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REVIEW ARTICLE
Factors affecting the ecology of tick-borne encephalitis in Slovenia

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SUMMARY

Recognition of factors that influence the formation of tick-borne encephalitis (TBE) foci is important for assessing the risk of humans acquiring the viral infection and for establishing what can be done (within reasonable boundaries) to minimize that risk. In Slovenia, the dynamics of the TBE vector, i.e. *Ixodes ricinus,* was studied over a 4-year period and the prevalence of infection in ticks was established. Two groups of tick hosts were investigated: deer and small mammals. Red deer have been confirmed as having a direct influence on the incidence of TBE and rodents have been recognized as important sentinels for TBE infections, although their role in the enzootic cycle of the virus still remains to be elucidated. Last, forest and agricultural areas, which are influenced by human activity, are suitable habitats for ticks, and important for TBEV transmission and establishment. Human behaviour is also therefore an important factor and should always be considered in studies of TBE ecology.

Key words: Ecology, epidemiology, tick-borne encephalitis, ticks, zoonoses.

INTRODUCTION

Tick-borne encephalitis (TBE) is the most important viral infection of the central nervous system in Europe, where more than 3000 human cases are reported annually. The tick-borne encephalitis virus (TBEV) is a member of the genus *Flavivirus* and is transmitted by *Ixodes* ticks. The first cases of TBE in Europe were recorded in the early 1930s; the disease was first recognized in Slovenia in 1946 [1, 2] and the incidence has varied significantly in different years [3]. Serological diagnostics were introduced in 1959, and the disease has been notifiable in Slovenia since 1976 [4, 5]. The main endemic areas have therefore been well known for decades. The majority of cases appear in the northern part of Slovenia and the endemic area then spreads through the central region down to the southwestern part of the country, excluding the coastal region [6]. Today, 200–300 cases of TBE are reported annually. The average incidence is 13·1/100 000, but it varies both regionally and temporally ranging from 1·2/100 000 in some areas in the southeastern part of the country to 31·1/100 000 in the endemic areas. Variation is evident also through the years ranging from 3 to 27 cases/100 000 in the years between 1970 and 2009 [3]. New endemic areas have been recognized in the past decade, especially in the border area between Slovenia and Italy, where there were virtually no cases of TBE 20 years ago [3].

The presence of TBEV in Slovenia is correlated with natural foci. The intensity of these can differ from very active foci to areas with less intense enzootic cycles or no circulation of the virus at all [7]. TBE is a

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focal zoonotic disease and it has been established by Pavlovsky that the pathogen, host and vector are required to form a biocoenosis in a biotope, with specific but complex environmental properties [8]. Several studies have been performed in the past to investigate the factors influencing the formation of a foci. Due to the number of factors implicated in the appearance of endemic areas, studies are usually very complex, but highly necessary for uncovering details in the transmission cycle of TBEV. The enzootic cycle of TBEV, as recognized today, is as follows: an infected *Ixodes* tick transmits the virus via saliva to the host within minutes of the tick bite [9], the competent host (usually rodents) develops viraemia within a couple of days, enabling the transmission of the virus to other ticks [6]. However, small mammals usually host a large number of ticks within a limited area, which enables direct transmission of the virus between the co-feeding infected nymphs and uninfected larvae [10, 11]. If the tick is infected with TBEV, it remains infected throughout its life [12]. Large hosts, such as ungulates or even humans do not develop sufficient viraemia to support direct viral transmission to ticks, but cows, sheep and goats can excrete the virus through milk during the viraemic phase [13]. Unpasteurized milk or dairy products from unpasteurized milk are a common source of infection for humans [14].

In previous studies performed in Slovenia, we investigated the TBEV vector distribution and seasonal dynamics of the *I. ricinus* tick, the characteristics of TBEV host infection, the abundance of the tick host species, and various additional factors influencing contact between humans and ticks. All these studies attempted to explain the variation in the incidence of TBE that we see in Slovenia and to explain the reasons for the establishment of endemic areas.

