NOT BITTEN BY IXODES TICKS OR BITTEN WITHOUT SYMPTOMS, WHY STILL TO WORRY?

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SUMMARY

Objectives: The purpose of the current study was to analyse the risks of Lyme borreliosis (LB) among 1,070 forestry workers, the influence of responsible behaviour (use of repellents, skin self-inspection) on Borrelia screening result status, and the occurrence of immediate and mid-term symptoms after tick bites and LB positive serological screening test.

Methods: The questionnaire was conducted as well as blood tests for LB disease by one-stage serological screening procedure using ELISA for specific *B. burgdorferi* IgM and IgG antibodies (EuroImmun AG company, Germany).

Results: While 39.6% of foresters were LB positive among bitten foresters, as many as 27.0% were LB positive among those, who did not recall any tick attacks at all. Individuals with known history of tick bites had significantly higher odds (1.770×) of being LB positive ($p < 0.05$), while the use of repellents or skin self-inspection after visiting woods had no influence on LB results. The odds of skin discolouration after tick bites was significantly lower (0.682×) in case of LB positive test compared to LB negative test ($p < 0.05$), which can be explained by the fact that foresters could be unaware about erythema migrans appearance and timing, considering tick bite and developed later rash as completely separate events. Moreover, 69.1% of the bitten foresters with LB positive result developed no secondary symptoms (excluding those related to the skin), and the most frequent clinical symptoms were arthralgia (24.9%), followed by myalgia (7.6%), headache (5.7%), and damage to facial nerve (2.7%), which are non-specific and can be present in other illnesses.

Conclusion: Therefore, the recommendations proposed would be the regular laboratory testing for LB of sensitive and at-risk population, who visits endemic woody areas, irrespective of all other factors involved.

Key words: Lyme borreliosis, risk, foresters

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INTRODUCTION

Woody areas, recreational parks and green zones are points of concern regarding attacks of Ixodes ticks, which are hard-bodied insects widespread in Europe and the USA. Carried by many mammals, both domestic and wild, they can attack humans and transfer many infectious agents, such as *Borrelia*, *Rickettsia*, and other bacteria, as well as protozoa and viruses, through bites and regurgitation (1, 2). Several tick species, including Ixodes ricinus, Ixodes scapularis, Ixodes pacificus, and Ixodes dammini, are responsible for tick-transmitted diseases worldwide (3–5).

Lyme borreliosis (LB) is the most widespread tick-associated disease in Ukraine, where significant portions of the territories are mixed forests and forest-steppe (6). Though cases of LB were recorded in Ukraine as early as in 1994, the official track of cases started only in 2000 (7). According to Shkilna et al., the incidence level of LB reached 10.62 per 100,000 in 2019 in Ukraine and continues to climb steadily (8). *B. burgdorferi* s.s., *B. garinii* and *B. afzelii* antibodies were identified in blood serum of Ukrainian patients, associated with different signs and symptoms (9). Most common short-term consequences of LB include migratory ring-shaped erythema and fever, while more dangerous long-term – pathological changes occur in the central and peripheral nervous system, joints and heart (1).

Though forestry workers belong to the group with a high tick bite risk and therefore LB (10), the awareness about dangers associated with ticks remains low among them (11). The same low awareness is present among Ukrainian general population, including youth (12, 13).

Wearing long-sleeved light-coloured clothing, which completely covers skin, use of insect repellents, and frequent self-inspection for ticks can decrease chances of tick bites (14), but such behaviour must be promoted through educational activities.

The purpose of the current study was to analyse the risks of LB among at-risk population, who consider themselves safe as not recalling having tick bites, the influence of responsible behaviour (use of repellents, skin self-inspection) on Borrelia screening result status, and the occurrence of immediate and mid-term symptoms after tick bites and LB positive screening test. The results of the data analysis will allow us to suggest proper behaviour regarding visiting woody areas in *Borrelia* spp. endemic areas and propose interferences to reduce negative effects of LB on communities.

