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

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Ticks and tick-born Disease (TTBD)

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Highlights:

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

Abstract:

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

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

Key-words:

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

Introduction

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

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

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

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

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

Indeed, the risk of Lyme borreliosis is a product of the hazard, exposure, and human vulnerability to the pathogen (De Keukeleire et al., 2015; Rousseau et al., 2017). The hazard is defined as the presence or abundance of infected questing *I. ricinus* ticks in the environment. It is influenced by the prevalence of infection in the reservoir host community, as well as environmental and meteorological factors. Human exposure is a function of the potential contact with an infected tick. The level of exposure often depends on the type of human activity (e.g., sitting on logs, gathering wood, brushing against trees, hiking) (Dantas-Torres, 2015; De Keukeleire et al., 2015, 2016). Finally, vulnerability depends on individual behavior and the actions taken to avoid infection (e.g., as taking account of prevention messages and following recommendations on clothing, repellent, etc.).Therefore, controlling the risk of this type of disease means evaluating the hazard, reducing exposure, and limiting vulnerability when exposed. These measures are affected by public prevention messages, the population's understanding of the risk, and the preventive behaviors they undertake. Unfortunately, public recommendations do not always achieve their objectives (Dernat and Johany, 2019a; Puppo and Préau, 2018) and can be anxiety-provoking for some individuals (Dernat and Johany, 2019b). Consequently, as shown by many studies, Lyme borreliosis preventive behaviors are often unevenly adopted by the population (Aenishaenslin et al., 2017, 2016; Butler et al., 2016; Hook et al., 2015). There is a need to move towards better management of prevention methods and public health messages. Specific procedures regarding the management of patients should be improved with respect to the organization and infrastructure of local healthcare systems and for the implementation of preventive healthcare measures. Several professionals are involved in this public health mission, including pharmacists (Jen et al., 2016). In fact, pharmacists are often the first health care professionals to be contacted by patients, particularly in medically underserved areas that have convenient accessibility to pharmacies in France, due to their expansive distribution throughout the country.

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

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

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

Materials and methods

Settings

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

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

Questionnaire development

A questionnaire (Appendix A) containing 22 multiple-true/false questions was divided into three sections. Participants responding to the questionnaire were asked to select all the proposed answers which correctly respond to the question. The feasibility and content validity of the questionnaire were checked by a group of scientists: a lecturer in pharmaceutical practices, a lecturer in veterinary parasitology, and a lecturer in medicine. The questionnaire was pilot tested with 15 people, including six pharmacists and five pharmacy assistants. Respondents' feedback from the pilot study was used to make minor adjustments to the final questionnaire. The first section, consisting of 8 questions, collected knowledge about ticks in general, the ability to identify ticks, and specific knowledge about the vector of Lyme borreliosis (*Ixodes* spp.) and its biology. The first two questions (Q1 and Q2) were based on the same pictures showing several arthropods: one insect, two spiders and ticks of different genera *Hyalomma*, *Dermacentor*, *Ixodes*, *Rhipicephalus* engorged or unfed. In Q1, respondents were asked which images show a tick, and in Q2, which images identified the *I. ricinus* tick, the vector of the bacterial pathogen responsible for Lyme borreliosis that is the primary vector in France and/or Europe. The second section, consisting of 8 questions, evaluated knowledge on tick prevention measures and the mechanism of Lyme borreliosis transmission. The third section collected descriptive information about the respondent: gender, age, function at the pharmacy (pharmacist or pharmacy assistant), location of the pharmacy (rural or urban, and postal code), and feedback on tick bites. After validation of the questionnaire by the respondent, a page identifying all the correct answers to the questions was available.

