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# Immunoglobulin G antibodies to *Borrelia burgdorferi* in game animals and small mammals in eastern Slovakia

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## Summary

In a survey of game animals and small mammals, the sera of 185 animals were examined for the presence of immunoglobulin G antibodies to the spirochete *Borrelia burgdorferi* sensu lato, which causes Lyme borreliosis. These animals comprised 59 fallow deer (*Dama dama*), 56 mouflons (*Ovis musimon*) and 70 small mammals of six different species. The sera of the fallow deer and the mouflons were examined by indirect haemagglutination assay. The sera of the small mammals were examined by modified enzyme-linked immunosorbent assay, which is available in a commercial kit. The sera of the 59 fallow deer demonstrated positivity of 40.77% (titres 1:40-1:80). The 56 mouflons demonstrated seropositivity of 17.8% (1:40-1:80). The sera of the small mammals were highly positive in the yellow-necked field mouse (*Apodemus flavicollis*) at 42.1% (titres 1:200-1:1,600), followed by the bank vole (*Clethrionomys glareolus*) at 14.3% (1:400-1:800), the common vole (*Microtus arvalis*) at 12.5% (1:200) and the black-striped field mouse (*A. agrarius*) at 10.0% (1:200-1:400-800).

The authors also report the rate of infestation of these small mammals by the tick *Ixodes ricinus*, as these mammal species are potential reservoirs for this vector. The study focuses on the relationship between the possibility of infestation by *I. ricinus* and the reservoir competence of the different species under study, as well as the possible spread of disease. The detected rate of seroprevalence indicates that all the investigated animals have had contact with infected ticks.

## Keywords

*Borrelia burgdorferi* – Enzyme-linked immunosorbent assay – Fallow deer – Indirect haemagglutination assay – *Ixodes ricinus* – Lyme borreliosis – Mouflons – Small mammals.

## Introduction

Lyme borreliosis (Lb), caused by the spirochete *Borrelia burgdorferi*, is the most common tick-transmitted disease of world-wide distribution. In central and western Europe, *Ixodes ricinus* ticks are the most important vectors for Lb. In some areas, there seems to be a specific association (10, 12) between *B. burgdorferi* sensu lato (s.l.) and vertebrate hosts, although the same tick vector, *I. ricinus*, occurs in all species. In Sweden, *I. ricinus* is the primary overwintering reservoir for

*B. burgdorferi*, rather than the bank vole, *Clethrionomys glareolus* (21). Specific antibodies to *B. burgdorferi* were detected in the sera of pheasants, roe deer (*Capreolus capreolus*), mouflons (*Ovis musimon*), red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and white-tailed deer (*Odocoileus virginianus*) (4, 13, 14, 15, 17, 22), as well as in other animals (1, 23).

Small mammals play an important role in the epizootiology of Lb among wild animals. Ticks of the genus *Ixodes* are the most important vectors for *B. burgdorferi* s.l. in central Europe. In the

Slovak Republic, the highest prevalences of *B. burgdorferi* s.l. in *I. ricinus* varied between 4.8% and 49% (3, 20). The isolation of *B. burgdorferi* from adult *I. ricinus* in the Czech Republic (7), from the larval (5), nymph and adult stages of ticks (8, 19) and mosquitoes (6), has been reported in southern Moravia.

This paper presents the results of a serological survey of mouflons, fallow deer and small mammals from an area of eastern Slovakia.

## Materials and methods

Samples of the blood sera of 59 fallow deer, 56 mouflons and 70 small mammals were examined for the presence of immunoglobulin G (IgG) antibodies to the spirochete *B. burgdorferi*. All the animals examined came from the same wooded area of 500 hectares (ha) in eastern Slovakia.

The sera from the fallow deer and mouflons were examined by indirect haemagglutination assay (IHA) with the *Borrelia* haemagglutination assay test. Rehydrated tanned sheep erythrocytes fixed in glutaraldehyde, bound with purified antigens from European/American *B. burgdorferi* strains, were used as test erythrocytes and non-sensitised sheep erythrocytes fixed in glutaraldehyde served as control erythrocytes. Titres of 1:40 and higher were taken as positive.