MATERIALS AND METHODS

The questionnaire was filled out by total of 1,070 forestry workers present at work during its conduction (972 males and 98 females), in seven regions of Ukraine – Chernigiv, Chernivtsi, Kchmelnytskyi, Ternopil, Volyn, Zakarpattya, and Zhytomyr. Refusals to fill out questionnaire were generally not observed. Questions 1 and 2 dealt with person's occupation, absences, or number of tick bites; questions 3, 4, and 5 with locations of tick bites, how ticks were removed, and if skin discolouration occurred; question 6 dealt with complains thereafter; while questions 9 and 10 with the use of insects repellents and skin self-inspection after visiting woods, respectively. Answers to the questions 7, 8, 11, 12 and 13 were irrelevant to the current data analysis. Therefore, all respondents, for the purpose of the current data analysis, were split into two large groups – those, bitten by the ticks at least one or more times (group n_1), and those, who did not remember such event (group n_2).

The respondents, irrespective of known events of tick bite, were tested for LB disease by one-stage serological screening procedure using ELISA for specific *B. burgdorferi* IgM and IgG antibodies (EuroImmun AG company, Germany). Following the manufacturer's manual, a value of ≤ 16 units/ml was considered as a negative result, 16 to 22 units/ml as an intermediate, while ≥ 22 units/ml as a positive result. Sensitivity and specificity for the ELISA (IgM) test kit are equal to 100.0% and 96.4%, respectively, and for the ELISA (IgG) test kit – 100.0% and 90.2%, respectively. Therefore, we considered only any positive (IgM or IgG) result as a presumptive positive LB result. Additionally, IgM-positive results were considered as well.

To analyse the serological screening result as influenced by the known event of tick bites, a 2 by 2 contingency table was built with serological result being either positive or negative, and influencing factor of tick bites history at two levels, represented by groups n_1 and n_2.

Similar 2 by 2 contingency tables were built to study influence of two influencing factors at two levels, namely, self-inspection (seldom or frequent) and no self-inspection after visiting woods, and use of repellents (seldom or frequent) or no use of repellents, in order to analyse behavioural consequences on Borrelia screening result status (either positive or negative for IgG plus IgM, or for IgM only).

Additionally, group n_1 responses were analysed for presence of immediate symptom of skin discolouration in connection with their LB screening status (positive vs. negative). Appearance of other individual clinical symptoms after the tick bites, such as arthralgia, myalgia, headache, etc., was analysed as percentages among responses in group n_1 of individuals, which tested positive during LB screening test.

A Pearson chi-squared test at $p < 0.05$ was performed as a test for association to analyse whether observed frequency distributions differ from expected ones. Odds ratios (OR), as well as confidence intervals (CI), were calculated (confidence level $\alpha = 95\%$) for all contingency tables to measure the associations and their significance ($p < 0.05$).

The study was carried out according to the principles of the Helsinki Declaration. All participants have given informed consent to participate in the research. All mandatory laboratory health and safety procedures have been complied within the course of conducting any experimental work reported in the manuscript. The manuscript was also approved by the University Ethics Committee.

RESULTS

Table 1 shows distributions of respondents split between groups n_1 and n_2 and their characteristics.

Interestingly, three clusters could be identified regarding percentages of foresters with confirmed known tick attacks: Zhytomyr, Ternopil, Kchmelnytskyi, and Chernigiv regions, where 70.8 to 76.7% of all forestry workers claimed being bitten by ticks; Volyn region with 60.5% claims; and Zakarpattya and Chernivtsi regions with 31.2 to 32.9% claiming tick bites (Table 1). Nevertheless, overall percentage of presumptive LB positive among all foresters was 32.5%, 33.3%, 34.5%, and 49.0% in Chernigiv, Kchmelnytskyi, Ternopil, and Zhytomyr regions, respectively, while it was only 21.9% and 31.2% for Chernivtsi and Zakarpattya regions, respectively. As one can see, though percentages of positives in the last two regions are smaller, they are not comparably smaller comparing to the recalls of tick bites incidences between regions. This can be explained by possible overall higher number of ticks present in the areas mentioned under group n_1 and by repetitive bites.

