PREVALENCE OF ANTIBODIES TO *Borrelia burgdorferi* IN HORSES OF EAST SLOVAKIA*

VÝSKYT PROTILÁTOK *Borrelia burgdorferi* U KONÍ NA VÝCHODNOM SLOVENSKU

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ABSTRACT: In studying the circulation of antibodies to *Borrelia burgdorferi* in animal population, sera of 207 clinically healthy horses from 11 districts of East Slovakia were examined by ELISA. Of all the sera examined anti-Borrelia IgG antibodies were detected in 99 horses, i.e. in 47.8%. The highest seroprevalence was observed in the districts Prešov (59.1%) and Košice (55.2%). A slightly lower seroprevalence was recorded in the districts Stropkov (43.7%) and Poprad (33.3%). A significantly lower seroprevalence relative to the districts Prešov and Košice was recorded from Michalovce district (23.8%) (Χ²-test, P < 0.05). In the remaining districts (Bardejov, Kežmarok, Levoča, Stará Lúbovňa, Rožňava and Spišská Nová Ves) the seroprevalence was 25–100%, but the number of animals examined was low (2–5 horses). An analysis of seroprevalence made with respect to the age showed that there was no significant difference between 1–3-year-old horses (40.9%) and older horses (48.5, or 48.7%). Seroprevalence showed the same pattern of its occurrence as regards the exploitation of horses. Considering the months of taking blood samples, a significantly lower seroprevalence was identified in spring season (40.0%) than in summer (62.0%) (Χ²-test, P < 0.05). The occurrence of *Borrelia burgdorferi* antibodies in horses in East Slovakia alerts veterinarians to consider this disease in their clinical practice and not to disregard it in differential diagnosis.

*Borrelia burgdorferi* sensu lato; antibodies; ELISA; horses

ABSTRAKT: Pri štúdiu cirkulácie *Borrelia burgdorferi* v animálnej populácii sme vyšetrili séra 207 klinicky zdravých koní z 11 okresov východného Slovenska metódou ELISA. Z celkového súboru vyšetrených sér sa antiboreliové IgG protitátky zistili u 99 koní, tj. 47,8 %. Najvyššia séroprevalencia bola v okresoch Prešov (59,1 %) a Košice (55,2 %). Mierne nižšia bola v severných okresoch Stropkov (43,7 %) a Poprad (33,3 %). Signifikantne nižšia séroprevalencia v porovnaní s okresmi Prešov a Košice bola v Michalovech (23,8 %) (Χ²-test, P < 0.05). V ostatných okresoch (Bardejov, Kežmarok, Levoča, Stará Lúbovňa, Rožňava a Spišská Nová Ves) séroprevalencia bola zistená u 25 až 100 % koní, avšak počet vyšetrených zvierat bol nižší (dvaja až päť koní). Na základe analýzy séroprevalencie podľa veku sa nezistili signifikantné rozdiely medzi jeden- až trojročnými (40,9 %) a staršími koními (48,5, resp. 48,7 %). Rovnaký priebeh výskytu antiboreliových protitátkov sme zistili pri hodnotení pracovného využitia koní. Signifikantne nižšiu séroprevalenciu sme zistili na jar (40,6 %) ako v lete (62,0 %) (Χ²-test, P < 0.05). Výskyt antiboreliových protitátkov u koní na východnom Slovensku je signálokom pre veterinárných lekárov, aby sa na toto ochorenie myslelo v klinickej praxi a nebolo opomenuté pri diferenciálnej diagnostike.

*Borrelia burgdorferi* sensu lato; protitátky; ELISA; kone

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INTRODUCTION

Lyme borreliosis is an important multisystemic infection of humans and animals. The main link in the epidemiology of this disease is an infected tick, harbouring borreliae for a long time, transmitting them through the developmental stages and in case of the overall infection most likely also transvariably (Lanen and Burgdorfer, 1986). Unlike in geographically defined areas with the focal occurrence of tick-borne encephalitis virus, *B. burgdorferi* is more dispersed and bound to the areas where ticks find suitable conditions for their existence (Zeman et al., 1990).

Our concern for the problems of Lyme borreliosis has been elicited by reports on its incidence in the neighbouring countries. In Austria, Stanek and Flamm (1985) reported the first findings of this infection in humans. The occurrence of *erythema chronicum migrans* (ECM) in humans in Hungary was reported by Bózsík et al. (1986), in Italy by Trevisan et al. (1986), in the Czech Republic by Doutilik et al. (1985). The initial surveys of ticks in the territory of Slovakia proved their extensive infestation with borreliae (Kněží et al., 1986, 1990). Borrelia infection rate in ticks in Slovakia has also been confirmed by other authors (Rehäček et al., 1991; Prokopčáková et al., 1992; Drogošová and Rehäček, 1995; Peklo et al., 1996). The first results about the presence of anti-Borrelia antibodies in dogs from urban and suburban areas of Košice suggest their considerable exposure to this zoonosis (Štefančíková et al., 1996).

Within the complex research of Lyme borreliosis in Slovakia in a veterinary field, we present the first findings on seropositivity of horses from selected districts of East Slovakia.

