A SEROLOGICAL SURVEY FOR BANJAN
AND TICK-BORNE ENCEPHALITIS VIRUSES IN SHEEP
OF EASTERN SLOVAKIA

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Abstract. 755 sheep from 41 farms in the districts of Trebišov, Michalovce, Humenné and Rožňava
in the East Slovakian region were investigated on the presence of antibodies to Bhanja (neutraliza-
tion test) and tick-borne encephalitis (haemagglutination-inhibition test) viruses. In respective
districts 2.2%, 0.4%, 1.7% and 26.7% of animals (total 5.3%) were found to be positive on
Bhanja virus, 4.4%, 1.5%, 1.7% and 3.0% (total 2.6%) on tick-borne encephalitis (TBE)
virus. The considerable infection rate in sheep with Bhanja virus in the district of Rožňava
(mean 26.7% — on one farm as high as 63.9% serologically positive animals) can be explained by
the local occurrence of the tick Haemaphysalis puncticollis, the main vector of this virus in Europe.
The serologic screening conducted indicates a low activity of natural foci of TBE in eastern Slo-
vakia today, and confirms the existence of a natural focus of infection with Bhanja virus in the
district of Rožňava. Recent circulation of Bhanja virus is indicated by antibodies detected in
several one-year-old sheep.

During the serologic screening of natural foci of diseases of bosquemiac (pasture)
type (sensu Rosický 1959), the free-ranging domestic animals (cattle, sheep, goats) are a very good indicator of the foci. This type of foci is also characteristic of the
tick-borne Bhanja (BHA) virus infection in Eurasia and Africa. After antibodies to
BHA virus had been demonstrated in goats, sheep (Bárdos et al. 1977) as well as
humans (Hubálek et al. 1982) we tried to localize the occurrence of this arbovirus
in Czechoslovakia. The present paper contains the results obtained by examining
a major number of sheep sera from the region of eastern Slovakia. Hitherto serologic
surveys for Bhanja virus in sheep in different countries of the world are given in
Table 1. We have included in our survey TBE virus as an additional antigen.

MATERIALS AND METHODS

Animals. During 1982–83 the blood samples of 755 sheep were collected from 41 farms in four
districts (Trebišov, Michalovce, Humenné and Rožňava) of the East Slovakian region. After
transport the sera were stored at −20°C. On each farm 15 to 20 sheep aged 1–9 years were
investigated; about two thirds of that number were over two years old. The mode of pasturing
practised in the region studied is such that in the spring and summer seasons the sheep on most
farms are grazed in more remote pastures, but in the autumn they are pastured on arable lands
(area under mixed crops, stubble-fields, winter crops etc.) in the vicinity of homesteads. In the
districts of Humenné, Rožňava and Michalovce (northern part) the pastures are in hilly terrain
with shrubs (Prunus spinosa, Crataegus, Rosa etc.), sometimes they are skirting the edges of
deciduous forests (Quercus, Carpinus etc.). In the districts of Trebišov and Michalovce (southern
part) the pastures are in lowlands and the sheep graze near homesteads, river arms, canals and
streams. The climate of the region studied belongs to the warmest in the Czechoslovakia.

Tube neutralization test (TNT). Antibodies to Bhanja virus were demonstrated in neutralization
test in tube cultures of the cell line Vero grown in Eagle's medium MEM (ÚSOI) with 10%, calf
serum (Bioveta) and antibiotics (100 i. units/ml of penicillin, 100 µg/ml of streptomycin). The
sera were inactivated (30 min/56°C), diluted for screening 1:8 and mixed sera with parts with
about 100 TCD50/0.1 ml BHA virus Bg 326 (5 passages in suckling mouse brains). This mixture
was incubated for 90 min/37°C and dosed per 0.2 ml on 2-day cell layer following exhaustion of growth medium. After 60 min/37°C 1.5 ml of medium MEM with 2% calf serum and antibiotics was added to each of the test-tube cultures and after 2-day incubation was replaced by 1.5 ml of medium MEM-GaPy (USOL: I. x. b. k. 1977) with 2% calf serum and antibiotics. All necessary controls (positive serum; negative serum; testing dose of virus and its titration) were included in the test. The final evaluation was carried out following 8–10 days, and the sera revealing neutralization effect in dilution 1: 8 were further diluted and titrated. As a serum titre was thereafter designated that dilution which showed 50% neutralization of virus test dose. As positive for the purposes of this study were considered those sera whose titre was 1: 16 or higher.