Formulation of questions and proposed answers

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

Statistical analysis

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

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

Ethical approval

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

Results

Questionnaire completion and respondents' characteristics

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

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

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

Global results

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

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

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

Knowledge about ticks

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

Among the images proposed for Q2, the pictures of two spiders and a louse were consistently identified correctly as not transmitting the agents that cause Lyme borreliosis (99.7%, 97.5% and 100% respectively), but among the pictures of a tick, the participants had more difficulty identifying the vector that transmits the pathogens responsible for Lyme borreliosis, *I. ricinus,* (Fig. 3). The picture of the engorged female tick, *I. ricinus* (H), was relatively well identified, selected correctly by 74.2% of respondents. But the unfed female *I. ricinus* (C) was only identified correctly by 47.3% of respondents. Other tick genera that do not transmit the agent responsible for Lyme borreliosis, *Rhipicephalus* (answer A), *Hyalomma* (D), and *Dermacentor* (F), were incorrectly identified as a Lyme borreliosis vector by 28.8%, 24.2% and 45.9% of respondents respectively.

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

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

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

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

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

Knowledge about Lyme borreliosis and prevention

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

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

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

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

Variables associated with different knowledge.

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

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

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

Discussion

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

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

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

Regarding adult ticks as vectors of the pathogens responsible for Lyme borreliosis, most participants correctly identified the female *I. ricinus* when engorged, but not when unfed. The nymphal stage, was correctly identified as a vector by only 25% of respondents. This very low rate may be related to question 2, where the *I. ricinus* vector was only illustrated by adult females (unfed (C) and fed (H)).

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

The advice to remove the tick capitulum if it has remained in the skin after a tick bite was poorly identified by pharmacists (62.5%). This result can be explained by the fact that, in a strictly morphological sense, this action is not necessary because the capitulum is not directly connected to the salivary glands containing the pathogens and it usually disappears after a few days (Figoni et al., 2019). The tick remover was clearly identified by 100% of the respondents, but surprisingly, the possibility of using tweezers was only validated by 8.2% of them. In the absence of a tick remover, tweezers, particularly the fine types used by entomologists, are an effective alternative for removing ticks, and especially larvae and nymphs. Such instruments should be clearly mentioned in public awareness efforts (Černý et al., 2020; Pitches, 2006). These findings highlight that PPA knowledge is similar to that of the general population, which results from each person's experiences and background (Dernat and Johany, 2019a; Slovic, 1987). In the study of a general population exercising some outdoor practices in Combrailles (sub-region of AURA), Dernat and Johany (2019a) found that the individual perception of tick bite risk depended on social dimensions (education, social groups), spatial dimensions (location of leisure practices or professional activities), and a cognitive perception or personal experience (tick bites, for example).This general population also held certain common perceptions or stereotypes about ticks and their consequences (bites, diseases) (Dernat and Johany 2019a; Dernat and Johany 2019b). Both populations interviewed in those studies, the PPA and the general population, gave an important place to the hardwood forest as a zone where ticks can be encountered, but very little precise knowledge about other more specific tick habitats. This perception that ticks are only significantly present in forested areas is inconsistent with the findings of Mathews-Martin et al. (2020) and suggests that It would have been interesting in our case to add a question about urban areas (without stipulating sidewalks).

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

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

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

The preventive role of PPA is all the more important for the local population as shown in a previous study performed in Combrailles (Letertre-Gibert et al., 2020). Indeed, although the natural areas at risk are familiar, the perception of risk is subjective and complex (Rohrmann and Renn, 2000) and the protective behavior variable (Brug et al., 2009; Herrington, 2004). Previous studies have noticed that even if people are aware of protective measures against tick bites, they do not always adopt them nor change their behavior (Aenishaenslin et al., 2016; Hook et al., 2015; Marcu et al., 2013; Puppo and Préau, 2018). This study was conducted in a region relevant to the risk of tick bites because the population is highly exposed due to professional and leisure activities and a high level of tick abundance (Dernat and Johany, 2019a; Sentinelles, 2020). Thus, it would be interesting to extend this study to other regions where the risk of ticks is similar in order to evaluate the knowledge of PPA in different geographical and social contexts. Before extending this study to other regions, a few modifications should be made to the two questions (what to do after a bite, and the likelihood of *Borrelia* bacteria transmission) that were difficult to understand and therefore interpretable in several ways. As seen in all surveys, the meaning and order of the questions may have influenced the answers provided.

Our findings are subject to several limitations.

