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Original article

Prevalence of tick-borne encephalitis virus in a highly urbanized and low risk area in Southern Poland

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ABSTRACT

The knowledge of the exact distribution of tick-borne encephalitis virus (TBEV) endemic foci is crucial to plan and implement the optimal prevention of tick-borne encephalitis (TBE), including a vaccination program. In Poland, however, there is still no data on the local distribution of TBEV in many areas of the country. Silesian agglomeration area (Southern Poland) is a highly urbanized and industrialized region of the country, where TBE cases are only sporadically recorded. In this study, a total of 4350 adult *Ixodes ricinus* were collected between September 2010 and June 2012 at twelve locations. The screening using real-time PCR was carried out on 854 tick pools of five specimens, and the positive pools were verified by pyrosequencing. TBEV was identified in 13 pools (1.52%) at 4 sites, of which 9 pools were verified by pyrosequencing. An overall pool prevalence was estimated at 0.31% ranging from 0.19% to 1.11% for positive locations [95% CI 0.16–0.52], which is comparable with regions with high number of TBE cases reported annually.

Introduction

Over the last 20 years, an upward trend in the number of tick-borne encephalitis (TBE) cases, an acute central nervous system disease, has been observed in many European countries and Asia (Süss, 2011). The rationale behind this phenomenon is not fully understood yet. It appears to be complex, and related to the numerous factors that influence both tick activity and human behavior. It is believed that the observed climate and ecological changes contribute to the increased activity of ticks and the expansion of their populations to new areas. On the other hand, the human intervention in the environment, e.g., by deforestation, leads to the reduction of natural ticks habitats and their hosts. Simultaneously, the natural biotopes serve as an attractive areas of leisure and recreation, which favors exposure to ticks and their pathogens. All those phenomena lead to the frequent exchange of fauna and consequently to blur the boundaries between the natural tick habitats and urban areas (Goldfrey and Randolph, 2011). It also seems that the increase in recorded cases of TBE can be in part due to increased awareness of the problem, and better monitoring thus more frequent diagnosis of TBE, especially in the risk areas (Kollaritsch et al., 2011).

Tick-borne encephalitis is caused by tick-borne encephalitis virus (TBEV) belonging to the family *Flaviviridae* (Thiel et al., 2005), and is transmitted to humans mainly by tick bites. There are three main subtypes of TBEV: European (TBEV-Eu), Far Eastern (TBEV-FE) and Siberian (TBEV-Sib) subtype (Ecker et al., 1999). Their geographic scopes are closely related to the range of their main vectors and reservoirs: TBEV-Eu subtype is associated with *Ixodes ricinus* – the dominant tick in Central, Eastern and Northern Europe; while TBEV-FE and TBEV-Sib subtypes are associated with *Ixodes persulcatus* – mainly distributed in Ural, Siberia and Far East (Süss, 2011).

Currently, approximately 10 000–12 000 new cases of TBE are reported annually, in 28 European countries (Donoso Mantke et al., 2011). In Poland, the regional sanitary-epidemiological stations record 300–340 new cases per year with the greatest infection rate observed in the north-eastern part of the country with smaller dispersed risk areas. What is more, the new cases of TBE are recorded in new areas each year (Stefanoff et al., 2013).

The formation of TBEV foci, further circulation and persistence of the virus in nature, depend on the density of tick population and their competent hosts in the given area, as well as the impact

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of environmental factors on their life processes. Importantly, non-viremic transmission between co-feeding ticks seems to play an important role in focal TBEV maintenance. This phenomenon is supported by the specific ecological features of ticks, including feeding process and synchronous seasonal activity, leading to aggregation of simultaneously feeding ticks in close proximity, which gives the possibility of TBEV transmission from infected to non-infected ticks on the competent host without systemic viremia, and even in the presence of neutralizing antibodies (Randolpg et al., 1996; Labuda et al., 1997). Humans are not competent hosts for ticks, and thus do not play a significant role in the natural transmission cycle of TBEV (Randolph, 2008). Therefore, the prevalence of TBEV in nature can be significantly distorted and incomplete, when it is based solely on an analysis of recorded TBE cases. The direct virus detection in ticks, depicts the main link in the transmission cycle and the main gateway to human infection, and provides a direct evidence for the presence of this pathogen in the given area. For this reason, the methods based on direct virus detection in ticks are considered to be the gold standard for identification of natural foci of TBEV by many authors (Süss et al., 2004; Klaus et al., 2010).

Since there is no specific treatment for TBE (Mansfield et al., 2009), the only way to minimize the problem is to develop and implement the optimal preventive measures, principally through its appropriate targeting for people living in risk areas. Therefore, the first step in reducing the incidence of TBEV infections should include construction of the complete map of risk areas, and its constant monitoring. The aim of this study is to evaluate the TBEV prevalence in tick populations residing in the Silesian agglomeration area, in Southern Poland. We believe that virus detection in ticks from investigated area would be indicative for introducing active vaccination plans and prevention measures for people living in this region.

