed, all false answers (Fig 2B, triangles) were correctly
229 identified more often (more than 80% of the time on average) than true answers (Fig 2B,
230 squares). True answers were correctly identified from 41 to 78% of the time, depending on
231 the question. In other words, participants were better at pointing out aberrant propositions
232 than appropriate ones. Among the questions where true answers were proposed, only two
233 were correctly identified more than 80% of the time, one which concerned advice to be given
234 to patients after a bite (Q12), and the other concerning when to refer the patient to a doctor
235 (Q14). Overall, the average of correct responses did not differ between the themes of
236 biology or prevention.

237
238 Knowledge about ticks

239 The ability to identify ticks in general and the ability to identify the tick that transmits the
240 pathogen responsible for Lyme borreliosis were assessed in the first two questions (Q1 and
241 Q2 respectively). In Q1, only 39.0% of the participants responded correctly to all eight
242 answers, identifying correctly all the pictures with a tick and those without a tick (Fig. 3).
243 More than 97% of the time, participants correctly identified each of the 3 non-tick images
244 (two spiders and the louse). However, the tick images (*Rhipicephalus sanguineus*, the unfed
245 and gorged *I. ricinus* Adult female, the *Hyalomma marginatum* and the *Dermacentor*
246 *reticulatus*) were not as well identified. Only 59.6 to 87.4% (depending on the image) of the
247 responses correctly identified them (Fig. 3).

248 Among the images proposed for Q2, the pictures of two spiders and a louse were
249 consistently identified correctly as not transmitting the agents that cause Lyme borreliosis
250 (99.7%, 97.5% and 100% respectively), but among the pictures of a tick, the participants had
251 more difficulty identifying the vector that transmits the pathogens responsible for Lyme

252 **borreliosis, *I. ricinus***, (Fig. 3). The picture of the engorged female tick, *I. ricinus* (H), was
253 relatively well identified, selected correctly by 74.2% of respondents. But the unfed female *I.*
254 *ricinus* (C) was only identified correctly by 47.3% of respondents. Other tick **genera** that do
255 not transmit **the agent responsible for** Lyme borreliosis, *Rhipicephalus* (answer A),
256 *Hyalomma* (D), and *Dermacentor* (F), were incorrectly identified as a **Lyme borreliosis vector**
257 by 28.8%, 24.2% and 45.9% of respondents respectively.

258 Concerning the habitats of *I. ricinus* tick (Q3), 15.1% of respondents correctly identified all
259 answers and 88.2% correctly identified 6 out of the 8 answers. The false answers (A: dry
260 areas; B: along the coasts; D: high mountain areas; and H: urbanized areas) were often
261 correctly identified as areas unfavorable to ticks (86%, 94.2%, 98.1% and 93.1%, correct
262 responses, respectively). However, the true answers (suitable habitats for ticks) were rather
263 poorly identified. For example, coniferous forests (answer C) were correctly identified in only
264 52.5% of responses, and temperate plains areas (answer E) in only 65.4% of responses.
265 Only two of the true answers, mid-mountains below 1500 meters above sea level (answer F)
266 and hardwood forests (answer G), were correctly identified with relatively comfortable
267 margins (75.5% and 86.3% respectively). This topic was complemented by question 4 on the
268 type of land cover that people encounter on an everyday basis and is also favorable to ticks.
269 Among the nine answers proposed, the five false answers were rather well identified while
270 three of the four true answers were less well known. All of the answers were correctly
271 identified by 12.9% of the respondents, and 7 out of 9 answers by 79.7%. The 5 false
272 answers that were often correctly identified concerned places where the *Ixodes* tick is rarely
273 encountered: treetops (answer A) identified correctly 86% of the time, middle of the rocks (B)
274 98.9%, houses (C) 99.2%, under the ground (G) 99.2%, and water (I) 99.5%. With respect to
275 suitable habitats for *Ixodes* ticks, only one, high grass (answer D) was known by almost all of
276 the respondents: 98.6% responding correctly. The 3 others true proposals were not as well
277 identified: only 63.2% of the answers were correct for pastures (answer E), 43.7% for the
278 paths (F), and 46.2% for dead leaves underneath trees (H).

279 With regard to the factors influencing tick activity (Q7, Fig. 4), 16.5% of respondents
280 correctly identified all of the answers and 45.3% correctly identified 6 out of 7. The two false
281 answers, the presence of insects (answer D) and blood type (G) were well identified as
282 having no known influence on tick activity, noted correctly by 95.1% and 98.4% of
283 respondents, respectively. The participants were less sure about the true answers. The
284 temperature (answer B) and season (answer F) were believed to be a suitable condition for
285 tick activity by nearly 89.8% and 77.5% respectively. However, correct responses identifying
286 humidity (answer A), altitude (C), and the presence of hosts (E) were only 57.7%, 51.1%,
287 and 58.5% respectively.