The sera of small mammals were examined by modified enzyme-linked immunosorbent assay (ELISA), which is available in commercial kits and used to diagnose Lb in humans (where it is known as Lyme disease). The method was as follows: 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 the wells three times with phosphate buffer (pH 7.2), 100 µl aliquots of sera were diluted at 1:200 in phosphate buffer with 0.05% tween. Tween and 1% bovine serum albumin were added to each well and incubated at 37°C for 30 minutes. After a triple washing, 100 µl of anti-mouse IgG peroxidase conjugate was added per well, diluted at 1:1,000. After 30 minutes of incubation and one subsequent washing, 100 µl of substrate solution (pH 5.0) with orthophenylene diamine was added per well. The reaction was stopped with 5% H<sub>2</sub>SO<sub>4</sub> after 15 minutes of incubation. Absorbance was measured at a wavelength of 492 nanometres. Mouse sera which had proved positive in repeated titrations were used as positive controls. Mouse sera which proved negative in repeated titrations, with an absorbance value of less than 0.4, served as negative controls. The cut-off point was determined as a value of three standard deviations above the mean optical density for negative serum samples. Sera with an absorbance value of higher than 0.5 were evaluated as positive.

In terms of the reproducibility of the ELISA, panel sera samples were repeatedly examined (10 times) with absorbance values in the ranges: > 1.2; 0.5-0.8; < 0.4.

## Results

The sera of the 59 fallow deer showed a total seropositivity of 40.7%. Among these sera, 13 samples demonstrated positivity at a titre of 1:40, whereas 11 sera samples showed positivity at 1:80. The sera of the 56 mouflons demonstrated a total seropositivity of 17.8%. Among these samples, sera from 6 animals showed positivity at a titre of 1:40, while sera from 4 animals showed positivity at a titre of 1:80 (Table I).

**Table I**  
Immunoglobulin G antibodies to *Borrelia burgdorferi* found in fallow deer and mouflons in eastern Slovakia in the years 1998 to 2000

| Species             | Year | Number of animals examined | Number of animals giving positive results (%) |                   |                   |
|---------------------|------|----------------------------|---|-------------------|-------------------|
|                     |      |                            | Titre   | 1:40              | 1:80              |
| <i>Dama dama</i>    | 1998 | 24                         | 6 (25.0)                                      | 5 (20.8)          | 11 (45.8%)        |
|                     | 1999 | 15                         | 4 (26.6)                                      | 3 (20.0)          | 7 (46.6%)         |
|                     | 2000 | 20                         | 3 (15.0)                                      | 3 (15.0)          | 6 (30.0%)         |
| <b>Total</b>        |      | <b>59</b>                  | <b>13 (22.0%)</b>                             | <b>11 (18.6%)</b> | <b>24 (40.7%)</b> |
| <i>Ovis musimon</i> | 1998 | 37                         | 2 (5.4)                                       | 2 (5.4)           | 4 (40.7)          |
|                     | 1999 | 2                          | 1 (50.0)                                      | –                 | 1 (50.0%)         |
|                     | 2000 | 17                         | 3 (17.6)                                      | 2 (11.7)          | 5 (29.4%)         |
| <b>Total</b>        |      | <b>56</b>                  | <b>6 (10.7%)</b>                              | <b>4 (7.1%)</b>   | <b>10 (17.9%)</b> |

Antibodies to *Borrelia* were detected in 13 of 70 small mammals which were examined in the study (18.6%) (Table II). The highest seroprevalence was found in yellow-necked mice (*Apodemus flavicollis*): in 42.1% of the sampled animals; followed by bank voles (*Clethrionomys glareolus*): 14.3%; common voles (*Microtus arvalis*): 12.5%; and striped field mice (*A. agrarius*): 10.0%. All sera from the house mice (*Mus musculus*) and pygmy field mice (*A. microps*) which were tested were found to be negative.

