

SEROPREVALENCE OF ANTI-*BORRELIA BURGDORFERI* ANTIBODIES IN SHEEP AND GOATS FROM MOUNTAINOUS AREAS OF SLOVAKIA

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Abstract: In the present study, domestic animals such as sheep and goats from eastern Slovakia were screened for the presence of anti-*Borrelia* antibodies. Seroprevalence in 181 sheep and 65 goats were carried out in 1999 and 2000. Modified ELISA method was used for detection of anti-*Borrelia* IgG antibodies. Seroprevalence obtained was 15.8% and 17.5% in 1999 and 2000 respectively in sheep, whereas in goats it was 17.2% and 19.4% respectively. The results suggest that these domestic species have potential to transmit the disease to other animals. Though the role of sheep and goats in Lyme disease has not yet been documented, there is great possibility of transmission of the causative agent via co-feeding to human beings.

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INTRODUCTION

Borrelia burgdorferi (Bb) is the most common tick-borne infection with worldwide distribution and occurs in domestic as well as in wild animals. In Europe, approximately 40 mammals and birds have been established as animal reservoir hosts [5]. This tick-borne disease is transmitted mainly by the *Ixodes ricinus* tick from the family *Ixodidae*. It has a zoonotic potential and causes Lyme disease in human beings, occasionally with serious consequences. In humans, Lyme borreliosis in its early stages is characterized by influenza-like symptoms, followed by erythema migrans in 60–80% cases [17]. The disease may proceed to neurological disorders and arthritis. In the case of small ruminants, the course of disease is chronic,

subclinical and sometimes without any pathological signs. Only exceptionally is it possible to observe lameness, arthritis with pain and persistent low fever.

Humair and Gern [10] suggested a specific association between *B. burgdorferi* sensu lato and vertebrate hosts. Domestic animals like sheep and goats are important hosts of borreliae. Positive levels of antibodies to Bb in sheep were detected in various countries: in Italy [3], Egypt [8], France [2], China [9, 13] and Turkey [20]. In the case of goats the antibodies to Bb was also confirmed in Italy [3, 4], Egypt [8], China [13, 14, 21] and Turkey [20].

Sheep are reported to feed all three stages of *I. ricinus*, i.e. larva, nymph and adults, and >20% of adult ticks are infected; yet, sheep do not appear to support systemic infection with *Borrelia burgdorferi*, but serve as a vehicle

Table 1. Anti-*Borrelia* IgG antibodies in sheep from eastern Slovakia.

Year	No. of examined animals	No. of positive % / Titres			Total
		1 : 200	1 : 400–800	1 : 1600	
1999	101	8 (7.9)	5 (4.9)	3 (3.0)	16 (15.8)
2000	80	6 (7.5)	3 (3.7)	5 (6.3)	14 (17.5)
Total	181	14 (7.7)	8 (4.4)	8 (4.4)	30 (16.5)

Table 2. Anti-*Borrelia* IgG antibodies in goats from eastern Slovakia.

Year	No. of examined animals	No. of positive % / Titres			Total
		1 : 200	1 : 400–800	1 : 1600	
1999	29	3 (10.3)	1 (3.4)	1 (3.4)	5 (17.2)
2000	36	4 (11.1)	2 (5.5)	1 (2.8)	7 (19.4)
Total	65	7 (10.7)	3 (4.6)	2 (3.1)	12 (18.4)

to transfer infection among co-feeding ticks [15]. *Borrelia* can be horizontally transmitted from all the stages of ticks to other vertebrate hosts. Nevertheless, possible transmission of *Borrelia* by other vectors is also reported. *Borreliae* were detected in larval [6], nymphal and adult stages of ticks [11, 16] as well as in other vectors such as mosquitoes [7]. The co-feeding might play an important way of horizontal transmission of *Borrelia burgdorferi* from ticks to hosts like sheep and goats to ticks and may pose a threat to transfer the disease to human being.

We tried to examine the antibody levels to *Borrelia burgdorferi* sensu lato in sheep and goats where human activity is maximum, environmental factors are favourable for the transmission of *Borrelia* and other competent reservoir hosts like rodents, and tick vectors are abundant. The study was conducted on the farms belonging to the university where the students, farmers and farm workers visit frequently.

MATERIALS AND METHODS

The study area consisted of grazing land, farms and small bushes adjacent to a forest. The average temperature in summer is $25 \pm 5^\circ\text{C}$ and $-5 \pm 5^\circ\text{C}$ in winter. Geographically, the area is situated 300 m above sea level. Tick population is noticeable in this area as the conditions are favourable for population growth of ticks. *Ixodes ricinus* is the major species of ticks found in this locality. The sheep and goats are confined on farms during the winter season, while in summer their activity is mainly out-of-doors on grazing land.

Blood serum samples of 181 sheep and 65 goats were collected during the years 1999–2000 and examined for the presence of anti *Borrelia* IgG antibodies. All investigated animals were from the farm located in the eastern part of Slovakia.

Preparation of antigen. Whole cell sonicated antigen was prepared from *B. garinii* (Ir 112, local strain isolated from *Ixodes ricinus* in Kosice). For strain identification SDS-PAGE electrophoresis and immunoblotting with monoclonal antibodies were used. *Borrelia* was cultivated in BSK II medium (sigma) at 7 day intervals. The culture was centrifuged at 10,000 g for 30 min., washed 3 times in PBS (pH 7.2 with 5 mM MgCl_2) and sonicated at 20 KHz for 3 min. on ice with 20–30 W (sonic Dismembrator, Dynatech. UK). The aliquot was again centrifuged at 10,000 g for 30 min. The supernatant was used as an antigen.