288 Ten images of animals were proposed (Q8, Fig.5) to evaluate the respondents'
289 knowledge about possible hosts for *Ixodes* ticks. Only 3.6% of respondents correctly
290 identified all answers, and 13.7 % correctly identified the validity of 8 answers out of the 10.
291 False answers, insects (D) and fish (I), were well identified as unsuitable hosts for ticks,
292 each with 98.9% correct responses. Dogs, rodents, and cattle were well identified as suitable
293 host animals of *I. ricinus* with participants responding correctly 90.7%, 73.1% and 72.8% of
294 the time. Knowledge expressed as a frequency of correct responses was weaker for hosts
295 such as snakes in answer B (7.1%), owls in G (22.5%), birds in A (23.9%), horses in J
296 (48.1%) and deer in answer E (53.0%).

297 Knowledge on *Borrelia* transmission by ticks was addressed with a question (Q5) about
298 the developmental stages when ticks can transmit the pathogen. In Q5, 5.5% of respondents
299 correctly identified all the questions and 45.1% correctly identified 3 out of 4. Among the
300 non-infective developmental stages of ticks when the bacteria are not transmitted, larvae
301 (answer A) were well identified as a false answer by 82.1% of participants contrary to the
302 adult male tick (answer C) which was only identified correctly by 41.5% of them. For the
303 infective stages, the female adult (answer D) was well identified 91.5% of the time, but only
304 24.5% of respondents recognized nymphs (answer B) as a stage involved in the
305 transmission of *Borrelia* pathogens.

306 Concerning the process of pathogen transmission by ticks (Q13, Q16), only 60.7% of the
307 respondents selected the true answer, "after 24 to 48 hours" (Q13). For 9.6% of
308 respondents, the risk of transmission occurred immediately after the bite, and for 6.3% of
309 them, the risk occurs one to two hours after the bite. In question Q16 on the microorganism
310 responsible for the transmission of Lyme borreliosis, 66.2% of respondents indicated that it
311 was indeed a bacterium, while 33.8% them believed it was a parasite (false answer).

312

313 Knowledge about Lyme borreliosis and prevention

314 The knowledge on tick bite prevention (Q9) was evaluated through 8 answers (appendix
315 B). For this question, 97.2% of respondents correctly identified 6 answers out of 8. All
316 answers were correctly identified between 93.9% and 99.5% of the time, except for "wearing
317 white clothing" (answer D) which was often misidentified. Only 24.5% of respondents
318 correctly chose "wearing white clothing" as one of the methods to prevent tick bites because
319 ticks could be detected more easily. In the question about the method of tick removal in case
320 of a tick bite (Q11), only 2 of the 6 answers were true: the tick remover (answer E) and the
321 tweezers (answer B). The tick remover was correctly identified by 100% of the respondents
322 but only 8.2% of them indicated the tweezers as a potential tool for removing ticks, perhaps
323 because they are more appropriate for larvae and nymphs. Almost all respondents knew that
324 (answer A), alcohol (C), fingers (D), and oil (F) are not recommended for removing ticks
325 (proportion of correct answers > 93.1%).

326 As for the part of the body that needs to be checked after being exposed to a bite risk
327 (Q10), the ten answers were all true. Only 10.4% of respondents correctly selected all the
328 answers, indicating they knew that all body part should be verified. Among respondents,
329 54.6% of them identified only one to four body parts. The legs (answer E, 84.3%), armpits
330 (D, 65.9%), scalp (B, 60.4%) or elbow crease (C, 51.6%) were selected by more than half of
331 the respondents, but chest (answer A, 21.1%), ears (G, 32.1%), and navel (J, 36.0%) were
332 selected by very few.

333 Concerning advice given to patients after a bite (Q12), only 34.9% of the respondents
334 correctly identified all the answers, but 82.7% correctly identified 6 out of 7. Monitoring the
335 location of the bite for the possible appearance of an erythema migrans defined as an
336 expanding red skin inflammation of at least 5 cm of diameter appearing around 3 days after
337 the tick bite (answer F) and the disinfection of the concerned area (answer A) were each
338 correctly identified by 97.5 % of the respondents. However, the advice to remove the
339 capitulum of the tick, commonly referred to as a tick's head if it remains in the skin (answer
340 E) was only identified by 62.5% of respondents.

341 There were 6 answers for the question on when they refer patients to a doctor (Q14). The
342 only true answer, "In case of a red halo (erythema migrans) greater than a 2€ coin" (answer
343 E), was correctly identified by 97.8% of the respondents. However, only 23.1% of
344 respondents correctly identified all the answers.

345

346 Variables associated with different knowledge.

347 No statistical associations by X^2 test were found between respondent's characteristics and
348 the quality of response, except in four questions. There was a significant difference (p-value
349 < 0.05) between the urban and rural profile about where *I. ricinus* ticks could be encountered
350 (Q4), with higher percentage of correct identifications among urban responders. There were
351 also significant differences between pharmacists and pharmacy assistants in three items:
352 pharmacists correctly identified more answers than the pharmacy assistants in the question
353 about the location of the disease agent in ticks (Q6), assistants indicated more often that
354 they refer the patient to a doctor (Q14, see Appendix B figure 40 for more details), and
355 pharmacists were better at identifying bacteria as the type of microorganisms implicated as
356 the Lyme borreliosis agent (Q16).

