The role of particular tick developmental stages in the circulation of tick-borne pathogens affecting humans in Central Europe. 2. Tick-borne encephalitis virus.

Grzegorz Karbowiak¹, Beata Biernat²

¹W. Stefański Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warszawa, Poland
²Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine, Medical University of Gdańsk, Powstania Styczniowego 9B, 81-519 Gdynia, Poland

Corresponding Author: Grzegorz Karbowiak; e-mail: grzgrz@twarda.pan.pl

ABSTRACT. Hard-bodied ticks transmit various pathogens, such as *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia* spp., *Babesia* spp., and carry numerous other microorganisms with an unknown pathogenic potential. Among them, tick-borne encephalitis virus has great importance. In Central European conditions all developmental stages of ticks participate in the zoonotic cycle of the TBE virus. According to pathogen and tick biology, the roles of larvae, nymphs and adults are different. Larvae and nymphs of *Ixodes ricinus* ticks are responsible for circulation in rodents and medium sized mammals; adults transfer the infection to ruminants and to next generations via transovarial transmission. All active developmental stages of *I. ricinus* can play role of the bridge vector, transmitting the infection to humans apart males which don’t feed. The late summer peak of human infectivity is caused by the summer peak of *I. ricinus* nymphs’ activity. The *Dermacentor reticulatus* tick attacks humans infrequently, but does participate in the circulation of the virus in the zoonotic foci; larvae and nymphs of the *D. reticulatus* ticks are responsible for circulation in rodents, mainly Microtinae, while adults transmit the infection to ruminants.

Key words: *Dermacentor reticulatus*, *Ixodes ricinus*, TBE virus, zoonotic foci

Introduction

Tick-borne encephalitis (TBE) is one of the tick-borne diseases with a great epidemiological importance in Central Europe. The agent of the illness is the RNA virus (TBEV) from the Flaviviridae family, genus *Flavivirus*. In Europe there is evidence of two of three known subtypes of the TBE virus: Western European, causing Central European brain fever (two-phase) and principally associated with the *Ixodes ricinus* tick, and Far-Eastern, causing Russian or spring brain fever [1], principally associated with the tick *Ixodes persulcatus*.

The European TBE virus occurs in the whole Central and Eastern Europe, up to the Ural Mountains to the east. TBE has become a growing public health concern in Europe and Asia and is the most important viral tick-borne disease in Europe [2]. Currently, 10,000–12,000 new cases of TBE are recorded annually in 28 countries around the world [3,4]. In Poland about 300–340 new cases per year are recorded with the greatest infection rate observed in Northeastern Poland. New cases of TBE are also noted each year in new areas [5].

Efficient vectors of TBE virus

The TBE virus is transmitted by ticks of the *Ixodes persulcatus* complex (called also as *Ixodes ricinus-persulcatus* complex). The most important vector in Central Europe is the *Ixodes ricinus* tick [6]. The infection was also noted in *Dermacentor reticulatus* [7–11]. Experimental infection was likewise developed in the *D. marginatus* and *D. silvarum* ticks [12–14]. Because of the limited...
host’s range, the probability of human infection with the TBE virus caused by the *D. reticulatus* tick is low. However, the *Dermacentor* ticks can support the maintenance of the TBE virus in nature and transmit the TBE virus to wild ruminants and grazing cattle [15]. Some other tick species, as *Ixodes hexagonus, I. trianguliceps, Haemaphysalis concinna, H. punctata*, and *H. inermis* are also able to be infected and transmit TBE virus [4], however, in Central European countries they can play the role of secondary vectors, due to their limited occurrence or in the case of species occasionally affected human, they support the transmission and circulation among wild mammals.

Systematic, direct TBE virus detection in ticks will help to develop further understanding of the prevailing epidemiological situation. The prevalence of TBE virus in ticks can be a suitable marker for current TBE risk analysis in natural foci [16,17].