THE VECTOR – TICK

TBEV isolates from Slovenia belong to the European subtype (TBEV-Eu) and the virus is transmitted by *I. ricinus* ticks [15–17]. *I. ricinus* can be throughout Europe, from Ireland to the Ural Mountains and from Scandinavia to northern Africa, although the spread of the virus is limited to endemic areas in central and eastern Europe, part of Scandinavia and the Balkan region [18, 19]. Over such an extensive area of distribution *I. ricinus* encounters a variety of environmental conditions, which challenge its endurance and adaptability. The life cycle of the tick ranges from 2 to 6 years, depending on the climate; the cold climates of the north provide a longer life cycle than the warm climates of the south, since the duration of favorable conditions in the latter is significantly shorter [20]. The three active stages each require a single host, from which they feed on blood for several days [20, 21]. After completing a bloodmeal, the tick then detaches, hides in the undergrowth and the larvae and nymphs moult, while the female adults produce eggs [21, 22]. Development from one stage to the next takes about a year but can be longer or shorter depending on environmental conditions. There have been changes in the distribution of the tick in Europe in past decades and there appear to be many driving factors for changes in the altitudinal and latitudinal spread of *I. ricinus* [23]. One of these is climate change, where increased temperatures and rainfall influence both the developmental period and winter survival of ticks, as well as causing changes in the forest and wildlife management and structure [24, 25] .

Even though Slovenia is a relatively small country in terms of its geographical area, there is considerable diversity of both climate and habitat types. The conditions influencing tick dynamics are consequently also very variable and are the cause of different patterns of seasonal activity [26]. Ticks were sampled monthly by flagging the vegetation at eight locations in Slovenia from April 2005 to September 2008 [26, 27]. The most common tick found throughout the country was *I. ricinus.* In only one location were two other species confirmed, *Dermacentor reticulatus* and *Haemaphysalis concinna.* The majority of the country has bimodal seasonal activity of *I. ricinus*, with the first peak occurring in the spring months (April, May, June) and the second peak occurring in the autumn months (September or October) (Fig. 1). Only the coastal region offers a climate that enables unimodal seasonal activity, with one peak extending from autumn, through winter, to the early spring months. Only occasionally can larvae be found during the early summer months in this region. *I. ricinus* ticks find their hosts by climbing vegetation and waiting for the host to walk by. In such a position, ticks are exposed to multiple environmental factors and are especially sensitive to desiccation when the relative humidity drops below 80% [28]. Saturation deficit was calculated since it gives an integrated measure of the drying power of the atmosphere [26, 29, 30] and its changes compared with the rate of change of nymph density during the period of decline. It was confirmed that warmer drier weather was associated

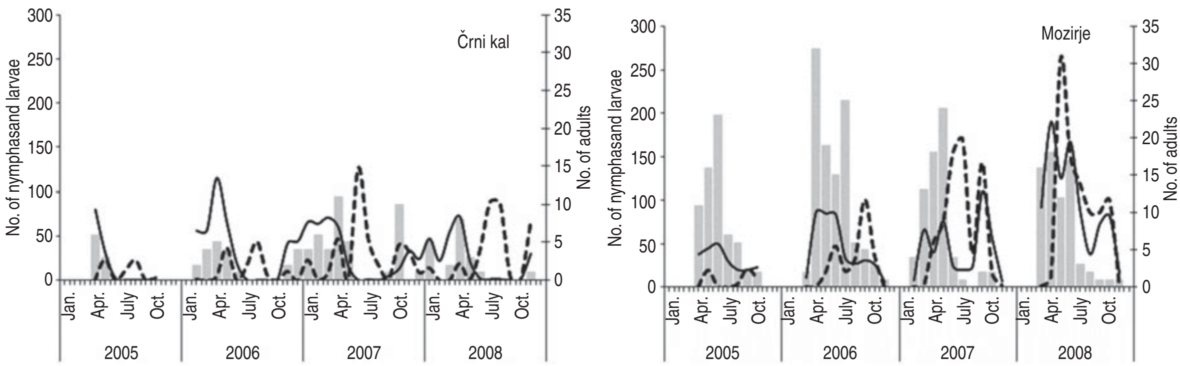

Fig. 1. Seasonal variation in the number of larvae (- - -), nymph (——) and adult (▒) *I. ricinus*/100 m^2 on two selected sampling sites in Slovenia. Črni Kal, a sub-Mediterranean coastal sampling site; Mozirje, continental Slovenia.

