Abstract

Serum samples from 123 cattle, 95 wild boars, and 43 deer (red deer, roe deer, and fallow deer) from the territory of eastern Poland were examined by the ELISA for the presence of specific antibodies against tick-borne encephalitis virus (TBEV). The rates of positive response in the animals were 4.1%, 16.8%, and 11.6%, respectively. Examination of 37 blood samples from deer with RT-PCR revealed only one positive result in a roe deer (2.7%). The relatively high serologic response rate in wild boars was due to a very high response rate (35.7%) in the Chełm district, which accounted for 94% of the total positive results. These findings seem to indicate that the Chełm district is most probably an endemic area of TBEV.

Key words: domestic animals, game animals, tick-borne encephalitis virus, seroprevalence, Poland.

Material and Methods

Animals. Blood serum samples from cattle and hunted game animals were examined. The samples from cattle were taken from 118 cows, three heifers and two bulls living on the territory of four districts of Lublin province (eastern Poland). Serum samples from game animals were taken from 95 wild boars (Sus scrofa), 12 red deer (Cervus elaphus), 11 roe deer (Capreolus capreolus), and 20 fallow deer (Dama dama). Game

animals were hunted on the territory of nine districts of Lublin province and two districts of Rzeszow province (eastern Poland).

Serological examination. The presence of specific TBEV antibodies of IgG class was determined with the commercial ELISA kit (Immunozym FSME IgG All Species, Progen, Biotechnik, Germany) following the producer’s instruction.

PCR examination. RNA was isolated from 200 µl of the blood taken immediately after hunting from 12 red deer, 11 roe deer, and 14 fallow deer using the commercial set Qiamp Viral Mini Kit (Qiagen, USA), following the producer’s instruction. The amount of RNA measured by spectrophotometry (NanoDrop ND1000, USA) ranged from 12.3 to 34.7 ng/mL. The isolated RNA was stored at -80°C until further examination. The reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR were performed using an earlier described procedure for the detection of TBEV-RNA in milk samples (4).

Statistical analysis. The data were analysed by \( \chi^2 \) test and Student’s \( t \)-test with the use of STATISTICA for Windows v. 5.0 package (StatSoft inc., Tulsa, USA). The value \( P<0.05 \) was considered as significant.

Results

The sera of examined cattle reacted positively to TBEV antigen in the range of 0%-10.0%, with a
The mean seroprevalence was recorded in the Zamość district and was significantly greater than in Biała Podlaska district (P<0.05). However, a total variability of the results between particular districts was not significant (P>0.05).

Mean seroprevalence to TBEV antigen among wild boars was high (16.8%) but it was only due to a very high positive response in the Chełm district (35.7%), while in the other districts, except for the single positive result in the Łuków district, the results were negative (Table 2). In wild boars, a total variability of the results between particular districts was significant (P<0.01), and the difference between the serological response of wild boars in the Chełm district (forming 94% of the total positive results) versus the response in all other districts was highly significant (P<0.00001).

Among deer, the mean seroprevalence to TBEV antigen was lower compared to wild boars and amounted to 11.6% (Table 3). A total variability of the results between particular deer species and particular districts was not significant (P>0.05).

The examination of 37 deer blood samples with RT-PCR revealed only one positive result, in a roe deer hunted in the Lubartów district. It constituted only 9.1% of the examined roe deer and 2.7% of all the examined deer samples.

<table>
<thead>
<tr>
<th>Province, district</th>
<th>Number (%) of sera with TBEV antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biała Podlaska</td>
<td>(N=12)</td>
</tr>
<tr>
<td>Chełm</td>
<td>(N=42) 15 (35.7%)</td>
</tr>
<tr>
<td>Hrubieszów</td>
<td>(N=2) 0</td>
</tr>
<tr>
<td>Łuków</td>
<td>(N=1) 1 (100%)</td>
</tr>
<tr>
<td>Parczew</td>
<td>(N=1) 0</td>
</tr>
<tr>
<td>Włodawa</td>
<td>(N=15) 0</td>
</tr>
<tr>
<td>Zamość</td>
<td>(N=11) 0</td>
</tr>
<tr>
<td>Total</td>
<td>(N=95) 16 (16.8%)</td>
</tr>
</tbody>
</table>

N=total examined.

<table>
<thead>
<tr>
<th>Province, district</th>
<th>Number (%) of sera with TBEV antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lublin</td>
<td>(N=23)</td>
</tr>
<tr>
<td>Lubartów</td>
<td>0/3 (0) 1/11 (9.1%) 3/9 (33.3%) 4/23 (17.4%)</td>
</tr>
<tr>
<td>Rzeszów Nisko</td>
<td>1/7 (14.2%) N.t. 0/5 (0) 1/12 (8.3%)</td>
</tr>
<tr>
<td>Stałowa Wola</td>
<td>0/2 (0) N.t. 0/6 (0) 0/8 (0)</td>
</tr>
<tr>
<td>Total</td>
<td>1/12 (8.3%) 1/11 (9.1%) 3/20 (15.0%) 5/43 (11.6%)</td>
</tr>
</tbody>
</table>

N=total examined; N.t.=not tested.
Discussion

The obtained results indicate that cattle in eastern Poland are infected by TBEV in relatively small proportion. Nevertheless, even such incidence could be of epidemiologic importance if the occurrence of TBEV in cow milk and a possibility of human infection by drinking raw milk, are considered (4). The stated seroprevalence of TBEV in cattle (4.1%) is lower compared to values obtained by Sikutová et al. (17) in Hungary (26.5%), those reported by Körenberg et al. (13) from the forests of the eastern part of the Russian Plain (17%), and to early data obtained in endemic focus of Bialowieża in north-eastern Poland (13.2%) (10), but it is higher compared to results obtained by Drągănescu et al. (6) in Romania (all negative), by Juceviciene et al. (9) in Lithuania (1.38%), and by Lvov et al. (14) in Russian tundra (all negative).

The seroprevalence of TBEV in wild boars distinguished itself by a very high incidence of positive findings in the Chełm district (35.7%) and almost negative response in the other districts (1.1%). This indicates that this district, which is an easternmost part of the Lublin province at the Polish-Ukraine border, contains most probably the natural focus (or foci) of TBE. The response of wild boars to TBEV antigen found in the presented study was higher compared to that noted by Van der Poel et al. (20) in the Netherlands (7%), and that reported by Hubálek et al. (8) from the Czech Republic (8%), but lower compared to values reported by Borcić et al. (2) from Croatia (39%).

The stated serologic response of deer to TBEV (11.6%) was similar to that reported by Skarphédinsen et al. (18) from Denmark (8.7%) and by Hubálek et al. (8) from the Czech Republic (8%), but lower compared to the data reported by other authors from Germany (22.9%-26%) (7, 11) and Croatia (24%-39%) (2). The relatively low response rate of deer to TBEV was confirmed by the examination of 37 deer blood samples by RT-PCR, which revealed only one positive result in roe deer (2.7%). Thus, our results suggest that wild boars are better sentinels than roe deer for the endemicity of TBEV in Poland.

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References


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