357 At the multivariate scale, a significant difference between assistants and pharmacists was
358 detected in the quality of the response on Q2 (identify the vector of pathogens responsible
359 for Lyme borreliosis) among those who recognized the unfed tick in Q1 (answer C, p-value=
360 0.01): pharmacists identified the vector that can transmit *Borrelia* better than assistants did.

361 The same difference was detected between rural and urban people among those who
362 recognized the engorged tick (answer H, p-value= 0.038): rural respondents were better
363 than their urban counterparts at identifying the engorged tick as a vector of pathogen that
364 causes Lyme borreliosis.

365 Discussion

366 In this study, the knowledge of pharmacists and pharmacy assistants (PPA) regarding tick
367 risk was evaluated through an online questionnaire. For this purpose, a large sample of
368 professionals were contacted in the Auvergne-Rhône Alpes region (AURA). No such
369 evaluation had been previously performed although the role of these professionals as a
370 health actor is of prime importance due to their proximity with the population.

371 The major results of this study highlight that PPA had solid knowledge of tick bite
372 prevention measures both before and after exposure to the risk of ticks, and after a bite
373 event. However, we noted weaker, even insufficient, knowledge on tick biology and ecology,
374 which could have a detrimental impact on the effectiveness of preventive measures. This
375 knowledge is fundamental to guide patients and reduce the risk of disease; indeed, to
376 engage a preventive behavior, people need to be aware of the risk involved (Beaujean et al.,
377 2013; Cartter et al., 1989; Herrington, 2004; Slovic, 1987). The PPA must therefore have a
378 broader public health approach with broader knowledge than that strictly and directly related
379 to prevention. This is necessary to be able to adapt and support the prevention discourse
380 according to specific conditions (environment, season, setting, human activity) and to alert
381 patients facing potential risks. It is all the more important because the risk associated with
382 ticks varies according to the season (Vollack et al., 2017), climatic factors (Alkishe et al.,
383 2017; Boehnke et al., 2017; Cayol et al., 2017; Furness and Furness, 2018), the presence of
384 hosts (Qviller et al., 2016), and the type of activities people are engaged in.

385 This study showed poor ability to recognize ticks other than *I. ricinus*. This could be
386 problematic. In fact, several tick species can be responsible for pathogen transmission to
387 humans in France, such as *Rhipicephalus* and *Dermacentor* ticks that can transmit
388 *Rickettsia* bacteria or *H. marginatum*, whose recent emergence highlight the risk of the
389 appearance of human cases of CCHF in France (Grech-Angelini et al., 2020).

390

391 Regarding adult ticks as vectors of the pathogens responsible for Lyme borreliosis, most
392 participants correctly identified the female *I. ricinus* when engorged, but not when unengorged. The

393 nymphal stage, **was** correctly identified **as a vector** by only 25% of respondents. This very
394 low rate may be related to question 2, where the *I. ricinus* vector was only illustrated by adult
395 females (unfed (C) and fed (H)).

396 From an epidemiological point of view, nymphs have a major role in the transmission of
397 the pathogen (Goldstein et al., 2018; Kjaer et al., 2019) because they are more numerous
398 than adults and much smaller in size, which makes them more difficult to detect. The risk of
399 transmission of the pathogen involved in Lyme **borreliosis** usually occurs between 24 and 48
400 hours after the bite (Kahl et al., 1998, Carriveau et al., 2019). This true answer was selected
401 by the majority of the responders (60.7%). Two of the false answers place the risk of
402 transmission after a longer period of time, one week after the bite (5.9%) or one month after
403 the bite (17.6%). These incorrect responses could be due to the misunderstanding of the
404 question: respondents may have confused the risk of transmission with the appearance of
405 the first symptoms of Lyme borreliosis.

406 **The advice to** remove the tick capitulum if it has remained in the skin after a tick bite was
407 poorly identified by pharmacists (62.5%). This result can be explained by the fact that, **in a**
408 **strictly morphological sense**, this action is not necessary because **the capitulum is not**
409 **directly connected to the salivary glands containing the pathogens and it usually disappears**
410 after a few days (Figoni et al., 2019). The tick remover was clearly identified by 100% of the
411 respondents, but surprisingly, the possibility of using tweezers was only validated by 8.2% of
412 them. In the absence of a tick remover, tweezers, particularly the fine types used by
413 entomologists, are an effective alternative for removing ticks, and especially larvae and
414 nymphs. Such instruments should be clearly mentioned in public awareness efforts (Černý et
415 al., 2020; Pitches, 2006). These findings highlight that PPA knowledge is similar to that of
416 the general population, which results from each person's experiences and background
417 (Dernat and Johany, 2019a; Slovic, 1987). In the study of a general population exercising
418 some outdoor practices in Combrailles (sub-region of AURA), Dernat and Johany (2019a)
419 found that the individual perception of tick bite risk depended on social dimensions
420 (education, social groups), spatial dimensions (location of leisure practices or professional

421 activities), and a cognitive perception or personal experience (tick bites, for example).This
422 general population also held certain common perceptions or stereotypes about ticks and
423 their consequences (bites, diseases) (Dernat and Johany 2019a; Dernat and Johany 2019b).
424 Both populations interviewed in those studies, the PPA and the general population, gave an
425 important place to the hardwood forest as a zone where ticks can be encountered, but very
426 little precise knowledge about other more specific tick habitats. This perception that ticks are
427 only significantly present in forested areas is inconsistent with the findings of Mathews-
428 Martin et al. (2020) and suggests that It would have been interesting in our case to add a
429 question about urban areas (without stipulating sidewalks).