The prevalence of TBE virus-infected ticks in *I. ricinus* populations is often low. In Central Europe, where TBE virus is endemic, the prevalence of the virus varies from 0.1 to 5.0% [2]. However, over the last few years, the TBE virus has been documented to be spreading into regions where it had not been endemic. It has been found at higher altitudes in the Giant Mountains and the Austrian Alps [2,18]. TBE virus strains were isolated from the *I. ricinus* ticks in Poland many times [19,21], e.g. in the years 2000–2001 one strain of TBE virus from *I. ricinus* ticks was isolated in the Radzyń Podlaski district (Eastern Poland); the minimum infection rate (MIR) in this district was estimated to be 4.2% [22]. The prevalence of TBE virus infection in questing *I. ricinus* ticks in Poland varies from 0.0% to 1.96%. Surprisingly, higher prevalence was noted in *D. reticulatus* ticks: between 0.33% and 10.8% (Table 1).

Transmission ways

Ticks can be infected with TBE virus at every active developmental stage. The virus localises in all tissues, among others in salivary glands and ovaries. Due to its presence in ovaries and developed oocytes, transovarial transmission of the virus to next generations of ticks is common. The virus’ presence in the whole organism makes transstadial transmission possible. This maintains the virus’ presence in the environment even in the absence of mammal susceptible to infection [1,4,23].

Larvae are infected by transvarial TBE virus transmission [24]. Larvae and nymphs acquire TBE virus during blood meal or by co-feeding on the same rodent host and maintain the infection after moulting into the next stage during transstadial transmission. Once infected, ticks carry the virus for the remainder of their lives. From an epidemiological point of view, infected nymphs are the most important stage because they are much more numerous than infected adult ticks [25,26]. The differences in infection rates of particular developmental stages of *Ixodes ricinus* ticks are not great – 2.2, 0.5, 1.77, 0.54% in larvae, nymphs, females and males, respectively [4,8–11,27–31] (Table 1).

Infection of mammals with the TBE virus is possible by tick-bite or by contamination of the tick-bite wound with virus-infected faeces. The virus is transferred with the saliva, the incubation period takes 7 to 14 days, depending on the host species and their immunological condition [32]. During that time the virus multiplies in the tissues around the infection place, and later spreads to the whole organism. This makes horizontal transmission between co-feeding tick specimens possible and has special importance in the absence of systemic viraemia in the host. The co-feeding transmission process requires that ticks feed contemporaneously in close vicinity on one host. In rodents, ticks localise mainly on the ears, sometimes in big numbers [33], but we observe a spatial distribution of species – *I. ricinus* larvae and nymphs usually attach to the inside and outside surfaces of the ears, and only when ticks are in a large number they attach also to the mouth, tail and between the fingers. *D. reticulatus* larvae attach to the whole surface of the ears when they are alone; however, in the presence of *I. ricinus*, they attach only to the edges of the ears. The nymphs of *D. reticulatus* prefer the acoustic duct [34]. Thus, it seems that co-feeding infection is possible only between specimens of one species.

Apart from transmission by ticks, the TBE virus is spread also by other ways, including oral – with milk, cheese or butter originating from infected cows and goats – inhalatory, with dust, and even by blood transfusion [32]. The alternative ways of transmission, without ticks’ participation, should have no influence on the seasonal dynamic of the incidences.
Table 1. Prevalence of TBEV RNA in field-collected questing ticks and in ticks removed from hosts in Poland, reviewed studies (2009–2016)