with a very rapid decline in nymph density [26], confirming the direct influence of weather on tick dynamics. The analysis was performed only for nymphs, since sampling by flagging is most efficient for this tick stage. Larvae quest closer to the ground, since their limited fat supply makes water stress a greater problem and they are consequently harder to reach by flagging [31]. Adult ticks are least sensitive to water stress. They are thus able to quest higher and can therefore be picked up quickly but also loosened from the flag faster. However, even when taking the sampling bias into account, we found that, during the 4-year sampling period, while all other sampling sites provided larvae and nymphs simultaneously (during the spring months), we never encountered large numbers of larvae and nymphs at the same time at the sub-Mediterranean, coastal location. The seasonal synchrony of immature stages has been established as the condition for tick co-feeding and therefore a potentially high virus transmission rate [32]. Our sampling data suggest that co-feeding of ticks on rodents is very likely to occur in most of Slovenia, while it is unlikely to occur at the coastal location. The virus transmission rate between tick stages is therefore significantly impaired at the coastal location (Fig. 1).

The sampled ticks collected during the 4-year period at eight different locations in Slovenia were screened for the presence of TBEV RNA by real-time RT–PCR [33]. Sampling sites were selected on the basis of the previously recognized incidence of TBE [27]. Studies performed in Europe have established that 0–5% of *I. ricinus* ticks are infected with TBEV, depending on the sampling area, screened tick stage and TBEV detection method [34–36]. Our study established that 0·3% of ticks were infected with TBEV (Table 1), although the infection rate varied from 2% in areas with a high incidence of the disease, to 0% where cases are never or only rarely confirmed [27]. Moreover, inter-annual variation was evident in all of the sampling sites at which TBEV was detected in ticks (Table 1).

Adult ticks (0·75%) had a higher infection rate than nymphs (0·18%; χ^2, $P = 8{\cdot}4 \times 10^{-5}$) and slightly more males (0·81%) harboured the virus than females (0·68%; χ^2, $P > 0{\cdot}05$). Since the analysed ticks were questing ticks, the rate of infection is connected to the probability of acquiring the virus during feeding in previous stages, which explains why the rate of infection is higher in adult ticks, which have fed twice before [21].

Ticks in which TBEV infection was confirmed were found only at locations at which analysis of seasonal dynamics showed that co-feeding of nymphs and larvae was possible due to seasonal synchrony of immature tick stages (Fig. 1). Co-feeding, in addition to direct TBEV transmission from a viraemic animal to the tick and transtadial and transovarial transmission, is recognized as one of the most important paths of TBEV transmission between ticks, and a high rate of infected ticks consequently causes higher TBE incidence [10, 37]. *I. ricinus* ticks are widely and densely distributed in most of Slovenia and exhibit a dynamic that makes co-feeding possible (Fig. 1). In areas in which the seasonal synchrony of nymphs and larvae does not occur, there were no or very few TBE cases (Črni Kal, Murska Šuma, Table 1) [27].

Additionally to *I. ricinus* ticks, *D. reticulatus* and *H. concinna* ticks were collected in one location in Slovenia. Although the virus has been demonstrated in *D. reticulatus* ticks in other countries and laboratory experiments have confirmed the ability of both

Table 1. *Rate of infection in* I. ricinus *ticks, number of positive and tested pools and tested individuals at eight sampling sites in Slovenia collected from 2005 to 2008*