ELISA. The sera of sheep and goats were examined by a modified ELISA method [18] as follows:

Microplates were filled with 100 μl antigen diluted in carbonate buffer at pH 9.6 (5 $\mu\text{g}/\text{ml}$) and incubated overnight at 4°C . After washing 3 times with phosphate buffer (pH 7.2) 100 μl of sera diluted at 1 : 200 in phosphate buffer with 0.05% tween and 1% BSA was added to each well and incubated at 37°C for 30 min. After triple washings, 100 μl of anti goat anti sheep IgG peroxidase conjugate (Sigma) was added per well, diluted at 1:1000. After 30 min. incubation and a subsequent washing, 100 μl of substrate solution (pH 5.0) with orthophenylene diamine was added per well. The reaction was stopped with 5% H_2SO_4 after 15 min. incubation. Absorbance was measured at a wavelength of 492 nm.

Positive controls were considered by repeated titration of serum samples belonging to animals from a geographical area with known high occurrence of infected ticks. For negative control, we obtained serum samples from young laboratory sheep and goats which had no contact with ticks. Considering previous experience with checker board titration we established the positive titres of anti-*Borrelia* antibodies (1 : 200 to 1 : 1600) on the basis of absorbance value.

Cut-off was determined as a value of 3 Standard deviations above the mean optical density (OD) for negative control serum samples. The reproducibility of ELISA: Panel sera samples were repeatedly examined (10 times) with the absorbance value in the ranges: <1.2 ; $0.5\text{--}0.8$; >0.4 .

ELISA reproducibility: Coefficient of the absorbance variation in all tested sera did not exceed 10%.

RESULTS AND DISCUSSION

Recent studies have shown that co-feeding in sheep has important epizootological significance, but the actual phenomenon of the transmission of Bb in the process is not yet clear. Sheep may not become actually infected with Bb, but allows co-feeding infection to take place, since sheep complement is partially borreliacidal [12]. The studies of Ogden *et al.* [15] on sheep suggest that nymphal ticks may become infected as a result of co-feeding.

According to Gern *et al.* [5], sheep (*Ovis aries*) is a species which does not infect feeding ticks with Bb, i.e. the sheep is not reservoir competent. Furthermore, they added that negative results do not necessarily exclude a species as having a role in infecting ticks.

In our study, the seroprevalences observed in sheep in 1999 (15.8%), and 2000 (17.5%) are comparable with the results of other authors (Tab. 1) and with the seroprevalence detected in other countries, viz. Italy - 14.1% of positivity [3], Turkey – 22.1% [20], Egypt – 23.8% [8] and China – 22.5% [9] and 15.8% [13].

The serological evidences of IgG antibodies in goats were 17.2% (1999) and 19.4% (2000) and total positivity was 18.4%. These seroprevalences are in accordance with the results published by other authors. In Italy, Ciceroni *et al.*, in one study, obtained 5% of prevalence [4] and in another 36.8% [3]. In Egypt, Helmy [8] confirmed 18.0% of prevalence and in China the prevalence was 22.2% [21], 19.1% [14], 20.3% [13]. The positivity obtained by Zhang *et al.*, [21] in the plain areas was 22.2%, but in the mountain district the positivity was 61.8%. They further stated that Lyme disease was endemic primarily in mountain areas, especially in goats, which may serve as a reservoir host for the disease. At this moment it is not possible to say if the goat has reservoir competence or not. In the review article published by Gern *et al.* [5], the role of goats as a possible reservoir host for Bb was not described.

In the epidemiology of Lyme disease the role of tick vectors is important. The species of ticks present in a particular area governs the maintenance of Lyme disease. The ticks are of different species in European countries from those in China. In Europe, the role of *Ixodes ricinus* is very well documented and confirmed. Apart from *Ixodes ricinus* ticks, Bb has also been detected in *Hemaphysalis punctata* in Europe [1]. It is not only necessary to consider the other species of ticks, but also other vectors such as mosquitoes and fleas should not be neglected in the ecology and transmission of borreliosis. Reports from Slovakia elaborate that infected *Ixodes ricinus*, a dominant tick species, was collected mainly in lowland habitats of oak woods with furze, grass and other small bushes, similar to our study area.

Total positivity of anti-*Borrelia* antibodies in sheep (16.5%) and goats (18.4%) signals the presence of *Borrelia* in these domestic animals. These domestic species may pose a threat to human beings by acting as hosts to Bb, especially by the means of co-feeding. The importance of different species of small mammals in the epidemiology of borreliosis is well known. In eastern Slovakia we have confirmed anti *Borrelia* IgG antibodies in game animals and small mammals [19], as well as the presence of all stages (larvae, nymph, adults) of tick, *Ixodes ricinus*. Reviewing all data on vectors, seropositivity in small mammals and rodents, favorable ecology of this region for Lyme disease transmission, and the sentinel nature of sheep and goats for Bb, we suggest that circulations of borreliae may occur in populations of sheep and goats and further intensive study is required to determine the exact role of these animal species in the transmission of Bb spirochetes to other mammalian hosts.

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