Haemagglutination-inhibition test (HIT). Antibodies to TBE virus were demonstrated by the standard HIT method (Clarke and Canas 1958) on microplates with 4 haemagglutinating units of TBE antigen (SEVAC). Sera for HIT were extracted by acetone, saturated by glycerehydrates and diluted twofold beginning with dilution 1: 10. As positive titre was considered 1: 10 and higher.

Table 1. Serologic surveys for Bhanja virus in sheep

<table>
<thead>
<tr>
<th>Country</th>
<th>Region</th>
<th>Number of animals examined</th>
<th>% pos.</th>
<th>Test*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Italy</td>
<td>Friuli</td>
<td>48</td>
<td>47.9%</td>
<td>HIT</td>
<td>Verani et al., 1970, 1977, 1979</td>
</tr>
<tr>
<td></td>
<td>Toscana</td>
<td>116</td>
<td>49.1%</td>
<td>HIT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grosseto</td>
<td>15</td>
<td>86.7%</td>
<td>HIT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lazio</td>
<td>32</td>
<td>21.9%</td>
<td>HIT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>eastern Sicily</td>
<td>45</td>
<td>51.1%</td>
<td>HIT</td>
<td>Castro et al., 1976</td>
</tr>
<tr>
<td></td>
<td>western Sicily</td>
<td>130</td>
<td>28.5%</td>
<td>HIT</td>
<td>Albanese et al., 1971</td>
</tr>
<tr>
<td>Yugoslavia</td>
<td>Island of Brac</td>
<td>80</td>
<td>100%</td>
<td>PRNT</td>
<td>Pata et al., 1977</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>Ahtopol</td>
<td>58</td>
<td>100%</td>
<td>PRNT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Checoslovakia</td>
<td>28</td>
<td>7.1%</td>
<td>PRNT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>France</td>
<td>10</td>
<td>30%</td>
<td>HIT</td>
<td>Ghastel et al., 1980</td>
</tr>
<tr>
<td></td>
<td>USSR</td>
<td>54</td>
<td>3.7%</td>
<td>HIT</td>
<td>Chiankin et al., 1971</td>
</tr>
<tr>
<td></td>
<td>Uzbek SSR</td>
<td>79</td>
<td>8.6%</td>
<td>HIT</td>
<td>Karasev et al. 1971</td>
</tr>
<tr>
<td></td>
<td>Tajik SSR</td>
<td>319</td>
<td>8.8%</td>
<td>HIT</td>
<td></td>
</tr>
<tr>
<td>Iran</td>
<td></td>
<td>?</td>
<td>&gt; 8%</td>
<td>HIT</td>
<td>Said et al., 1975</td>
</tr>
<tr>
<td>Pakistan</td>
<td></td>
<td>46</td>
<td>13.9%</td>
<td>CFT</td>
<td>Darwis et al., 1963</td>
</tr>
<tr>
<td>India</td>
<td></td>
<td>149</td>
<td>0.7%</td>
<td>CFT</td>
<td>Shumagum et al., 1974</td>
</tr>
<tr>
<td>Egypt</td>
<td></td>
<td>531</td>
<td>4.1%</td>
<td>CFT</td>
<td>Darwis et al., 1978</td>
</tr>
<tr>
<td>Nigeria</td>
<td></td>
<td>?</td>
<td>0.9%</td>
<td>HIT</td>
<td>Theiler et al., 1973</td>
</tr>
</tbody>
</table>

* HIT — Haemagglutination-inhibition test; PRNT — plaque reduction neutralization test; CFT — complement-fixation test