<table>
<thead>
<tr>
<th>Tick species</th>
<th>Sentinel</th>
<th>Sample size</th>
<th>Prevalence (%) and/or MIR* (%)</th>
<th>Study area Voivodeship: district</th>
<th>Detection method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>2383 (a, n)</td>
<td>MIR 0.58</td>
<td>Wielkopolskie: Nowy Tomyśl, Oborniki; Zachodniopomorskie: Police; Pomorskie: Starogard Gdański</td>
<td>reverse transcription PCR</td>
<td>Makówka et al. 2009 [28]</td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>875 (a, n): 510 (a) and 365 (n)</td>
<td>MIR 1.6</td>
<td>Lubelskie: Włodawa, Lublin, Krasnystaw, Zamość, Lubartów</td>
<td>reverse transcription PCR</td>
<td>Wójcik-Fatla et al. 2011 [8]</td>
</tr>
<tr>
<td><em>D. reticulatus</em></td>
<td></td>
<td>147 (a)</td>
<td>10.8</td>
<td>Lubelskie: Włodawa, Parczew</td>
<td>Real-time PCR</td>
<td></td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>7436 (a, n)</td>
<td>0.0</td>
<td>Dolnośląskie; Kujawsko-Pomorskie; Małopolskie; Wielkopolskie; Zachodniopomorskie</td>
<td>Real-time PCR</td>
<td>Stefanoff et al. 2013 [5]</td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>7270 (a, n): 3605 (a) and 3665 (n)</td>
<td>MIR 0.21</td>
<td>Podlaskie: Hajnówka, Suwałki, Siemiatycze, Kolno, Mońki, Białystok</td>
<td>Real-time PCR</td>
<td>Katargina et al. 2013 [9]</td>
</tr>
<tr>
<td><em>D. reticulatus</em></td>
<td></td>
<td>600 (a)</td>
<td>0.33</td>
<td></td>
<td>Real-time PCR</td>
<td>Biernat et al. 2014 [10]</td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>2075 (a, n, l): 1231 (a), 799 (n), 45 (l)</td>
<td>MIR 0.96</td>
<td>Podlaskie: Białystok, Hajnówka; Pomorskie: Puck, Gdańsk, Gdynia, Kartuzy, Kościerzyna, Nowy Dwór Gdański, Starogard Gdański, Sopot, Wejherowo; Warmińsko-Mazurskie: Braniewo, Pisz, Elbląg, Gizożcko, Kętrzyn, Mragowo, Olsztyn, Ostróda, Węgorzewo</td>
<td>reverse transcription PCR</td>
<td>Biernat et al. 2014 [27]</td>
</tr>
<tr>
<td><em>D. reticulatus</em></td>
<td>Questing ticks</td>
<td>471 (a)</td>
<td>2.12</td>
<td>Mazowieckie: Warszawa; Podlaskie: Hajnówka, Mońki; Warmińsko-Mazurskie: Mragowo</td>
<td>reverse transcription PCR</td>
<td>Biernat et al. 2014 [10]</td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>4350 (a)</td>
<td>MIR 0.31</td>
<td>Śląskie: Katowice, Chorzów, Będzin, Tarnowskie Góry, Bytom, Tychy, Dąbrowa Górnicza</td>
<td>Real-time PCR</td>
<td>Drellich et al. 2014 [29]</td>
</tr>
<tr>
<td><em>D. reticulatus</em></td>
<td>Questing ticks</td>
<td>92 (a)</td>
<td>7.6</td>
<td>Mazowieckie</td>
<td>Real-time PCR</td>
<td>Mierzejewska et al. 2015 [30]</td>
</tr>
<tr>
<td><em>I. ricinus</em></td>
<td>Questing ticks</td>
<td>1750 (n)</td>
<td>MIR 0.11</td>
<td>Śląskie: Lubliniec, Myszków, Tarnowskie Góry, Sosnowiec, Zawiercie, Dąbrowa Górnicza, Piekary Śląskie, Gliwice, Świętochłowice, Chorzów, Katowice, Jaworzno, Tychy, Rybnik, Racibórz, Pszczyña</td>
<td>Real-time PCR</td>
<td>Cuber et al. 2015 [31]</td>
</tr>
<tr>
<td><em>D. reticulatus</em></td>
<td>Collected from European bison (Bison bonasus)</td>
<td>114 (a)</td>
<td>18.42</td>
<td>Podlaskie: Hajnówka</td>
<td>reverse transcription PCR</td>
<td>Biernat et al. 2016 [11]</td>
</tr>
</tbody>
</table>