430 Both the PPA and the general population in the Dernat and Johany studies rarely
431 mentioned weather and seasonality as parameters affecting tick activity. In our study no
432 significant difference in the answers was observed according to the location (rural or urban)
433 and the professional status (pharmacists or assistants). This is concordant with the results of
434 Dernat and Johany (2019b) which showed that general knowledge was similar among urban
435 and rural respondents. However, this differs from the results of Bayles et al. (2013), who
436 observed different preventive behaviors between populations in rural areas versus suburban
437 parks.

438 In this study, 91.2 % of PPA respondents were aware of the presence of ticks because of
439 customer enquiries or because they or someone close to them have been affected. These
440 results were similar to those observed in Canada, where only 12% of the general population
441 had never heard of Lyme borreliosis (Aenishaenslin et al., 2016). Since pharmacies are
442 numerous and uniformly distributed throughout the territory, PPAs constitute an important
443 link in the coordination of health care and should be fully involved in risk prevention.
444 Consequently, it is necessary for PPA to know how to recognize the early signs of erythema
445 migrans so that they can refer patients to a physician and possibly avoid the progression of
446 the disease. They can provide advice in the selection of effective repellents, the purchase
447 and use of a tick remover, and how to protect oneself using appropriate clothing. Actually,
448 the most commonly practiced prevention behaviors are the use of repellent (Butler et al.,

449 2016) and checking for ticks on pets and humans (Niesobecki et al., 2019). In the present
450 survey, checking for ticks on the body was not well identified. This confirms the necessity to
451 increase specific knowledge in pharmacies to improve attitudes and behaviors towards tick
452 bites. As health professionals, PPA have a mission of advice and information (Jen et al.,
453 2016) to provide a better knowledge about risk (Bonner, 2017; Jen et al., 2016). Although
454 the topic of ticks and tick-borne diseases has been included in teaching courses of
455 pharmacy students for many years in France, results obtained in this study highlight the
456 need to improve the global knowledge among PPA. PPA should therefore be active in
457 maintaining and updating their knowledge on tick risk (Puppo and Préau, 2019, 2018), on
458 the evolution of that risk, and on their position as a principle channel for disseminating
459 information about prevention. The transfer of knowledge from researchers to health
460 professionals, health authorities, citizens, and students by continuous training is therefore
461 essential (Ahmed et al., 2018). To be effective, knowledge transfer by researchers must offer
462 a message adapted to local beliefs and stereotypes. One possibility is to rely on the concept
463 of a living lab that involves people with different interests and backgrounds (Malmberg et al.,
464 2017). The Living Lab is defined as an open and citizen-centric approach for innovation
465 where the participants bring their own specific wealth of knowledge and expertise to the
466 collective and can propose new way to share knowledge (Bergvall-Kåreborn and Ståhlbröst,
467 2009). In this type of organization, partners bring their own specific wealth of knowledge
468 and expertise to the collective (Bergvall-Kåreborn and Ståhlbröst, 2009). **And they thus**
469 **improve** the base of **knowledge** about **the subject** being studied, in our case, **ticks and the**
470 **prevention of Lyme borreliosis.**

471 The preventive role of PPA is all the more important for the local population as shown in a
472 previous study performed in Combrailles (Letertre-Gibert et al., 2020). Indeed, although the
473 natural areas at risk are familiar, the perception of risk is subjective and complex (Rohrman
474 and Renn, 2000) and the protective behavior variable (Brug et al., 2009; Herrington, 2004).
475 Previous studies have noticed that even if people are aware of protective measures against
476 tick bites, they do not always adopt them nor change their behavior (Aenishaenslin et al.,

477 2016; Hook et al., 2015; Marcu et al., 2013; Puppo and Préau, 2018). This study was
478 conducted in a region relevant to the risk of tick bites because the population is highly
479 exposed due to professional and leisure activities and a high level of tick abundance (Dernat
480 and Johany, 2019a; Sentinelles, 2020). Thus, it would be interesting to extend this study to
481 other regions where the risk of ticks is similar in order to evaluate the knowledge of PPA in
482 different geographical and social contexts. Before extending this study to other regions, a
483 few modifications should be made to the two questions (what to do after a bite, and the
484 likelihood of *Borrelia bacteria* transmission) that were difficult to understand and therefore
485 interpretable in several ways. As seen in all surveys, the meaning and order of the questions
486 may have influenced the answers provided.