Sampling site	2005			2006			2007			2008			Total		
	Positive pools/ all pools	Tested individuals	Rate of infection (%)	Positive pools/ all pools	Tested individuals	Rate of infection (%)	Positive pools/ all pools	Tested individuals	Rate of infection (%)	Positive pools/ all pools	Tested individuals	Rate of infection (%)	Positive pools/ all pools	Tested individuals	Rate of infection (%)
Črni Kal	0/17	133	0·00	0/49	420	0·00	0/36	320	0·00	–	–	–	0/102	873	0·00
Sodražica	3/23	157	2·05	4/38	293	1·37	5/43	362	1·40	0/48	276	0·00	12/152	1088	1·13
Rakovnik	0/37	215	0·00	4/63	458	0·90	0/43	336	0·00	0/50	438	0·00	4/193	1447	0·28
Mozirje	0/41	293	0·00	4/68	499	0·83	0/50	416	0·00	0/98	889	0·00	4/257	2097	0·21
Kamniška Bistrica	0/38	249	0·00	1/69	493	0·20	0/18	143	0·00	0/19	136	0·00	1/144	1021	0·10
Štefanja gora	5/42	314	1·67	0/75	601	0·00	0/41	334	0·00	0/27	201	0·00	5/185	1450	0·35
Osolnik	0/19	118	0·00	1/47	344	0·29	0/17	143	0·00	0/20	154	0·00	1/103	759	0·13
Murska Šuma	0/13	36	0·00	0/33	154	0·00	0/10	45	0·00	–	–	–	0/56	235	0·00
Total	8/230	1515	0·54	14/442	3262	0·43	5/258	2099	0·24	0/262	2094	0·00	27/1192	8970	0·30

types of ticks to transmit the virus [38, 39], TBEV was not detected in these two species in Slovenia. In contrast to the *I. ricinus* ticks, only adult *D. reticulatus* quest for hosts by climbing vegetation. Nymphs and larvae are thus rarely found by flagging vegetation. Even the life cycle of this species is significantly different. Since the development from egg to adult animal only takes 1 year, there is less overlap between the ticks active stages during their life cycle, so co-feeding is unlikely to occur [39, 40]. *H. concinna* also has a specific scheme of seasonal dynamics, which does not support co-feeding transmission [38, 41]. The importance of these two species of ticks for transmission of TBEV in the foci is probably therefore minimal.

THE TICK HOSTS

Each of the three active stages of the *I. ricinus* tick requires a vertebrate host to provide a bloodmeal, which is necessary for further development. The selection of tick host may be determined by the tick's questing height [21]. Larvae quest close to the ground and feed mostly on small mammals, which are also good hosts for the nymphs. All three tick stages can be found on larger hosts, such as wild cervids, cattle and other animals [42]. Furthermore, these large hosts are the most important host for adult reproductive ticks [42]. Host density directly influences the density of ticks, whereby the density of adult female tick hosts in particular (e.g. deer) can be a limiting factor or can influence the expansion and abundance of the *I. ricinus* population [20, 42].

Small mammals are important hosts in the tick life cycle and also play an important role in facilitating TBEV transmission between ticks. They enable non-viraemic transmission by co-feeding infected nymphs and uninfected larvae [10] and also develop sufficient viraemia to support direct transmission of the virus to feeding ticks [43–45]. By contrast, deer are not competent hosts for TBEV transmission, since they do not develop sufficient viraemia for transmission, although they are an important factor in the TBEV enzootic cycle since they support tick populations [13, 25, 46]. Theoretical and empirical studies have demonstrated that TBEV amplification can occur when a certain host threshold density is achieved [47, 48]. Transmission of TBEV by co-feeding has been suggested, due to a large number of ticks feeding on a single deer in very close proximity, but no experimental or direct evidence exists to support such a hypothesis [49, 50].