More troubling, while overall 39.6% of foresters were serologically positive for LB (39.6% of males and 39.0% of females) among those who claimed tick bites, overall 27.0% of foresters (27.1% of males and 25.6% of females) were serologically positive for LB, who, according to the questionnaire, did not recall any tick attacks at all (Table 1). These data give some expectations, that the primary risk factor of contracting LB may be not an event of tick bite, but frequent visits to the woody areas where LB is endemic. Even more troubling, no matter of tick bites, overall 34.9% of foresters presumptively tested positively for Lyme disease, which corresponds to 34,860 cases per 100,000, which is alarmingly higher than previously reported incidence rate of 10.62% per 100,000 among general population, as mentioned by Shkilna et at. (8).

As previously shown, forestry workers with low awareness about LB, who do not use insect repellents or perform skin self-inspection, are less likely to seek professional help for tick removal, while many Ukrainian foresters remove ticks with bare hands without use of disinfectant (15).

Therefore, it can be speculated, that not only "high at risk" population, but also general population is at risk, who do not pay attention to consequences of visiting woody areas, irrespective if they were bitten by ticks or not.

It was shown that Ixodes ticks upon their bites inject stabilizing cement, and the bite itself is not painful and therefore less likely to be noticed by a victim (16). Additionally, ticks of the earlier development stages (larva and nymphs) are smaller than adults and may not be noticed on the skin at all. Specifically, nymph is a size of a poppy seed and is usually accounted for the majority cases of LB transmissions (17).

Pearson chi-squared value and p-value significance of tick bite events (groups n_1 and n_2) and behavioural habits influencing result of LB screening test, either positive or negative, as well as odds ratios and confidence intervals are shown in Table 2. Over-

Table 1. Analysed groups of respondents in different regions of Ukraine (N = 1,070)

Region	Category	Group n_1 (n)	Group n_2 (n)	Percentage of n_1 in ($n_1 + n_2$)	Group n_1 LB positive (%)	Group n_2 LB positive (%)	Average age (SD)
Chernigiv	Total	92	28	76.7	35.9	21.4	45.3 (11.1)
	Males	85	27	75.9	34.1	22.2	45.3 (10.8)
	Females	7	1	87.5	57.1	0.0	44.3 (14.4)
Chernivtsi	Total	24	49	32.9	25.0	20.4	38.0 (10.7)
	Males	23	43	34.8	26.1	20.9	37.7 (10.5)
	Females	1	6	14.3	0.0	16.7	40.9 (12.3)
Kchmelnytskyi	Total	109	41	72.7	31.2	39.0	39.8 (13.1)
	Males	96	39	71.1	32.3	41.0	40.3 (12.9)
	Females	13	2	86.7	23.1	0.0	35.3 (14.7)
Ternopil	Total	240	99	70.8	37.9	26.3	42.3 (11.2)
	Males	235	97	70.8	37.9	25.8	42.2 (11.2)
	Females	5	2	71.4	40.0	50.0	48.0 (10.1)
Volyn	Total	89	58	60.5	44.9	31.0	39.1 (11.5)
	Males	73	47	60.8	46.6	29.8	39.1 (11.7)
	Females	16	11	59.3	37.5	36.4	39.3 (10.4)
Zakarpattya	Total	44	97	31.2	47.7	23.7	41.0 (12.3)
	Males	41	85	32.5	48.8	23.5	41.7 (12.0)
	Females	3	12	20.0	33.3	25.0	34.9 (13.2)
Zhytomyr	Total	72	28	72.0	55.6	32.1	40.0 (10.6)
	Males	58	23	71.6	56.9	34.8	38.3 (10.3)
	Females	14	5	73.7	50.0	20.0	47.2 (8.3)
Overall	Total	670	400	62.6	39.6	27.0	41.6 (11.5)
	Males	611	361	62.9	39.6	27.1	41.5 (11.5)
	Females	59	39	60.2	39.0	25.6	42.3 (11.9)

Group n_1 – claiming tick bites; Group n_2 – no tick bites at all; LB positive – serologically positive for Lyme borreliosis

Table 2. Events of tick bites or no tick bites and behavioural habits influencing outcome of Lyme disease screening result