487 Our findings are subject to several limitations.

488 Regarding the questionnaire itself, two main limits could be pointed out. First, the meaning
489 and order of the questions may have influenced the answers provided. We did not propose
490 the nymphal stage as a vector in question 2. This may be why *that stage was not* correctly
491 identified (Q5) as infectious *and harboring the pathogens responsible for Lyme borreliosis*.
492 This could be taken into account in subsequent questionnaires by randomly ordering the
493 questions within the survey.

494 Secondly, despite pretesting the questionnaire with 15 people, there were four questions that
495 were problematic and would benefit from clarification. The question concerning the
496 *probability of developing Lyme borreliosis after being bitten by a tick infected with the*
497 *pathogen* (Q15) was poorly formulated, leading to responses with multiple interpretations. *As*
498 *a consequence, we did not take* this question into account in the analysis. The questions
499 about advice after a bite (Q12), about the time frame when the risk of transmission increases
500 (Q13), and finally, about when to refer the patient to a doctor (Q14) were not precise
501 enough.

502 These questions should be modified for future applications of the questionnaire. Question 12
503 should specify that it is advice to be given immediately or very soon after a tick bite.
504 Question 13 should clarify that the context is one where the tick is a carrier of a *Borrelia*

505 *pathogen*. Finally, for question 14, it would be necessary to indicate that it refers to a case
506 where the person has been bitten by a tick. Moreover, the present study analysed
507 information only from pharmacists and preparers working mainly in AURA region in France,
508 therefore, all the results may not be generalizable to pharmacists at an international scale
509 where the risk of Lyme borreliosis and lifestyle habits are very different. However, this study
510 gives us ideas for directions to take, and in particular, points to the need to further
511 understand the knowledge of all health professionals. A survey with these goals could be
512 completed by semi-directed interviews to better understand the individual and societal
513 origins of our main findings and identify relevant training tools to be developed.

514

515

516 Conclusion

517 This study showed that in a region where tick risk is present, general knowledge about ticks
518 was mastered by pharmacists and pharmacy assistants, but weaknesses were observed
519 regarding the vector's **life cycle**. This can lead to difficulties in disseminating relevant and up-
520 to-date information about tick risk. This study highlights the need to improve the knowledge
521 of pharmacists, particularly through post-graduate training. This is particularly important for
522 pharmacists because these health care professionals are often the first and most accessible
523 points of contact with the public. The construction of an effective policy of prevention
524 involving citizens, health professionals, health authorities, and scientists via a living lab
525 **where each one brings his or her knowledge, experiences, and questions**, could partly meet
526 these challenges.

527

528

529 Appendices:

530 Appendix A: Questionnaire with proposed answers in English and in the original French
531 version.

532 Appendix B: Document with all descriptive analysis of survey data.

533

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545

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547 The authors declare that they have no competing interest.

548

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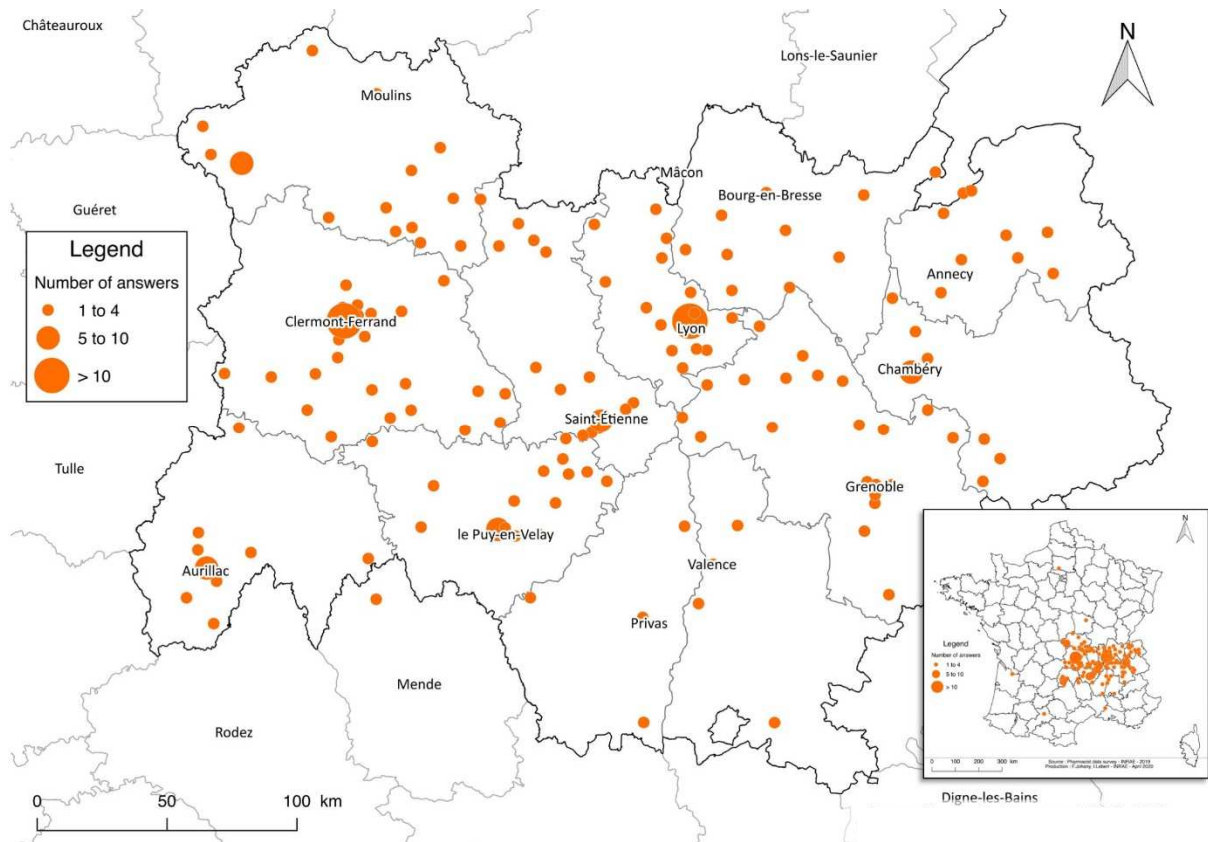


