HAEMAGGLUTINATION-INHIBITING ANTIBODIES AGAINST ARBOVIRUSES OF THE FAMILIES TOGAVIRIDAE AND BUNYAVIRIDAE IN BIRDS CAUGHT IN SOUTHERN MORAVIA, CZECHOSLOVAKIA

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Abstract. A total of 295 birds belonging to 10 species of 7 families of wild Passeriformes were examined by haemagglutination-inhibition test. The birds were caught for an international research program "Balt" at the time of autumn migration (August—September 1984). Their sera were examined for antibodies against 4 arbovirus antigens of the genera Alphavirus (Sindbis — SIN) and Flavivirus (tick-borne encephalitis — TBE, West Nile — WN) and family Bunyaviridae (Takyrna — TAH, Caloro — CVO and Bhanja — BHA). Antibodies against all studied viruses were detected at different frequencies: SIN 6.4 %, TBE 7.1 %, WN 9.7 %, TAH 16.5 %, CVO 12.1 %, and BHA 1.9 %.

In the years 1978—1984, small birds were caught for the international research program "Balt" in the littoral of the Nysyt fish pond near Sedlec (Břeclav district) at the time of autumn migration (July 15 — September 12). For a more complex utilization of the scientific material obtained, the birds were serologically examined for the presence of antibodies against arboviruses transmitted by mosquitoes (TAH, CVO, WN, SIN) and ticks (TBE, BHA). The present study is a continuation of previous papers published by E knife et al. (1971, 1975) and K oIm an et al. (1975, 1976).

MATERIALS AND METHODS

Wild small birds were caught in the littoral of the Nysyt fish pond (48°45' N; 16°42' E) in southern Moravia. This locality is situated in the southern part of the valley of the rivers Dyje and Svratka, in one of the most warm and dry places in Czechoslovakia (average yearly temperatures 8–9 °C, average yearly precipitations 500—600 mm). Until recently, the rivers Dyje and Morava formed here numerous meanders and blind branches. The rivers are bordered by inundated forests consisting mainly of poplars (Populus alba, P. nigra), ash-trees (Fraxinus excelsior), willows (Salix sp.), oaks (Quercus robur), and characteristic herbs (Arthrochloa clausitidis, Clematis vitalba etc.). The inundations, which occur every year even at the present time, form optimal conditions for a mass occurrence of various mosquito species. In the littoral zone of the pond Nysyt, there are wide reed belts (Phragmites communis, Typha angustifolia, T. latifolia) which are a suitable biotope for both mosquitoes and birds living in reedwamps.

The birds were caught by "Kokett" and "Z" nets (GDR). After the species determination, they were weighed, measured and ringed and their sex and age were assessed. Blood samples were taken under aseptic conditions. After disinfection with 95 % alcohol, vena ulnaris cutanea was pierced with a sharp small needle and the flowing blood was sucked by means of narrow-piston sucking pump into haemocytost microcapillaries (Silko Union Glass Works Kavalier). The wound was treated by Acutol. The capillaries were immediately sealed by gas flame and after 24 h coagulation the serum was separated from blood samples by centrifugation in a haemocytost centrifuge (Mechanika przemysław, Warszawa, type 316) at 10 000 g for 4 min. The capillary was filled at the site between the serum and erythrocytes and the serum was transferred into a tube with physiological saline up to
final serum concentration of 1 : 10. Non-diluted serum was stored at —25°C until serological examination. The serum was extracted by acetone, saturated with gadder ethyeloxides and tested by haemagglutination-inhibition test (HIT) after Ullarke and Casals (1958) modified for Takatsy (1956) microtiter.

TBE antigen was a commercial product (Inma), other antigens (SIN, WN, TAH, CVO, BHA) were prepared by macro-acectate extraction from brains of infected sucking mice after Ullarke and Casals (1958). Always 4 units of haemagglutinin were used in the test. Titres of 1 : 20 and higher were taken as positive.

RESULTS

A total of 295 birds belonging to 19 species were examined (Table 1). No antibodies against any of the studied viruses were detected in six species (Acrochophalus r parsermatus, Phylloscopus trochilus, P. collibibo, Hirundo rustica, Serinus serinus, and Emberiza schoeniclus).

Sera of 13 bird species were positive containing antibodies against the following viruses: SIN — 7 species (Acrochophalus scirpaceus, A. schoenobenus, Locustella luscinoides, Panurus biarmicus, Parus coeruleus, Remiz pendulinus, and Miliaria calandra), TBE — 5 species (Silvia atricapilla, Acrochophalus scirpaceus, A. schoenobenus, Parus coeruleus, and Carduelis carduelis), WN — 5 species (Locustella luscinoides, A. schoenobenus, A. palustris, Parus coeruleus, and Remiz pendulinus). Two of 194 specimens examined contained antibodies against BHA virus (Acrochophalus scirpaceus and Parus biarmicus).

A cross reaction with TBE and WN antigens was observed in one bird.

DISCUSSION

The birds examined were mostly infected with the viruses TAH and CVO transmitted by mosquitoes (16.3% and 12.1%, respectively) and WN and SIN (0.7% and 6.4%, respectively). This is quite understandable in the bird species living in the litoral pond ecosystem. The records of antibodies against TAH and CVO viruses corresponded with the observations by Ernek et al. (1971, 1973), who detected antibodies against these viruses in the same region in the past. Similarly, the antibodies against WN and SIN viruses were previously found in 5 of 13 birds examined in western Slovakia (Ernek et al. 1975) and later in 12 of 35 bird species in western, southern and southwestern Slovakia (Ernek et al. 1977). Antibodies against WN were recorded in Austria (Aspöck et al. 1973) in 4 of 27 bird species examined. In Bulgaria (Gréshková et al. 1962), antibodies against WN virus were found in 19 of 234 birds captured in the vicinity of the locality Iskra and in 20 of 89 birds captured in the vicinity of Burgas. The detection of antibodies against SIN and WN viruses suggests that these viruses may circulate in nature in central Europe. This assumption is supported by the isolation of SIN virus (Ernek et al. 1973) and WN virus (Gréshková et al. 1975) from birds and seroconversion against SIN virus in sentinel chickens and ducklings in western Slovakia (Ernek et al. 1973/74). It is possible, however, that the seronegative birds (not born in the same year) could get the antibodies during their overwintering or migration.