MATERIAL AND METHODS

Serum samples

Over the years 1996–1998, serum samples from 207 horses were examined for anti-Borrelia antibodies. Blood sera were collected from 39 herds from 11 districts of East Slovakia: Košice sera 76 from 10 herds, Prešov 54/9, Stropkov 16/2, Poprad 30/7, Michalovce 21/5, Bardejov 2/1, Rožňava 2/1, Spišská Nová Ves 2/1, Lovoča 5/1, Stará Lubovňa 5/1 and Kežmarok 4/2. The sera were provided by State Veterinary Institutes in Košice and Prešov, Veterinary Department of the University of Veterinary Medicine in Košice and the Military Training Camp in Poprad district.

Antigen

Our own endemic strain of *Borrelia burgdorferi* sensu lato isolated from ticks *I. ricinus* in Košice city was used as antigen, prepared by the method of Tresová et al. (1997). The protein content was assessed by the method of Lowry et al. (1951). The working dilution of antigen was determined by box titration.

Enzyme – linked immunosorbent assay (ELISA)

The sera were examined by a modified ELISA, commercially provided by the Institute of Sera and Vaccines (USOL, Praha) as a medical kit for human diagnosis of Lyme borreliosis in the following way: Microplates were filled with 100 µl antigen diluted in carbonate buffer at pH 9.6 (5 µg/ml) and incubated overnight at 4 °C. After washing three times with phosphate buffer (pH 7.2) 100 µl portions of sera diluted at 1 : 400 in phosphate buffer with 0.05% Tween 20 and 1% BSA were added to each well and incubated at 37 °C for 30 min. After a triple washing of the plates 100 µl portions of anti-horse IgG peroxidase conjugate (Sigma) were added per well, diluted at 1 : 2000. After 30 min of incubation and a subsequent washing 100 µl of substrate solution (pH 5.0) with orthophenylene diamine were added per well. The reaction was stopped with 5% H2SO4, after 15 min of incubation. Absorbance was measured at a wavelength of 492 nm. Horse serum that proved positive in repeated titrations was used as a positive control. Horse sera that proved negative in repeated titrations, with their absorbance value less than 0.4, served as negative controls. Cut-off was determined as a value of 3 standard deviations above the mean optical density (OD) for negative serum samples. Sera with the absorbance value higher than 0.6 were evaluated as positive. Anti-horse peroxidase conjugate (Sigma) was used.

The reproducibility of ELISA

Six sera samples were repeatedly examined (10 times), two of them being positive with absorbance value higher than 1.2, two with absorbance 0.7–0.9 and two with absorbance less than 0.4. All the sera from 207 horses were duplicately examined after 6 months’ time.

Statistical evaluation was done by χ²-test and coefficient of variation (V%) (Reisenauer, 1970).

RESULTS

In the set of sera from 207 horses IgG antibodies were detected in 99 horses, i.e. 47.8% (Tab. I). The highest seroprevalence was found in Prešov and Košice districts, 59.1 and 52.6%, respectively. A lower seroprevalence was recorded in Stropkov and Poprad districts, 43.7 and 33.3%, respectively. A significantly lower seroprevalence relative to districts Prešov and Košice was observed in Michalovce district (23.8%) (χ²-test, P < 0.05). In the districts Bardejov, Kežmarok, Lovoča, 228
I. Seroprevalence in horses for *B. burgdorferi* in regions studied

<table>
<thead>
<tr>
<th>Region</th>
<th>1996</th>
<th>1997</th>
<th>1998</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>examined</td>
<td>positive</td>
<td>%</td>
<td>examined</td>
</tr>
<tr>
<td>Banďajov</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Kežmarok</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Košice*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>38</td>
</tr>
<tr>
<td>Levoč</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Michalovce*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Poprad*</td>
<td>15</td>
<td>5</td>
<td>33.3</td>
<td>5</td>
</tr>
<tr>
<td>Prešov*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>29</td>
</tr>
<tr>
<td>Rožňava</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Spišská Nová Ves</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Stará Ľubovňa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Širokýov</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>5</td>
<td>33.3</td>
<td>85</td>
</tr>
</tbody>
</table>

Michalovce differs from Košice, Prešov and Poprad (P < 0.05)

Stará Ľubovňa, Rožňava and Spišská Nová Ves the seroprevalence was 25–100%, but the number of animals examined was low. An analysis of seroprevalence by age (Tab. II) showed that there was no significant difference between 1–3 year-old horses (40.9%), compared with the groups of older horses (48.5, or 48.7%). Seroprevalence showed the same pattern of occurrence as regards the utilization of horses. A significantly lower seroprevalence was recorded in spring (40.9%) than in summer time (62.0%), (X²-test, P < 0.05) – Tab. III.

ELISA reproducibility: Coefficient of absorbance variation in highly positive sera reached higher values (15.4–20.2%) than in less positive (11.6–16.4%) or negative sera (12.6–14.9%). Duplicate examinations of all sera after 6 months' time showed a 100% reproducibility (positive sera remained positive and negative remained negative).