Figure 1: Map of the respondents' locations in France. The size of point is proportional to the number of respondents at each location.

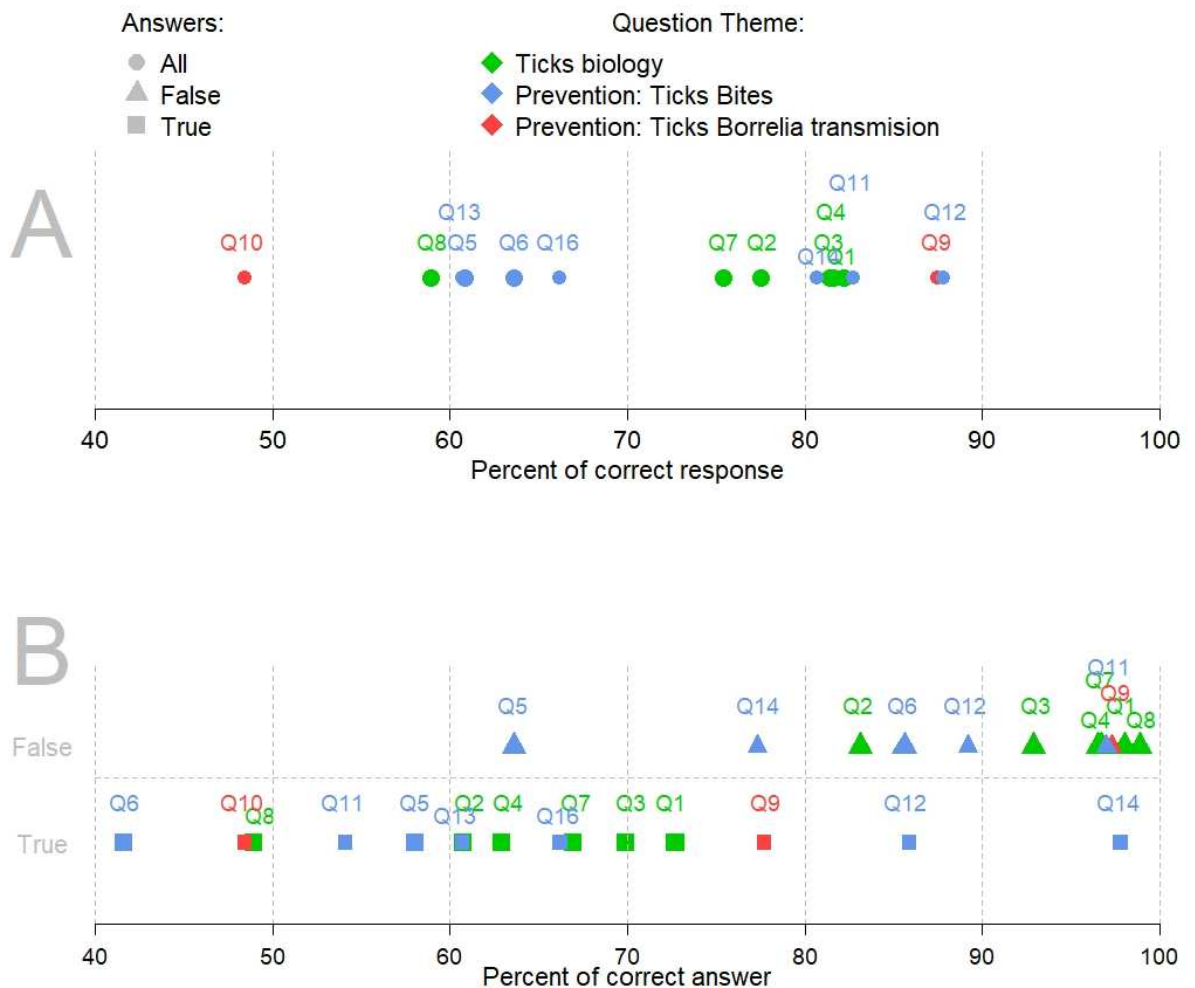


Figure 2: Distribution of questions according to the average of correct identification for all proposed answers in A and distribution of questions according to the average of correct identification for true answers (squares) and false answers (triangles) in B; questions regarding ticks and their biology are in green; questions regarding prevention associated to ticks *Borrelia* transmission are in red; questions regarding prevention bites are in blue.