The occurrence of tick-borne viruses was somewhat lower. Ti infection with TBE virus was found in 7.1%, whereas BHA virus was detected only in 1.0% of birds. An inspection of the areas surrounding the pond Nesy did not reveal any tick in the local vegetation (Ernek et al. 1975). However, inundated forests with rather numerous populations of ticks of the family Ixodidae occur in the wider vicinity of the pond, which may explain the finding of antibodies. Another possibility of the contact with ticks occurs during the migration or overwintering of birds.

The infection with BHA virus was low. Similar results were obtained also by Verrani et al. (1970) in Italy. The authors examined sera of 635 wild birds and 1.9% of them were positive in HIT. With respect to the results obtained by Hübäke and Rödd (1980) it is little probable that the birds may serve as hosts of this virus, but they may play a role in the introduction of infected ticks into other regions during migration (Hübäke et al. 1982). The presence of antibodies shows that the birds get into contact with both arboviruses types, those transmitted by ticks as well as those transmitted by mosquitoes, and that they participate in their ecology.

The birds living primarily in the litoral pond vegetation are attacked mostly by the mosquitoes, whereas the birds living also in the forests are infested also by ticks (particularly Ixodes ricinus).

Acknowledgements. Our thanks are due to Mrs. D. Serbajová, Mr. J. Peško and Mrs. L. Ševčíková for their excellent technical assistance in blood collection.

Table 1. Survey of wild bird species tested by HIT for the presence of antibodies against some arboviruses

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>SIN</th>
<th>TBE</th>
<th>WN</th>
<th>TAH</th>
<th>CVO</th>
<th>BHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sylviae</td>
<td>Silvia atricapilla</td>
<td>0/4</td>
<td>1/4</td>
<td>0/4</td>
<td>1/2</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Locustella luscinoides</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Locustella naevia</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acrochophalus scirpaceus</td>
<td>4/7</td>
<td>5/8</td>
<td>3/10</td>
<td>11/8</td>
<td>9/34</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>Acrochophalus schoenobenus</td>
<td>2/41</td>
<td>5/44</td>
<td>1/20</td>
<td>7/45</td>
<td>5/33</td>
<td>0/9</td>
</tr>
<tr>
<td></td>
<td>Acrochophalus palaetus</td>
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<td>0/6</td>
<td>1/4</td>
<td>2/4</td>
<td>1/2</td>
<td>0/6</td>
</tr>
<tr>
<td>Timaliidae</td>
<td>Parus biarmicus</td>
<td>1/8</td>
<td>0/11</td>
<td>0/11</td>
<td>0/11</td>
<td>1/2</td>
<td>1/9</td>
</tr>
<tr>
<td>Paridae</td>
<td>Parus coeruleus</td>
<td>1/4</td>
<td>2/6</td>
<td>2/2</td>
<td>2/6</td>
<td>2/2</td>
<td>0/6</td>
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<tr>
<td>Remizidae</td>
<td>Remiz pendulinus</td>
<td>1/18</td>
<td>0/25</td>
<td>2/16</td>
<td>5/23</td>
<td>2/17</td>
<td>0/25</td>
</tr>
<tr>
<td>Fringillidae</td>
<td>Carduelis chloris</td>
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<td>0/3</td>
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<tr>
<td></td>
<td>Carduelis cotriculus</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
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<td>0/3</td>
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<tr>
<td>Emberizidae</td>
<td>Miliaria calandra</td>
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<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

Total of positive sera | 11/171 | 14/196 | 10/103 | 32/196 | 24/198 | 2/194 |
Total %            | 6.4    | 7.1    | 9.7    | 16.3   | 12.1   | 1.0   |

x — number of positive sera
y — number of examined sera
ИГНИБИРУЮЩИЕ ГЕМАГГЛЮТИНАЦИЮ АНТИТЕЛА ПРОТИВ АРБОВИРУСОВ СЕМЕЙСТВ ТОГАВИРИДАЕ И БУНЬЯВИРИДАЕ У ПТИЦ, ОТОБЛЯНЕННЫХ В ЮЖНОЙ МОРАВИИ В ЧЕХОСЛОВАКИИ

3. Юржинова, З. Губко, П. Халоука, Й. Пелланова и Й. Хитял

Резюме. С помощью теста комбинации гемагглютинирования исследовано 295 птиц, относящихся к 19 видам 7 семей птиц, в которых наблюдалось 10 видов птиц в рамках международного научного мероприятия (штамм гемагглютинина 1984 г. Их кровь, выщепленная из птицы, против антител к арбовирусам, относящимся к роду *Alphavirus* (Sinivirus, Borna) и роду *Flavivirus* (клещевой энцефалит - TBE, West Nile - WN) и семейства Bunyaviridae (Tithonia, Choro, CVO и Enhemlia - BNA). Были обнаружены антитела против всех исследованных арбовирусов в следующих концентрациях: SIN 6,4 %, TBE 7,1 %, WN 9,7 %, TAH 16,3 %, CVO 12,1 %, BNA 1,0 %.

REFERENCES


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284