Previous studies on wildlife and the abundance of ticks have produced inconclusive results concerning a connection between TBE incidence and deer density [25, 50, 51]. A study performed in Slovenia demonstrated a link between the abundance of deer and the number of TBE patients [46]. Roe deer and red deer numbers have been increasing in past decades, as well as the incidence of TBE. A temporal connection was significant in cases of both host groups. Since such connections may be a result of coincidental factors, an additional spatial analysis was performed. The analysis established that roe deer density is not associated with the incidence of TBE but that the red deer density is significantly connected to TBE incidence in Slovenia. The study confirmed that fewer people contract TBE in areas with a lower red deer density. The density of roe deer in Slovenia is very high compared to neighbouring countries, such as Italy, where a connection between roe deer and TBE incidence has been established [25, 46]. In Slovenia, the average density of roe deer is 8·79 head/km^2, which might exceed the threshold beyond which host density has a measurable effect on the incidence of TBE [46, 52]. Nevertheless, roe deer should not be neglected in further research, since evidence from studies in other countries supports their important role in the circulation of the virus in nature [25, 42, 46]. However, it is important to note that the density of red deer has been increasing since the 1970s, both in Slovenia and in other European countries [46]. Red deer, too, which almost became extinct in the 19th century, are currently increasing in abundance due to shooting restrictions and artificial restocking [46]. Why the connection between TBEV and red deer is more pronounced than the connection with other deer species remains unknown but further studies elucidating the red deer–tick host characteristics and analysing the preferred red deer habitat and behavioural features are needed to explain the connection.

Rodents are the most studied vertebrate host group in connection with the TBEV enzootic cycle, especially the yellow-necked mouse (*Apodemus flavicollis*) and the bank vole (*Myodes glareolus*). These species are most abundant in areas in which TBE incidence is high and both are excellent hosts for the nymphal and larval tick stages. They are often considered bridge hosts for TBEV between the two subadult tick stages due to co-feeding transmission [10, 11, 53].

Table 2. *Prevalence of infection of rodent species captured in Slovenia from 1990 to 2009*

Species	Prevalence of infection *n*/*N* (%)
Apodemus agrarius	4/164 (2·44)
Apodemus flavicollis	33/853 (3·87)
Apodemus sylvaticus	7/73 (9·59)
Arvicola terrestris	0/2 (0·00)
Chionomys nivalis	1/6 (16·67)
Crocidura leucodon	1/3 (33·33)
Crocidura suaveolens	0/2 (0·00)
Glis glis	1/83 (1·20)
Microtus agrestis	0/7 (0·00)
Microtus arvalis	0/6 (0·00)
Microtus lichtensteini	0/2 (0·00)
Microtus subterraneus	0/1 (0·00)
Mus musculus	0/7 (0·00)
Myodes glareolus	39/311 (12·54)
Rattus norvegicus	0/11 (0·00)
Rattus rattus	0/11 (0·00)
Sorex araneus	0/24 (0·00)
Total	86/1556 (5·53)

A study was performed in Slovenia analysing TBEV infection patterns in naturally infected wild rodents [44]. Rodents were trapped from 1990 to 2009 throughout Slovenia. Live traps were used and animals were euthanized and the internal organs and blood harvested, and stored at -70 °C. Rodent sera were screened for the presence of TBEV antibodies as described previously and samples from almost 700 rodents were screened for the presence of TBEV RNA [44]. TBEV-specific antibodies were detected in 5·53% of investigated rodent sera but the prevalence differed significantly according to the rodent species (Table 2) and the investigated region (Fig. 2).

The prevalence of infection was highest in bank voles (12·54%); a lower prevalence was detected in *Apodemus* sp. rodents (4·04%). Several of the animals in which TBEV antibodies were present were still in the viraemic phase or had detectable viral RNA in their organs [44]. Although previous studies indicated that the viraemic phase only lasts a couple of days, more recent studies have shown that viraemia can be detected for weeks or month after the initial infection. Recent studies have shown that some vole species (*M. glareolus* and *Microtus arvalis*) can develop persistent TBE infection, without apparent clinical symptoms in the majority of infected animals [43, 54]. Especially prolonged continuation of TBEV in the brain tissue of infected animals (both naturally and experimentally) [43, 44, 54] might indicate a more