Materials and methods

Study area and collection of ticks

Ticks were collected in spring and in autumn between September 2010 and June 2012 (four seasons) at twelve sites (Fig. 1), and each site was visited at least once in each following season. The study was focused on adult ticks (both male and female) because they were dominant in number over nymphs in study area during the sampling period (data not shown). The study area covered urban and adjacent suburban areas situated within about 18 km radius of the central part of the Upper Silesia province (Silesian agglomeration, Southern Poland), the largest urban region with the highest population density in Poland. To date, based on the reports of surveillance of TBE cases, Silesian province was considered to be a low-risk area. The study sites included the recreational areas within and in the vicinity of the major cities, where the risk of human contact with tick is increased, i.e., sports and entertainment facilities, hiking and biking trails, camping and picnic areas (Table 1).

Ticks were collected by flagging with a white flannel blanket (1.0 m × 0.7 m) through low vegetation and litter as earlier described by Falco and Fish (1992). Ticks were placed in Eppendorf tubes and transported to the laboratory for taxonomy identification (Siuda, 1993). Collected adults were selected in randomly mixed male and female pools of five according to site and sampling season, and stored at −86 °C for further investigation.

RNA extraction

Each frozen tick pool was transferred to a new tube placed in an ice-box and grounded thoroughly by using hand micro-homogenizer (Bionovo Inc., Poland). Extraction of total RNA was performed by using the Universal RNA Purification Kit (Eurx, Poland), according to the manufacture's protocol. Tick powder resulting from the homogenization was resuspended in RL lysis buffer (provided in the kit). Finally, extracted RNA was eluted in 40 μl RNase-free water available from the kit and stored at −86 °C. The concentration (ng/μl) and purity (proportion of the absorbance at 260 and 280 nm, $A_{260/280}$) of extracted RNA was determined for each pool using Beckman DU 530 Life Science UV-Vis (Beckman Coulter Inc., USA).

Reverse transcription and detection of Ixodes ricinus 16S ribosomal RNA

The reverse transcriptase (RT) reaction was performed with 5 μl (750 ng) of total RNA extracted from each pool of ticks, using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystem, CA, USA) with RNase Inhibitor and random primers according to the manufacture's instruction. The quality of RNA and efficiency of

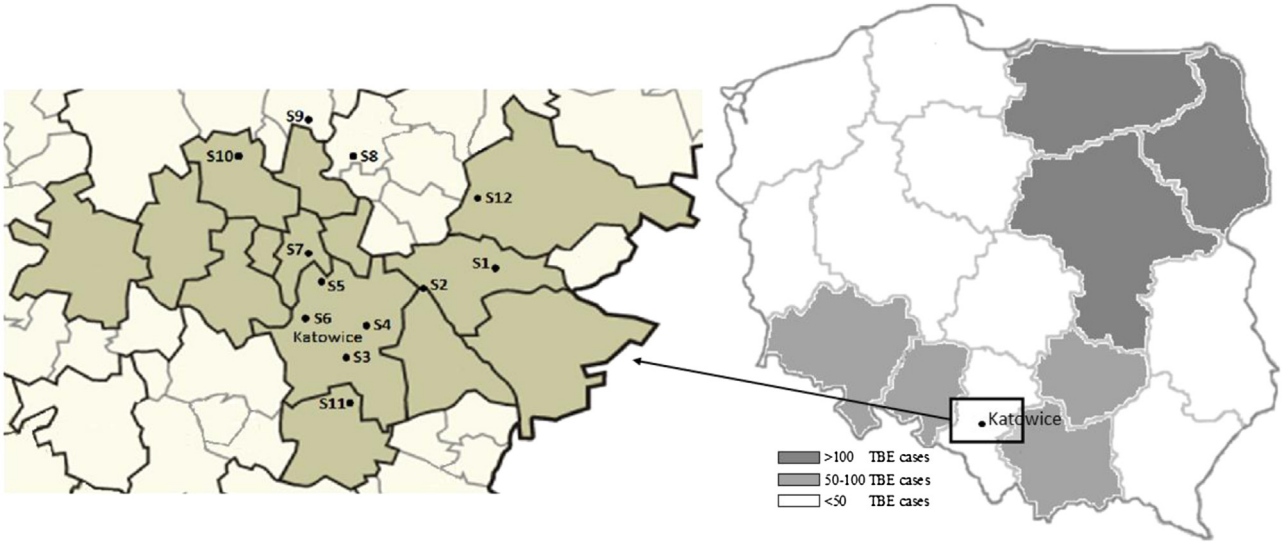

Fig. 1. Map of Poland with the main TBE risk areas (according to data recorded cases of TBE in the epidemiological surveillance), and Silesian agglomeration region (Southern Poland) with the locations of ticks collections.