**Table II**  
Immunoglobulin G antibodies to *Borrelia burgdorferi* found in small mammals in eastern Slovakia in 2000

| Species                        | Number of animals examined | Number of animals giving positive results (%) |                |                |                  |
|--------------------------------|----------------------------|---|----------------|----------------|------------------|
|                                |                            | Titre   | 1:200          | 1:400-800      | 1:1,600          |
| <i>Apodemus flavicollis</i>    | 19                         | 5 (26.3)                                      | 2 (10.5)       | 1 (5.3)        | 8 (42.1)         |
| <i>Apodemus agrarius</i>       | 30                         | 2 (6.7)                                       | 1 (3.3)        | 0              | 3 (10.0)         |
| <i>Apodemus microps</i>        | 5                          | 0   | 0              | 0              | 0                |
| <i>Microtus arvalis</i>        | 8                          | 1 (12.5)                                      | 0              | 0              | 1 (12.5)         |
| <i>Clethrionomys glareolus</i> | 7                          | 0   | 1 (14.3)       | 0              | 1 (14.3)         |
| <i>Mus musculus</i>            | 1                          | 0   | 0              | 0              | 0                |
| <b>Total</b>                   | <b>70</b>                  | <b>8 (11.4)</b>                               | <b>4 (5.7)</b> | <b>1 (1.4)</b> | <b>13 (18.6)</b> |

The reproducibility of the ELISA was good: the co-efficient of absorbance variation in all the sera tested did not exceed 10%.

The mean intensity of infestation of *I. ricinus* and *I. trianguliceps* collected from small mammals was highest in the case of *A. flavicollis* (12.3), followed by the species *C. glareolus* (9.5), *A. agrarius* (3.0) and *M. arvalis* (1.0) (16). No ticks were observed on *A. microps* and *Mus musculus* (Table III).

## Discussion

The tested fallow deer and mouflons came from one area (Rozhanovce) in eastern Slovakia, enclosed by fences, with a surface area of 500 ha. This biotope is characterised by woods with bushes, meadows and valleys with springs. This area is situated at 550 metres (m) to 650 m above sea level and the geographical orientation is south west. The climate is the typical continental climate, with temperatures reaching 30°C to 34°C in summer, and from -15°C to -20°C in the most extreme winter conditions. The number of large mammals living in this area oscillates between 260 and 360 during the year. Approximately two thirds of these large mammals are fallow deer and the remaining third are mouflons. Because of the high density of fallow deer and mouflons in this area, these animals are partially fed by government workers, particularly during winter, but from April to November there is sufficient naturally occurring food available. The main reasons for maintaining these animals are as follows:

- repopulation of hunting areas
- export

- for teaching purposes
- hunting.

The fallow deer and mouflons are kept separate from predators, e.g. wolves and dogs, as well as from large ungulates, e.g. roe deer (*C. capreolus*). However, they are not separated from other smaller wild animals. Thus, their contact with birds and small mammals is uninterrupted.

As *B. burgdorferi* antibodies were detected in fallow deer and mouflons in the year 1998 (in 45.8% and 10.7%, respectively) and in 1999 (46.0% and 50.0%), the authors decided to include a serosurvey of small mammals in 2000. Small mammals are recognised as an important reservoir for *B. burgdorferi* (2). Such small mammals are mainly parasitised by larvae and nymphs, whereas large game animals are parasitised by adult ticks. Free-living large game animals are the principal source of blood for *I. ricinus* ticks. However, according to some authors (2), fallow deer are not competent reservoirs of *B. burgdorferi*, that is, these hosts are not able to infect engorging ticks. In Denmark, specific antibodies to *Borrelia* were detected in 30% of fallow deer (23), while others have found 46.2% positivity in this species (2). In the Czech Republic, antibodies against *B. burgdorferi* were detected in 50.7% of tested fallow deer, using IHA (14). The results obtained by the authors, of positivity in 40.7% of fallow deer, are comparable with those results mentioned above.