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

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

These questions should be modified for future applications of the questionnaire. Question 12 should specify that it is advice to be given immediately or very soon after a tick bite. Question 13 should clarify that the context is one where the tick is a carrier of a *Borrelia* *pathogen*. Finally, for question 14, it would be necessary to indicate that it refers to a case where the person has been bitten by a tick. Moreover, the present study analysed information only from pharmacists and preparers working mainly in AURA region in France, therefore, all the results may not be generalizable to pharmacists at an international scale where the risk of Lyme borreliosis and lifestyle habits are very different. However, this study gives us ideas for directions to take, and in particular, points to the need to further understand the knowledge of all health professionals. A survey with these goals could be completed by semi-directed interviews to better understand the individual and societal origins of our main findings and identify relevant training tools to be developed.

Conclusion

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

Appendices:

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

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

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The authors declare that they have no competing interest.

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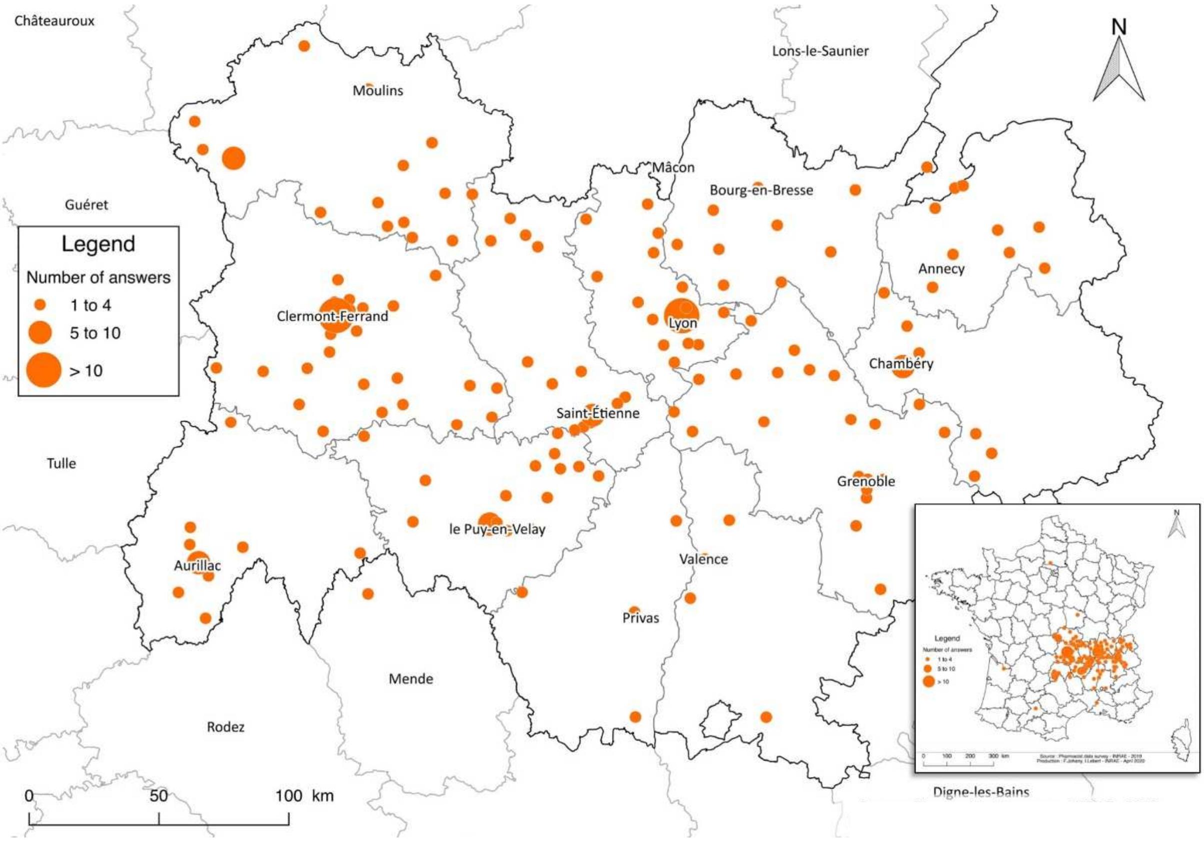


Figure 1: Map of the respondents' locations in France. The size of point is proportional to the number of respondents at each location.