Correct identification for an answer corresponds to an answer selected by the respondent when that answer was true and to an answer not selected by the respondent when that answer was false. For example, in graph A, for question 6, approximately 65% of respondents correctly identified the answers as a whole (circles). In graph B, for question 6 again, the wrong answers (blue triangle) were correctly identified by 85.6% of respondents compared to only 41.2% for the right answers (blue square).

Q1 - Which pictures below show a tick? Q2 - Which photos below show the Lyme disease vector, *Ixodes ricinus*? Q3 - In which regions do ticks *Ixodes ricinus* live? Q4 - In everyday life, where do we encounter *Ixodes ricinus* ticks? Q5 - What are the infective stages of tick development in terms of Lyme disease? Q6 - Where in the tick is the pathogen that causes Lyme disease? Q7 - What can affect tick activity? Q8 - Which of the animals below can be hosts for *Ixodes ricinus*? Q9 - What can be done to prevent tick bites? Q10 - Which body parts should be checked first after potential exposure? Q11 - What are the current recommendations for removing a tick in the event of a bite? Q12 - After a bite, what other advice can you give? Q13 - How long after the bite does the risk of transmission increase? Q14 - When do you refer the patient to a doctor? Q16 - To what group of microorganisms do you think the agent for Lyme disease belongs?







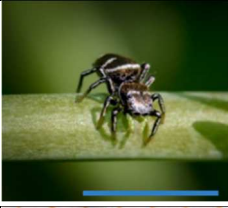

		Image	Q1		Q2	
			Answer	% correct response	Answer	% correct response
A	<i>Rhipicephalus sanguineus</i> tick, adult female		T	68.7	F	71.2
B	spider		F	98.6	F	99.7
C	unfed <i>Ixodes ricinus</i> tick, adult female		T	69.5	T	47.3
D	<i>Hyalomma marginatum</i> tick		T	59.6	F	75.8
E	louse		F	96.7	F	97.5
F	<i>Dermacentor reticulatus</i> tick, adult female		T	78.6	F	54.1
G	spider		F	98.6	F	100.0
H	<i>I. ricinus</i> tick, engorged adult female		T	87.4	T	74.2

Figure 3 Percent of correct responses for questions 1 and 2: Q1 – Which picture below shows a tick? Q2 – Which picture below show the Lyme disease vector *Ixodes ricinus*? T=True answer; F=False answer; The blue line on pictures represents the scale at 0.5cm.