RESULTS AND DISCUSSION

Table 2 shows the prevalence of antibodies to both viruses in sheep in individual districts. Antibodies to Bhanja virus were detected sporadically in Trebíkov (2.2 % of animals, titres 1: 16, 1: 64, 1: 64), Michalovce (0.4 % titre 1: 16) and Humenné (1.7 % titres 1: 32, 1: 64, 1: 1, 024.1 4, 096). On the other hand, the sheep from the district of Rožňava revealed antibodies in considerable frequency (20.7%) and with marked titres (as high as 1: 2,048); on some farms of this district the infection rate with the virus was very high (Kočové 63.9%) and antibodies were detected even

in one-year-old sheep. All positive cases were verified in HIT with four units of saccharose-acetone antigen of BHA virus Bg 335/6, and the results of both tests (TNT, HIT) were in a very good correlation.

While considering the distribution of tick-borne viruses it is important to know their main and secondary vectors and the tick fauna in the region studied. The main vector of Bhanja virus in Europe is Haemaphysalis punctata, the secondary vectors are H. sulcata, Dermacentor marginatus, Hypermachthus burus and Hyaenomma marginatum. The occurrence of ixodids of arbovirological importance in the studied districts of eastern Slovakia is indicated in Table 3 (Rosicky 1953, Nosek 1971, 1972, Nosek et Krippel 1974). These data make it possible to explain the higher infection rate with Bhanja virus in animals from the district of Rožňava (in comparison with other districts) by the presence of tick species H. punctata (and D. marginatus).

Table 2. Frequency of antibodies to Bhanja (BHA) and tick-borne encephalitis (TBE) viruses in sheep

<table>
<thead>
<tr>
<th>District</th>
<th>Month of collection</th>
<th>Number of farms</th>
<th>Altitude (m)</th>
<th>Race</th>
<th>Number of animals examined</th>
<th>BHA pos.</th>
<th>TBE pos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trebiakov</td>
<td>III, IV, IX</td>
<td>8</td>
<td>100–200</td>
<td>merino</td>
<td>137</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Michalovce</td>
<td>II, III, IV</td>
<td>14</td>
<td>100–300</td>
<td>merino</td>
<td>259</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Humenné</td>
<td>II, III, IV</td>
<td>13</td>
<td>200–600</td>
<td>cipay</td>
<td>238</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Rožňava</td>
<td>III, IV, X</td>
<td>6</td>
<td>250–600</td>
<td>merino</td>
<td>129</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>41</td>
<td>100–600</td>
<td></td>
<td>753</td>
<td>40</td>
<td>20</td>
</tr>
</tbody>
</table>

Table 3. Occurrence of ixodid ticks in the region studied

<table>
<thead>
<tr>
<th>District</th>
<th>Trebiakov</th>
<th>Michalovce</th>
<th>Humenné</th>
<th>Rožňava</th>
</tr>
</thead>
<tbody>
<tr>
<td>Izodes ricinus (L.)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Dermacentor reticulatus (fab.)</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>D. marginatus (Sulzer)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Haemaphysalis concinna Koch</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H. truncatellus Birula</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H. puconis Can. et Fanx.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+, species present; —, species absent; ? occurrence vague

In connection with the serologic indication of the Bhanja virus circulation in the territory of Czechoslovakia there is the problem of pathogenicity of this virus for sheep, and its epizootological importance in general. Semaško et al. (1973) briefly report that experimental intracerebral injection of 2–3-month-old lambs with BHA virus produces meningoencephalitis, but they do not mention the number of animals tested nor other details (dose of virus etc.). Canic et al. (1981) infected one sheep intravenously with 10^6 SMICLD_50, but did not observe any clinical symptoms and detected no virus in either. Mårdr et al. (1984) infected 7 young sheep subcutaneously or intracerebrally; clinical symptoms (meningoencephalitis) were observed only after i.e. application of a large dose (10^6–10^8 SMICLD_50) of virus, including also weak viremia; immune response of animals, however, was very distinct even after applica-

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