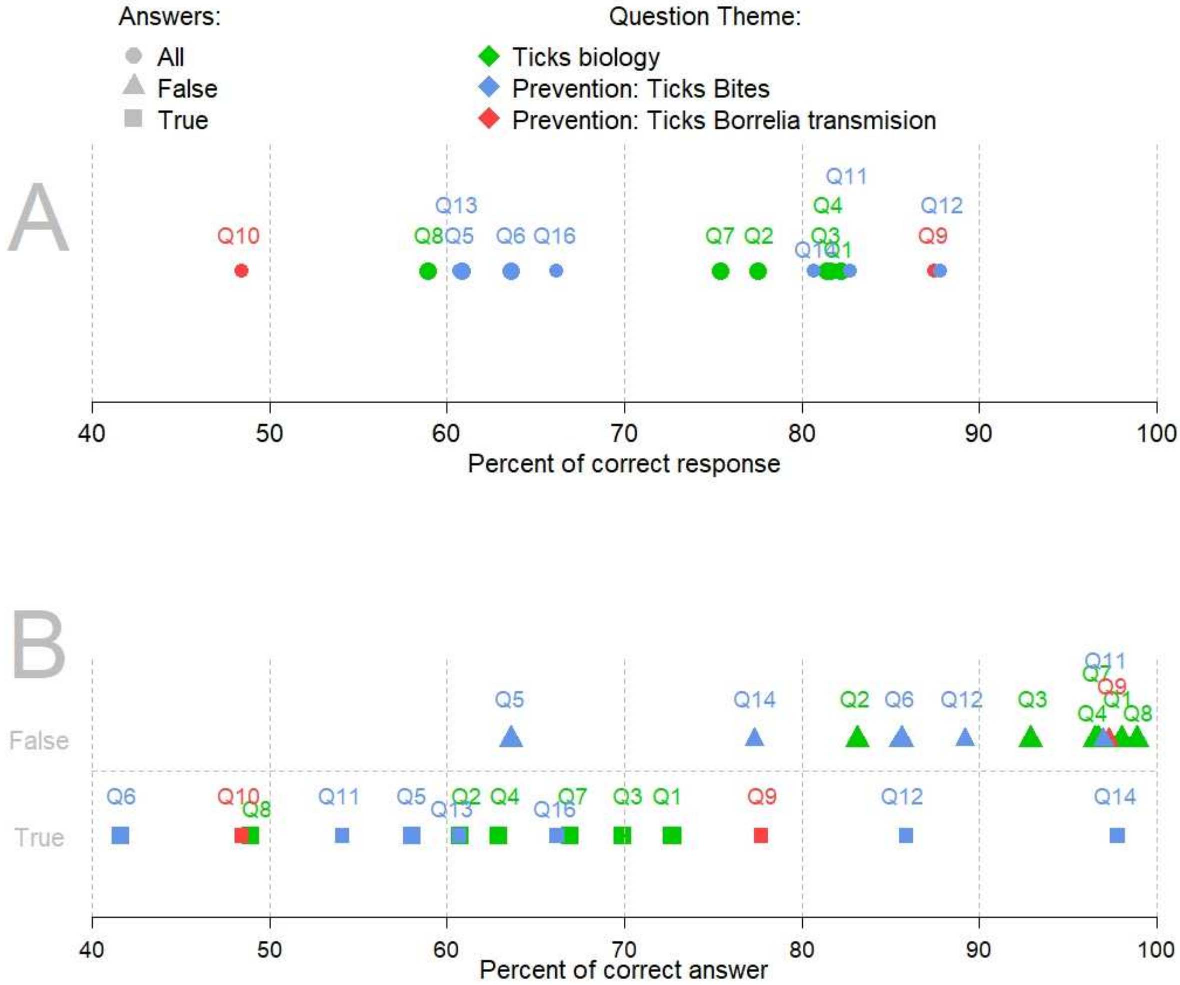


Figure 2: Distribution of questions according to the average of correct identification for all proposed answers in A and distribution of questions according to the average of correct identification for true answers (squares) and false answers (triangles) in B; questions regarding ticks and their biology 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?