II. Seroprevalence in horses for *B. burgdorferi* by age and their utilization

<table>
<thead>
<tr>
<th>Age/years</th>
<th>Examined</th>
<th>Positive</th>
<th>%</th>
<th>Group of horses</th>
<th>Examined</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–3</td>
<td>22</td>
<td>9</td>
<td>40.9</td>
<td>Breeding</td>
<td>15</td>
<td>5</td>
</tr>
<tr>
<td>4–9</td>
<td>103</td>
<td>50</td>
<td>48.5</td>
<td>Race</td>
<td>55</td>
<td>32</td>
</tr>
<tr>
<td>10–16</td>
<td>82</td>
<td>40</td>
<td>48.7</td>
<td>Draft</td>
<td>137</td>
<td>62</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>99</td>
<td>47.8</td>
<td>Total</td>
<td>207</td>
<td>99</td>
</tr>
</tbody>
</table>

DISCUSSION

Our results on seroprevalence of Lyme borreliosis in horses suggest that out of 207 serum samples taken in East Slovakia districts almost the half was positive (47.8%), with seroprevalence differing with individual districts. Although it is the dog that plays a very important epidemiological role in this serious disease as a constant companion of man to tick-infested areas (Eng et al., 1988), the international literature also presents data on such exposure of free-living animals (Iosogi et al., 1991) and farm animals, including horses (Burgess and Mattison, 1987; Bernard et al., 1990; Parker and White, 1992). A seroepidemiological survey conducted in the U.S.A. has proved that in the north-eastern part of the
country some 14–25% of horses are seropositive (Marcus et al., 1985; Magnarelli et al., 1988), in the western U.S.A. 6–35% or more (Magnarelli and Anderson, 1989).

In Japan seropositivity in horses is only 2.6–4.6% (Tassai et al., 1993) and in the Czech Republic 7.8% (Treml, 1993). Horses in Texas have no record of seropositivity (Cohen et al., 1992). Clinical symptoms were recorded in only 10% of seropositive horses (Marcus et al., 1985; Magnarelli and Anderson, 1989).

The differences found in seroprevalences have been caused by several factors. They may include the use of different methods (indirect fluorescent-antibody assay – IFA, indirect enzyme-linked immunosorbent assay – ELISA and Western immunoblot – WB) or their various modifications, which give different interpretations of results in terms of determining the reaction positivity. Furthermore, it is the geographical variability of Borrelia burgdorferi sensu lato that is reflected in the antigenic diversity of isolates used as antigens for serological diagnosis of this serious zoonosis and consequently in a different sensitivity of the method (Anderson et al., 1989; Baranton et al., 1992; Park et al., 1993). Most of the previous studies (Bunikis et al., 1995; Norman et al., 1996; Štefančíková et al., 1998) have proved that antigens prepared from local Borrelia isolates give a higher sensitivity of reaction, disclosing a higher seroprevalence. We found seropositive horses in 35 out of 39 herds. Considering the fact that sonicated antigens contain more than 100 proteins (Lüft et al., 1989) some of which are equivalent to antigens of more than 60 different bacterial species, it is not possible to rule out cross reactions if clinical symptomatology is unavailable. Nevertheless, the prevalence of anti-Borrelia IgG antibodies in almost every herd examined suggests a distribution of agents and their possible contact with horses from the eastern part of Slovakia.

The differences in seroprevalence are extensively influenced by the occurrence and different degree of tick infection in the particular territory. Ticks I. ricinus are infected with Borrelia burgdorferi almost all over the territory of Slovakia with differences between individual localities (Kometa et al., 1990) as well as between individual localities within the particular district (Peško et al., 1996). In addition to ticks, the secondary role in transmission of Lyme borreliosis may also be played by other blood-sucking insects such as horseflies, mosquitoes, mites (Magnarelli et al., 1988).

Our results on the seroprevalence of anti-Borrelia IgG antibodies in horses obtained in individual months of the years suggest that this prevalence is connected with the activity of ticks. Considering the fact that this activity peaks in spring and autumn and that IgG antibodies generally start emerging about 1–2 months post infection, this could account for their increased prevalence in summer time as detected by our study. Since we have not determined any IgM antibodies, the presence of which would indicate an early stage of infection, the evidence of IgG antibodies can only serve as an indicator of a mere contact with the agent of Lyme borreliosis, but it will not determine whether it is the case of acute infection or reinfection. Only scarce data are available on the duration of spirochaetemia and persistence of antibodies in horses (Magnarelli and Anderson, 1989), however, in dogs with subclinical infections or reinfections they were found to persist for 1–2 years or even more (Magnarelli, 1990). It is assumed that titres of anti-Borrelia antibodies in horses in endemic areas persist as a result of frequent reinfections (Parker and White, 1992).

The occurrence of Borrelia burgdorferi antibodies in horses in East Slovakia alerts veterinarians to pay attention to this disease in their clinical practice and not to disregard it in differential diagnosis. Moreover, knowledge of Borrelia burgdorferi circulation in animal population helps detect new foci of this disease, thus also contributing to the protection of human health.

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