* Minimum Infection Rate, assuming that only one tick in each positive pool carried the pathogen
The role of developmental stages of ticks in circulation of TBE virus

Generally, immature stages of ticks, larvae and nymphs, feed on small mammals and birds, and adults on larger animals. The competent animal reservoirs of the TBE virus are mainly small mammals, i.e., small rodents and insectivores. Other mammals, as wild boars, hares, bats, deer, goats, cows, and sheep, become infected, but levels of viraemia may be low [4,35], thus their role is rather the supporting of virus circulation by enabling tick reproduction. The role of birds in the ecology of TBE viruses has not been established. Their significance as a virus reservoir is probably less important, but birds and bigger animals can spread infected ticks to new areas [36]. Migratory “traffic hubs” such as the Danube Delta, may be favorable grounds for the establishment due to the abundance and diversity of potential new hosts [37]. Many different vertebrates have been implicated in both the maintenance and circulation of TBE virus. Large wild mammals, such as deer, are accidental hosts of TBE virus but have an important role in the transmission of the vector. They distribute ticks across their foraging areas [38], but the viraemia in
cervids is short and low-grade, and their contribution to the maintenance of the TBE virus is therefore assumed to be minor [39]. Small mammals (mostly rodents) are considered to be both amplifiers and reservoir hosts for TBE virus, and, according to Bakhvalova et al. [40], they may maintain a persistent infection with TBE virus throughout the year. The major role in TBE virus circulation belongs to wood mice (Apodemus sylvaticus) and bank voles (Myodes glareolus) because they are abundant and they are excellent hosts for nymphal and larval tick stages [21,41–42]. Studies from Germany, Hungary and Slovenia have assessed the presence of TBE virus in small mammals (rodents) many times; TBE virus RNA was detected also in wood mice (A. sylvaticus), yellow-necked mice (A. agrarius), common voles (M. arvalis) and field voles (M. agrestis) [41–44]. Interestingly, that Microtinae Myodes glareolus demonstrated higher post-infection anti-body titres and levels of viraemia than Murinae (A. sylvaticus and A. flavicollis) [39,41]. Among domestic animals the infection was detected in goats, sheep, cattle and dogs [45–47].

The seasonal dynamic of TBE incidences is similar to other tick-borne diseases. The peak occurs between August and September; however, in other months cases are noted as well [48–50]. Human infection by I. ricinus ticks is possible from early spring to early winter, because every active developmental stage is able to transmit the virus, and all stages are active during the whole season. The early autumn peak is the result of the I. ricinus nymphs’ activity in July–August. The nymph seems to be the developmental form most capable of transmitting the pathogen from wild animals to humans, owing to their aggressiveness and number in the environment. Moreover, their small size makes difficult their perceiving and removes.

The D. reticulatus tick, though it is also able to transmit the TBE virus, attacks humans very rarely, plays a part in its circulation in the environment. Biology determines their participation in detail. Adult ticks attack big mammals, thus can be infected with the virus or be its source in spring and autumn. Larvae and nymphs are active only in the summer, and their participation is limited to infecting small rodents. The role of the Dermacentor ticks in TBE circulation has not studied so far and needs to be investigated. Transmission between small rodents, deer and Dermacentor ticks without a risk for humans is possible. Such circulation is proven by records of a rather high prevalence of the virus in the Dermacentor ticks [10]. Further proof is provided by higher prevalence of the virus in Microtinae rodents than in Murinae [39], in accordance with the Dermacentor larvae and nymphs preference to voles [33-34].

The proposed scheme of TBE virus circulation in environment, with the visualised role of I. ricinus and D. reticulatus tick’s developmental stages is presented in the Fig. 1.

Acknowledgements

Results of the following projects were used in the review: MNiSW N303 047 31/1487 and NCN 2011/01/B/NZ7/03574.

References


The role of particular tick developmental stages


Received 15 January 2016