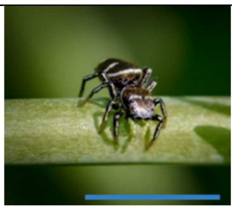
			Q1		Q2	
		Image	Answer	% correct response	Answer	% correct response
A	*Rhipicephalus sanguineus* tick, adult female		T	68.7	F	71.2
B	spider		F	98.6	F	99.7
C	unfed *Ixodes ricinus* tick, adult female		T	69.5	T	47.3
D	*Hyalomma marginatum* tick		T	59.6	F	75.8
E	louse		F	96.7	F	97.5
F	*Dermacentor reticulatus*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

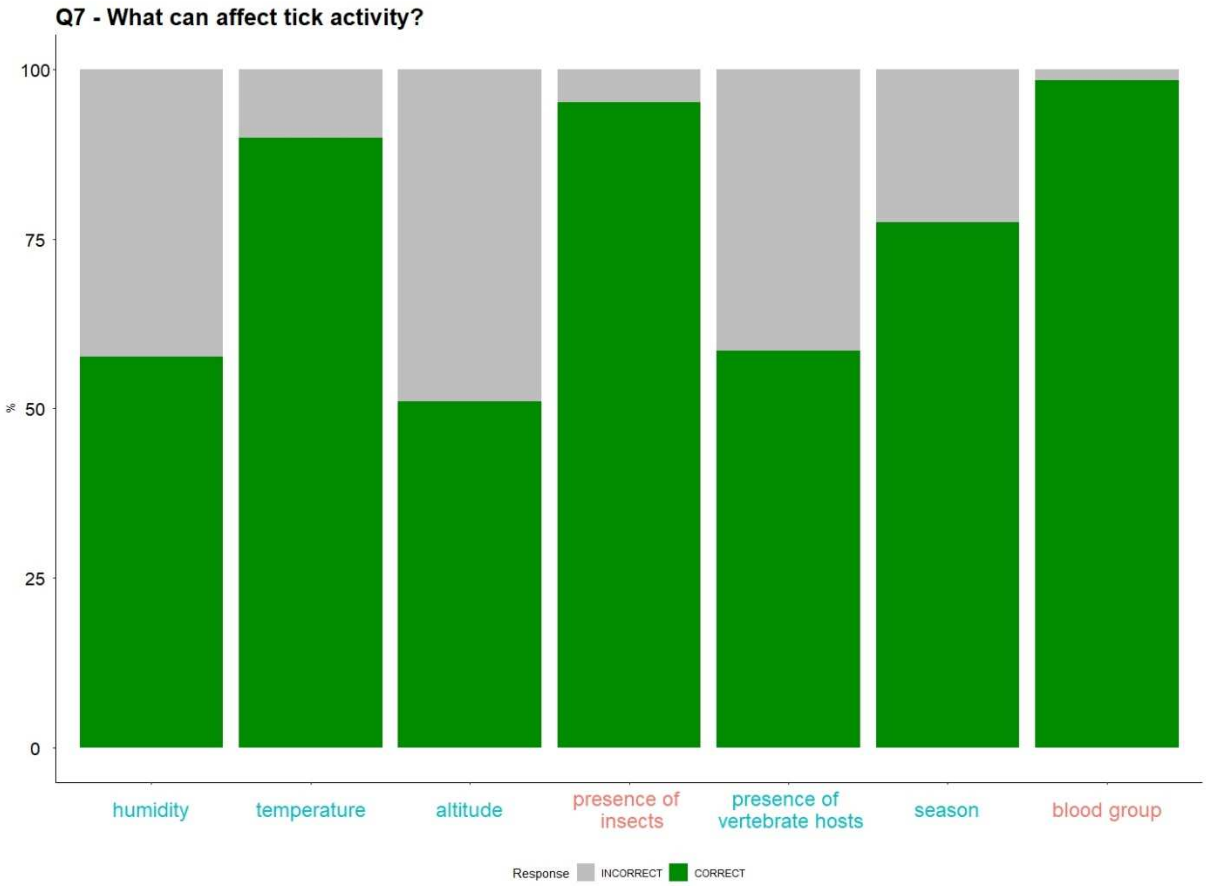


Figure 4. Percent of correct (green) and incorrect (gray) responses for each answer proposed for question 7: What can affect tick activity? x-axis: true answers in blue, false answers in red.