Levels of influence on LB screening test result (positive/negative)			χ^2 (1, N = 1070), p-value	OR (95% CI) z-statistic p-value
Tick bites	LB+	LB-	17.379 **<0.001**	1.770 (1.351–2.317) 4.147 **<0.001**
Bitten	265	405		
Not bitten	108	292		
Use of repellents	LB+	LB-	0.043 0.836	0.967(0.707–1.324) 0.207 0.836
Repellents (frequent or seldom)	74	142		
No repellents	299	555		
Inspection	LB+	LB-	1.908 0.167	1.267 (0.905–1.772) 1.379 0.168
Self-inspection (frequent or seldom)	314	563		
No self-inspection	59	134		

LB – Lyme borreliosis; χ_2 – Pearson chi-squared test; OR – odds ratios; CI – confidence intervals
Numbers in bold indicate statistically significant values.

all, 20.2% and 82.0% of all respondents used repellents seldom or frequently and performed at least seldom skin self-inspection after visiting woods, respectively. Contrary, only 3.2% and 40.1% of all respondents used repellents and performed skin inspection frequently, respectively.

Interestingly, only individuals with claimed tick bite events had significantly higher odds (1.770×) of being serologically positive for LB compared to those who did not recall tick bites ($p < 0.05$), while use of repellents (frequent or seldom) vs. no use, and self-inspection (frequent or seldom) vs. no self-inspection, both had no influence on LB positive results (Table 1). Rearranging levels of factors (use of repellents: frequent vs. seldom or no use, and self-inspection: frequent vs. seldom or no self-inspection) did not produce significant odds ratio as well (OR = 1.020, CI: 0.500–2.085, p = 0.957 and OR = 0.991, CI: 0.766–1.281, p = 0.943) as influencing status of LB serological result (p > 0.05).

Positive result for blood serum IgG to *Borrelia* spp. may be a sign of older infection that could persist in human body for years, while presence of IgM usually indicates current or very recent infection. It was observed that self-inspection (frequent or seldom) vs. no self-inspection marginally approximated to have significant influence on LB IgM positive results (OR = 1.516, CI: 0.931–2.471, p = 0.095), with skin self-inspection decreasing odds of being IgM LB serologically positive, while repellents use (frequent or seldom) vs. no use did not have such effect (OR = 1.200, CI: 0.800–1.801, p = 0.379). This can mean that many cases related to LB are old infections, and improved behavioural habits (more frequent self-inspection) influence only recent infections, related to IgM. However, there are some limitations to this model, as this model is somewhat simplified and artificial, as positive IgM without positive IgG may mean false positive IgM, and ongoing infection can be also expressed as both positives – IgG and IgM, as well as higher than usual level of IgG with negative IgM. High sensitivity and specificity, as claimed by the manufacturer of the used ELISA kits, allowed using a single-step verification without second step confirmation by the immunoblot for the research purposes only.

Pearson chi-squared value and p-value significance of appearance of skin discolouration or no discolouration on Borrelia positive screening status, in foresters bitten by ticks (group n_1), as well as odds ratios with confidence intervals are shown in Table 3. Overall, 26.3% of foresters developed skin discolouration after tick bites (group n_1, n = 670), while only 21.9% of LB positive tick-bitten foresters had this symptom. Oddly, 29.1% of bitten respondents who were tested negative for LB had skin discolouration as well.

DISCUSSION

Erythema migrans is a bull-eye's patterned skin rash related to the early infection by *Borrelia* spp., which appears one or two weeks after the bite at the site of the bite and expands ca 2 to 3 cm per day, making a circular shape with 5–70 cm in diameter (an average of 16 cm) (18, 19). According to numerous research reports, it appears in ca. 80% of all LB cases (18, 19).

It was observed that the odds of skin discolouration after the tick bites was significantly lower (0.682 times) in case of LB positive test compared to LB negative test. Such results can be explained because questionnaire did not specifically mention migratory erythema, its appearance and timing as a characteristic sign of LB. Additionally, as it was shown before in the same cohort of foresters, almost half (44.6 %) of the respondents claimed to remove ticks with bare fingers and only 32.2% used disinfectants after tick removal (15). Such behaviour could cause secondary bacterial infection and local inflammation irrespective of *Borrelia* spp. introduction into the wound. Moreover, foresters could be unaware that erythema migrans appears typically one or two weeks after the bite and could consider tick bite and developed later rash as completely separate events.

