Paradigm Burgenland: Risk of *Borrelia burgdorferi* sensu lato infection indicated by variable seroprevalence rates in hunters

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**Summary.** Background: This study concerns the prevalence of antibodies against *B. burgdorferi* sensu lato as an indicator of previous borrelial infection among hunters, a group of occupationally exposed persons. In order to define associated risk factors and preparing data for future comparisons, a study was performed in the eight districts of Burgenland, the most eastern state of Austria.

Methods: Blood samples of 1214 men (median age 51 years, range 18 to 89 years) and 39 women (median age 44 years, range 21 to 69 years) were collected during autumn 2002 and winter 2003. Demographic data regarding age, sex, profession, residence, duration of employment (hunters), hunting-ground, animals in the environment, and history of tick bite were obtained by a questionnaire. A two-step testing strategy was used in which sera were screened for antibodies against *B. burgdorferi* sensu lato by a commercially available recombinant enzyme-linked immunosorbent assay (Biotest Anti-Borrelia IgG ELISA; Biotest AG, Dreieich, Germany). Reactive sera were then subjected to immuno blot testing (recomBlot Borrelia; MikroGen, Munich, Germany) for confirmation of specificity.

Results: A total of 673 (54%) sera tested positive for IgG antibodies to *Borrelia burgdorferi* sensu lato: 663 (55%) men and 10 (26%) women. Seropositivity was clearly related to age and duration of hunting activity; it was 33% among persons younger than 29 years and 83% in those older than 70 years. Further, there was also a difference in the distribution of seroprevalence within the districts; the highest was found in hunters from the most southern district of Burgenland, Jennersdorf, (69%) and the lowest was noticed in the most northern district, Neusiedl (39%).

Conclusions: We found an overall seroprevalence of 54% in asymptomatic hunters of Burgenland. Infectious risk exists in the entire state but the prevalence rate differs in the various districts indicating a variable risk which peaks in the south. The nearly linear increase of seroprevalence with age and duration of hunting activity reflects repeated tick exposure.

**Key words:** Lyme borreliosis, seroprevalence, tick exposure.

**Introduction**

Lyme borreliosis is a tick-transmitted illness that occurs worldwide and is associated with areas inhabited by ticks. *Borrelia burgdorferi* sensu lato infection causes a progressive or episodic disease with variable clinical presentations which range from asymptomatic infection to significant illness, affecting skin, musculoskeletal and the nervous system [1, 2]. Only one of the clinical manifestations of disseminated Lyme borreliosis is pathognomonic: the only specific sign of a *B. burgdorferi* s.l. infection is erythema migrans, a transient local skin infection that usually manifests in early disease [3–5]. Diagnosis of Lyme borreliosis is primarily based on the recognition of clinical signs. Supporting evidence is provided by laboratory investigation, usually antibody tests. In 1995 the Centers for Disease Control and Prevention recommended sequential two-step antibody testing to improve the accuracy of serological tests for Lyme disease [6, 7]. A similar recommendation is given by the 'Quality Standards for the Microbiological Diagnosis of Infectious Diseases' MIQ 12, 2000 [8].

Nevertheless, serological results are neither supportive for the diagnosis of erythema migrans nor a parameter for successful antibiotic treatment of infection with *B. burgdorferi* s.l. [9].

According to a 1995 WHO report [10], all of Europe should be regarded as an endemic area of Lyme borreliosis, although the number of infections varies in particular countries and even within the same geographical area [11, 12]. The disease occurs with relatively high frequency in southern Scandinavia, The Netherlands, parts of Germany, the Czech and Slovak republics, Austria and Slovenia. The highest incidence rates are reported from central-eastern Europe, and in local areas in Austria the incidence rate of Lyme borreliosis is up to 1000/100000 population. However, the number of actual Lyme borreliosis cases is estimated to be higher than the reported cases [13]. The risk is associated with residence in rural areas and with occupational activities in forested areas. Serologic studies in Europe have shown that populations exposed to contact with ticks more often have specific antibodies against *B. burgdorferi* s.l. than the rest of the population [14].