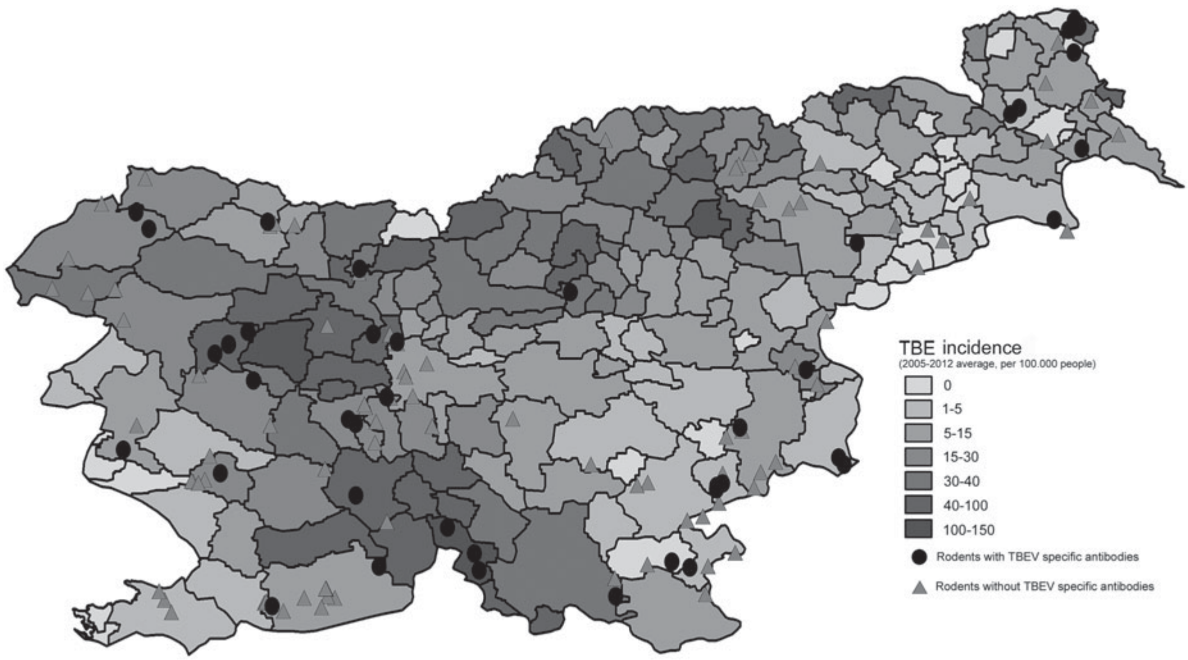

Fig. 2. Map of Slovenia indicating the incidence of tick-borne encephalitis (TBE) in Slovenian municipalities and the sampling sites of rodents, where specific antibodies against TBEV were detected (●) or not detected (▲) in rodent sera.

crucial role of certain rodent species in the enzootic cycle of TBEV, a role that extends beyond the important tick host function.

HUMANS AND THE ENVIRONMENT

Two sets of factors influence TBE endemicity. One set influences the increased ability of the virus to be transmitted between different hosts while the other set increases the relative risk, namely the risk of humans coming into contact with the infected tick [55]. Both sets of factors are influenced by human interventions and activity in nature.

Direct interventions, which include modifications of the forest area, and an increase or abandonment of agricultural areas, can influence both tick and tick hosts [56]. Abandoned agricultural fields are good habitats for rodents and increased *A. flavicollis* and *M. glareolus* populations are an important step in the TBEV enzootic cycle. Additionally, more forests again provide more suitable habitats for the TBEV vector, the tick, *I. ricinus*, as well as its hosts, i.e. deer, rodents and other vertebrate animals [56]. More than 60% of Slovenia is covered by forests. Analysis of statistical data in past decades has shown that the forest area has been increasing and agricultural land has been slightly decreasing. We were able to confirm a correlation between forest area and TBE incidence (Pearson's correlation coefficient: $r = 0{\cdot}640$, $P < 0{\cdot}0001$), but were unable to confirm a connection between agricultural land and TBE (Pearson's correlation coefficient: $r = -0{\cdot}201$, $P = 0{\cdot}204$) (Fig. 3). Although a negative correlation between decreasing agricultural areas and the incidence of TBE is indicated, no straightforward correlation could be confirmed. The influence of deer abundance control was described in the previous section but there are other influences of human interventions on host populations that need to be investigated. One example is changes in rodent populations due to forest and agricultural land management. Changes in farmed crops could influence small mammal populations. On the other hand, pesticide use could influence tick populations directly. Many of these factors have already been researched and their influence established [57]. It is therefore important to recognize these factors, which synergistically act to increase the zoonotic risk of infection as well as influencing the exposure of humans to risk.