Percentages of different clinical symptoms experienced among group n_1 foresters, besides skin discolouration, which were bitten and later tested positive for LB by screening test, are shown in Figure 1. Moreover, 69.1% of the foresters from this group developed no secondary symptoms at all, according to the analysed questionnaire data.

The most frequent clinical symptom was arthralgia (24.9%), followed by myalgia (7.6%), headache (5.7%), and damage to facial nerve (2.7%) (Fig. 1). As those signs and symptoms are non-specific and can accompany many pathological conditions, LB is frequently diagnosed at a very late stage when the damage to the nervous system and joints is already irreversible (1).

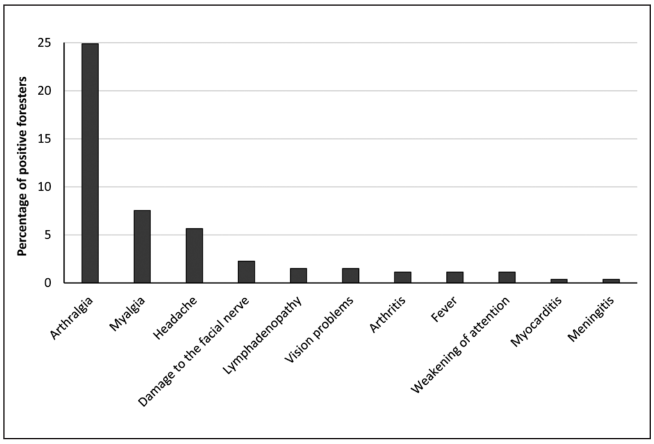

Fig. 1. *Clinical symptoms experienced among group n_1 foresters – bitten and tested positive for LB.*

Table 3. *Skin discolouration in foresters with Lyme positive screening test*

Levels of influence on Lyme borreliosis screening test result			χ² (1, N = 670), p-value	OR (95% CI) z-statistic p-value
	Skin discolouration	**No skin discolouration**	4.346 0.038	0.682 (0.475–0.978) 2.079 **0.037**
LB+	58	207		
LB−	118	287		

LB – Lyme borreliosis; χ_2 – Pearson chi-squared test; OR – odds ratios; CI – confidence intervals
Numbers in bold indicate statistically significant values.

CONCLUSION

It was shown that among high risk-population, namely foresters of Ukraine, high percentage of serologically LB positive individuals (27.0%) was present among those who did not recall any tick bites at all, though it was still lower comparing to those recalling them (37.0%). Therefore, major factor influencing LB infection can be frequent visit to LB endemic forests, irrespective of event of the tick bites.

While odds of those bitten by ticks to contract LB were significantly higher compared to those who were not, skin self-inspection for ticks after visiting woods and use of insect repellents in our study did not have such effects. While skin self-inspection factor can be neglected if proper clothing is used, such as long pants and long sleeves covering entire skin, data on inefficiency of repellent use can be explained by low use of repellents by forestry workers at all (27.0% seldom use overall, and 3.0% frequent use overall).

Low awareness about migratory erythema appearance and timing and possible secondary bacterial infections irrelevant to LB due to the unsanitary conditions of tick removal may devalue skin discolouration as a reliable factor for LB diagnosis.

Generic symptoms observed in bitten foresters, who later tested positive to LB in this study, together with their low frequency (for e.g., arthralgia at 24.9%, myalgia at 7.6%, headache at 5.7%, and damage to facial nerve at 2.7%), makes them impossible to be used as reliable diagnostic criteria. Such clinical signs and symptoms may be related to other diseases in aging individuals. Moreover, 69.1% of the foresters who were bitten and tested LB positive, did not mention any clinical symptoms listed in the questionnaire.

Therefore, the recommendations proposed by using the data analysed in this study would be the regular laboratory testing for LB of sensitive and at-risk population, who visits LB endemic woody areas, irrespective of all other factors involved.

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Conflicts of Interest

None declared

Data Availability Statement

The data used to prepare this manuscript are available upon individual requests to the corresponding author.

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