This study concerns the prevalence of antibodies against *B. burgdorferi* s.l. as an indicator of previous bor-
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**Materials and methods**

Members of the hunters association of Burgenland (Burgenländischer Jagdverband) were recruited by the association’s executives for participation in a study to establish the seroprevalence of the tick-borne zoonosis Lyme borreliosis. All who participated gave informed consent.

In total, samples of 1253 human sera (1214 men, 39 women) were collected during autumn 2002 and winter 2003. Demographic data on age, sex, profession, residence, duration of employment as hunter, hunting-ground, animals in the environment, and tick bite history were obtained by questionnaire.

In accordance with the MIQ 2000 [8], a two-step testing strategy was used in which sera were screened for anti-*B. burgdorferi* s.l. antibodies with a commercially available enzyme-linked immunosorbent assay (ELISA), and reactive sera were then subjected to Western blot testing for confirmation of specificity.

**ELISA**: serum samples from each hunter were extracted from blood by centrifugation and aliquots of the sample were frozen at –20 °C until examination. After thawing, the sera were screened with an anti-*Borrelia* recombinant ELISA (Biotest Anti-Borrelia ELISA, Biotest AG, Dreieich, Germany) for the presence of IgG antibodies against *B. burgdorferi* s.l. The borrelial antigens used in this assay are p100, OspC, p41 and p18 (Osp17). The p41 antigen is a fusion protein from the internal flagellin regions of two different *Borrelia* species, *B. afzelii* and *B. garinii*. All other antigens in this test originate from a *B. afzelii* strain [15].

An automated system (Minilyser, Tecan, Crailsheim, Germany) was used to prepare the dilution steps; all other steps were made by hand. The negative and positive controls were measured in duplicate with a spectrometer at 450 nm as for the test samples at a final dilution of 1:21. The cut-off value was calculated from the mean absorbance of the negative controls plus a factor of 0.250 for IgG.

If the absorbance of a sample was greater than or equal to the cut-off value, it was considered positive for IgG antibodies. For quantitative determination of antibodies against the borrelial antigens the optical density (OD) values were expressed as relative quantification units (RU/ml). These quantitative values are test-specific and their definition is based on a *Borrelia* ELISA-specific serum standard provided with the test kit. According to the manufacturer the specificity of the Biotest Anti-Borrelia IgG is 91%.

**WESTERN BLOT**: a commercial Western blot/immunoblot (recomBlot Borrelia IgG, Mikrogen GmbH, Martinsried, Germany) was used for confirmation of the specificity of positive ELISA results. This immunoblot uses the recombinant proteins OspA, OspC, p100, p39, p18 and the p41/internal fragment as antigens. A negative control and a weak positive control were included in each immunoblot. The weak positive control provided the cut-off and was used for checking the reactivity of the blot. A score system was used for measuring the intensity of each recombinant antigen, and the test strips for each serum sample were given a total score by adding up the scores for each band. The results were recorded on an evaluation form. A serum was considered positive if the total score was ≥6.

**Table 1.** Frequency of *B. burgdorferi* sensu lato IgG antibodies among male and female hunters in Burgenland in relation to age and duration of employment (mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seroprevalence</td>
<td>673 (54%)</td>
<td>663 (54%)</td>
<td>10 (26%)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>55 (±13)</td>
<td>55 (±13)</td>
<td>44 (±12)</td>
</tr>
<tr>
<td>Duration of employment (years)</td>
<td>27 (±13)</td>
<td>27 (±13)</td>
<td>13 (±12)</td>
</tr>
<tr>
<td>Negative Sera</td>
<td>580 (46%)</td>
<td>551 (46%)</td>
<td>29 (74%)</td>
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</tbody>
</table>

**Fig. 1.** Numbers of sera positive for *B. burgdorferi* sensu lato IgG antibodies by age group in hunters in Burgenland