Human behaviuor influences TBE incidence, since it can trigger actions that increase the contact of people with infected ticks. One example is good weather, which increases the time spent outdoors, therefore the chance of an encounter between ticks and humans is significantly increased [58]. This happened in 2006, when a long warm autumn provided favourable

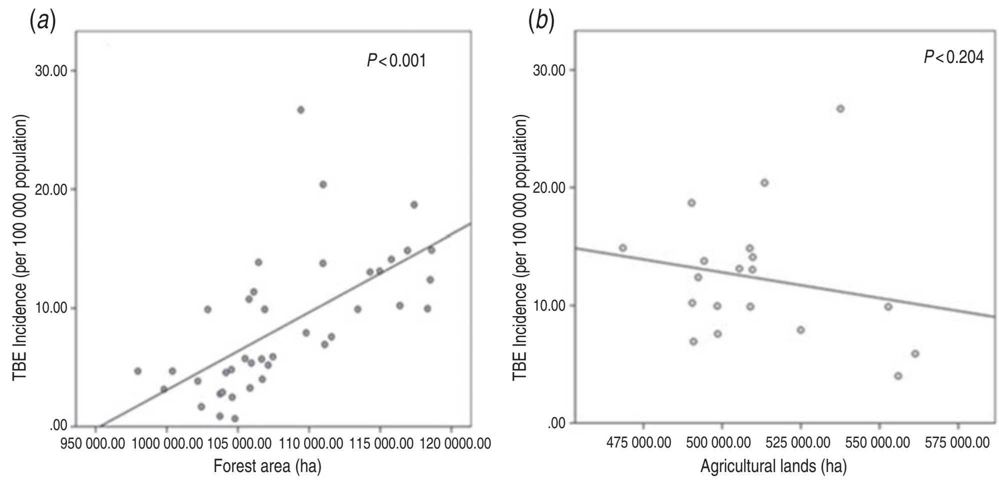

Fig. 3. Correlation between tick-borne encephalitis (TBE) incidence and (*a*) forest areas (m^2) and (*b*) agricultural land areas (m^2).

weather for outdoor recreational activities and a spike in TBE incidence can be observed during that period [58]. Additionally, it has been indicated that background socioeconomic conditions also impact on TBE incidence [59]. For example, both unemployment and an increase in household expenditure on food can generate an increase in the number of TBE cases [59]. A study performed in Slovenia on vaccination against TBE indicated that being in middle- or high-income groups increases the chances of people being vaccinated [3]. People also tend to be more aware of the risk of TBE and, consequently, increasingly cautious when coming from a higher-income population. High-risk behaviour in Slovenia has been shown to include leisure-time activities, mushroom and berry picking, and farming [60].

The genetic variability of TBEV was studied in Slovenian patients, as well as in rodents and tick populations. TBEV E and NS5 protein gene sequences were obtained and the correlation between phylogenetic and geographical clustering was investigated and confirmed [16]. At the same time, it was established in this study that the majority of patients become infected in relative proximity to their homes [16, 61]. Therefore, human behaviour should not be neglected in studies of TBE, since it can significantly alter the incidence. The ecological factors that influence such changes should also be identified as far as possible.

CONCLUSIONS

A complex mixture of factors is involved in the formation of TBEV foci. None of these factors should be neglected; while some directly influence TBE incidence, the influence of others is more indirect or is connected with the intensity of specific foci. Further studies should concentrate on the importance of tick hosts. It needs to be established whether tick hosts play an important role 'only' as reproductive hosts for the ticks, or if they can also serve as TBEV reservoir hosts.

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DECLARATION OF INTEREST

None.

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