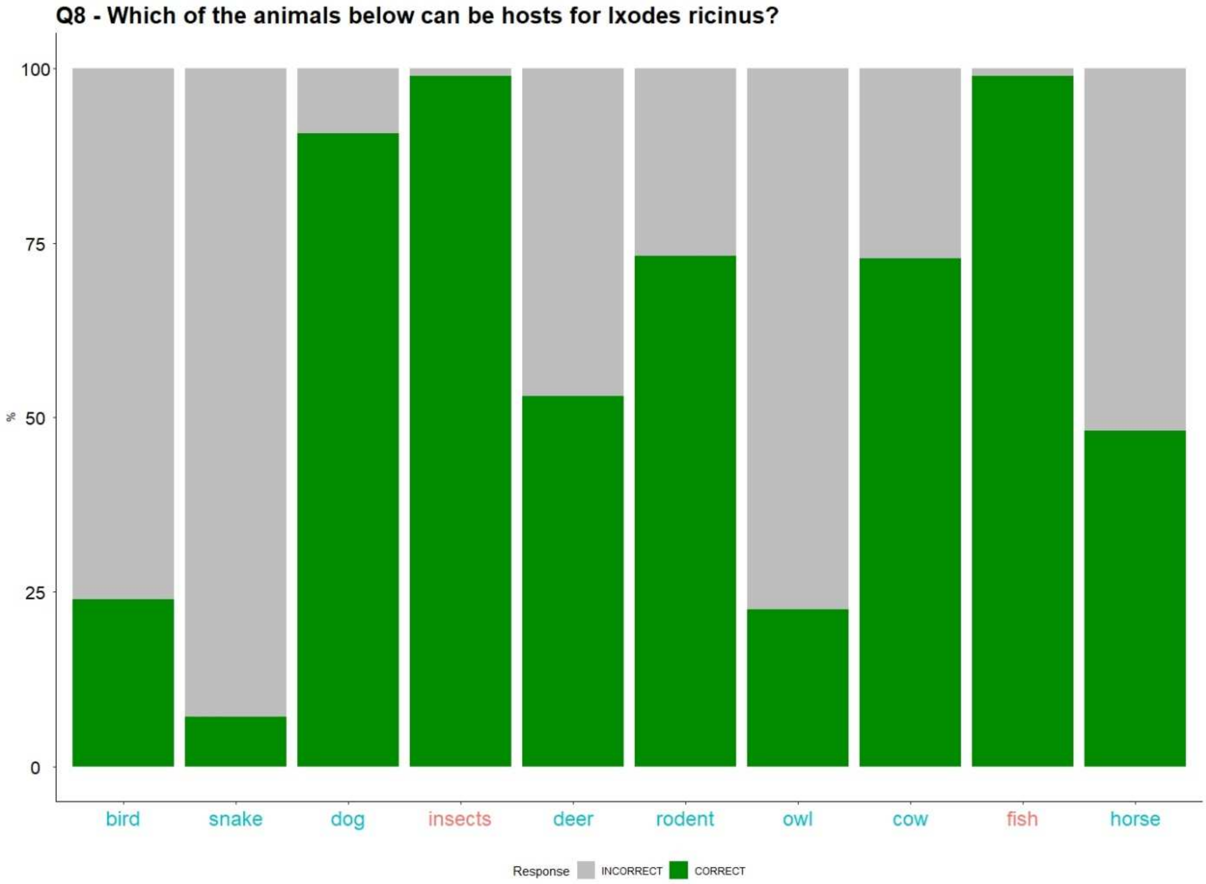


Figure 5. Percent of correct (green) and incorrect (gray) responses to each answer for question 8: Which of the animals below can be hosts for *Ixodes ricinus*? x-axis: true answers in blue, false answers in red.