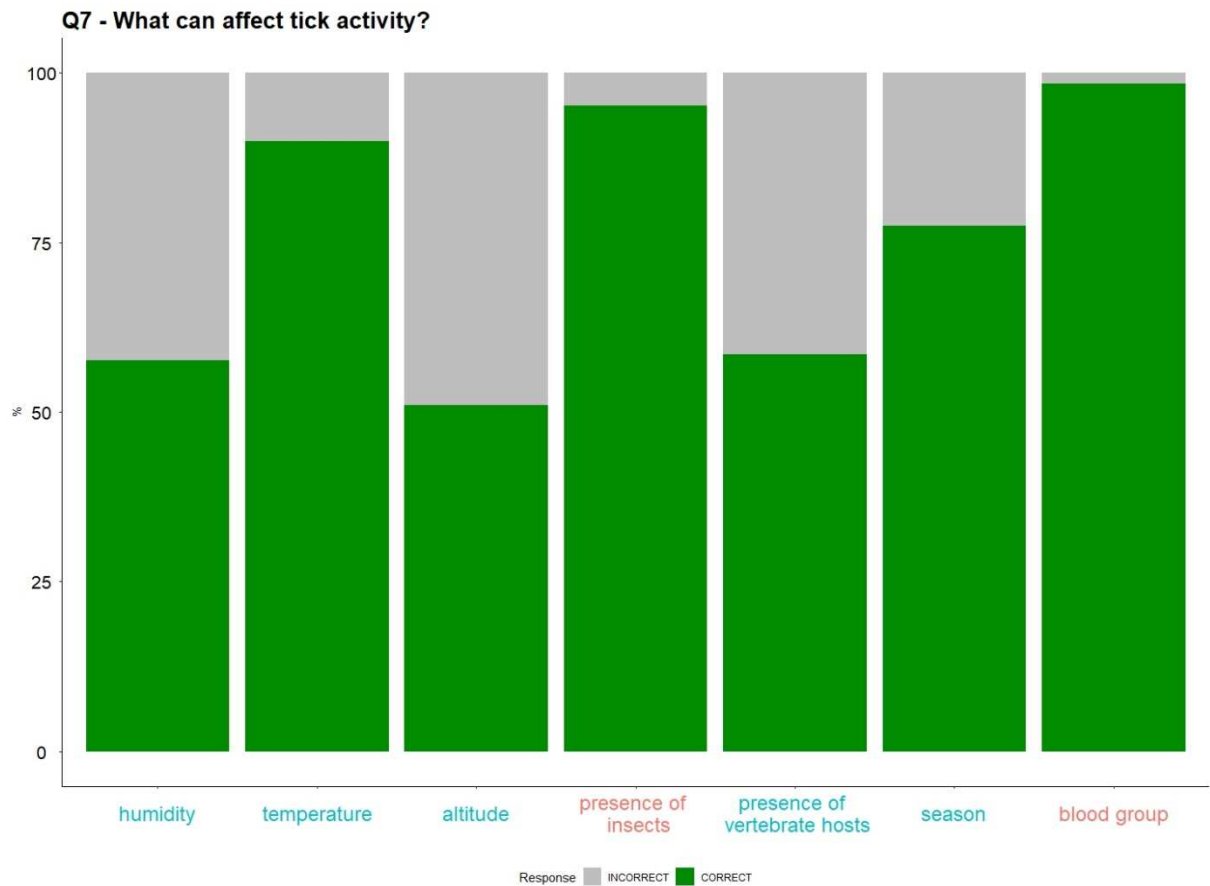


Figure 4. Percent of correct (green) and incorrect (gray) responses for each answer proposed for question 7: What can affect tick activity? x-axis: true answers in blue, false answers in red.

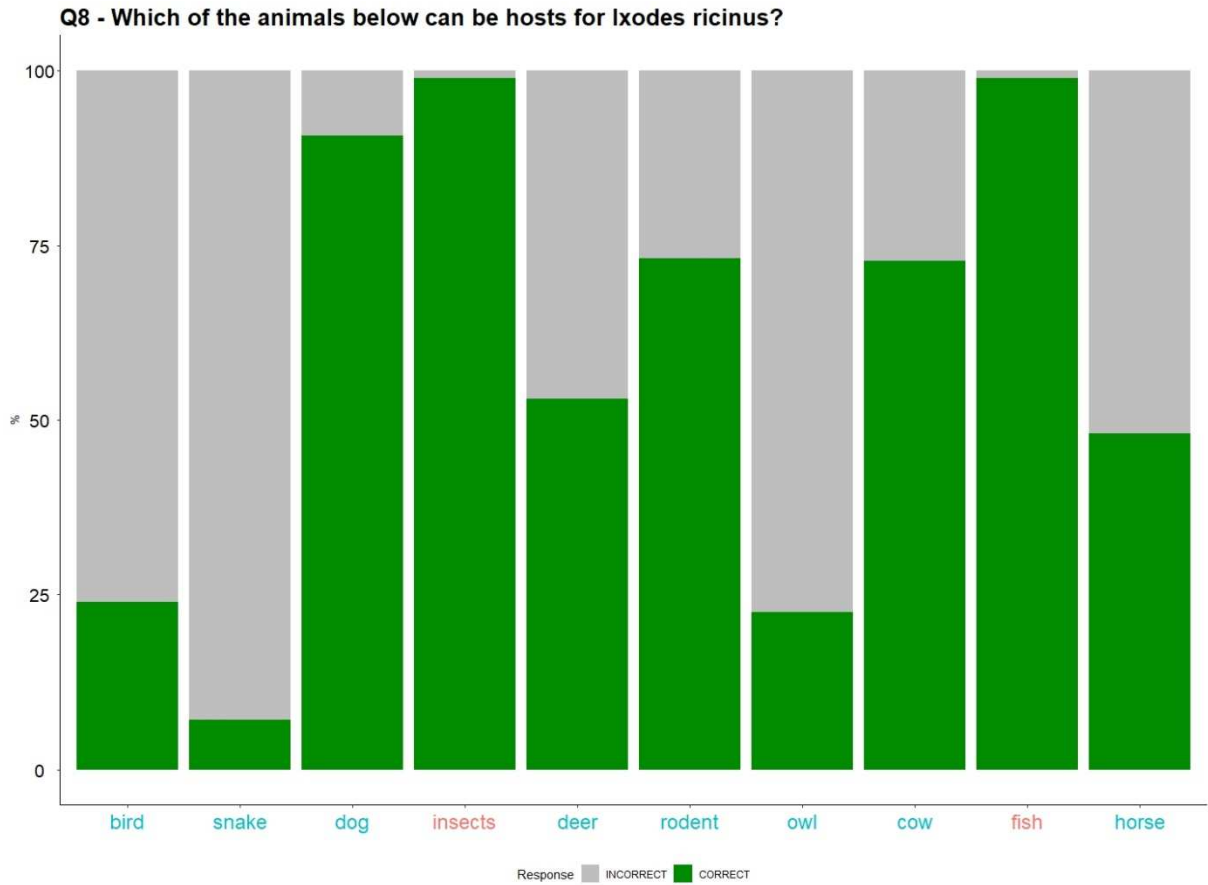


Figure 5. Percent of correct (green) and incorrect (gray) responses to each answer for question 8: Which of the animals below can be hosts for *Ixodes ricinus*? x-axis: true answers in blue, false answers in red.