In this study, the seroprevalence in large mammals, that is, in fallow deer and mouflons, was found to be consistently higher than that in small mammals (Tables I and II). This may be due to the fact that small mammals are the principal hosts for larvae,

**Table III**  
Infestation of small mammals by the tick species *Ixodes ricinus* and *I. trianguliceps* in eastern Slovakia in 2000

| Species                        | Number of animals examined | Number of animals giving positive results | Total number of ticks found on small mammals   | Mean number of ticks per animal |
|--------------------------------|----------------------------|---|--|---------------------------------|
| <i>Apodemus flavicollis</i>    | 19                         | 12  | <i>I. ricinus</i><br>- larvae (141)<br>- nymphs (6)<br><i>I. trianguliceps</i><br>- nymphs (1) | 12.3                            |
| <i>Apodemus agrarius</i>       | 30                         | 12  | <i>I. ricinus</i><br>- larvae (27)<br>- nymphs (7)<br><i>I. trianguliceps</i><br>- larvae (2)  | 3.0                             |
| <i>Apodemus microps</i>        | 5                          | 0   | None   |                                 |
| <i>Microtus arvalis</i>        | 8                          | 2   | <i>I. ricinus</i><br>- larvae (2)  | 1.0                             |
| <i>Clethrionomys glareolus</i> | 7                          | 6   | <i>I. ricinus</i><br>- larvae (56)<br>- nymphs (1)   | 9.5                             |
| <i>Mus musculus</i>            | 1                          | 0   | None   |                                 |

which are usually not infected as unfed ticks. The frequency of transovarian transmission of *B. burgdorferi* from the adult female tick to the larval offspring is very low (2). Thus, larvae are usually unable to infect small mammals. In contrast, large mammals are the main hosts for the nymphs and adults. These stages have a higher infection rate than that of larvae, which means that contacts between large mammals with infected ticks (nymphs and adults) are more frequent than contacts of small mammals with infected ticks (larvae).

According to Gern *et al.*, the mouflon is not a competent reservoir host for *B. burgdorferi* (2). In one study, conducted in the Czech Republic, seropositivity was detected in 37.5% of mouflons, whereas, in central Bohemia, seropositivity was detected in 76.5% of the tested animals (24). In this study of eastern Slovakia, the authors detected seropositivity in 17.8%.

In this region, infection of *I. ricinus* by *B. burgdorferi* oscillates between 4.8% and 28.4% (20). Small mammals are known to be reservoir hosts. According to Gern *et al.* (2), the following small mammals are strong reservoirs for *B. burgdorferi* s.l.:

- *A. flavicollis*
- *C. glareolus*
- *A. agrarius*.

In another study, Humair *et al.* (11) have described differential transmission of *B. afzelii* from *Apodemus* mice and *Clethrionomys* voles to *I. ricinus* ticks. In the central part of the Carpathian region of Slovakia, a higher rate of infestation by *I. ricinus* was found in *A. flavicollis* (1880/1488) (18) than in *C. glareolus* (1054/450). The *Apodemus* sp. belongs to the group of rodents (mice) with a big home range, so these animals have more chance of coming into contact with *I. ricinus*. On the other hand, the *Clethrionomys* sp. belongs to the group of rodents (voles) which live mostly underground. These animals have a small home range, so there is less possibility of them coming into contact with ticks such as *I. ricinus*. In the group of small mammals under study (Table III), the mean intensity of infestation detected in *A. flavicollis* was 12.3, which was a higher rate of infestation than that found in *C. glareolus* (9.5).

The seroprevalence in *A. flavicollis* was found to be much higher than that in other species of small mammals under study. The level of seroprevalence in *Apodemus* sp. is different from that found in *Clethrionomys*. This may be because there is a lower degree of tick infestation in *Clethrionomys* than in *Apodemus* sp.,

as observed in this study. It may also be the case that *Clethrionomys* has more resistance to ticks (9).

There is a relative difference, in terms of reservoir competence, between the presence of antibodies against *B. burgdorferi* in game animals and their presence in small mammals. During its ontogenic development, i.e., during the progression from the larval stage to the adult stage, *I. ricinus* changes its hosts from competent reservoir hosts to non-competent reservoir hosts. Larvae, which feed principally on small mammals, become highly infected. Nymphs and adult ticks, which feed mostly on large mammals (i.e., in this particular study, on fallow deer and mouflons), lose their infectivity as they change from competent reservoir hosts (i.e., small mammals) to non-competent hosts (i.e., large mammals). This suggests a possible explanation for the dynamics and equilibrium of tick infectivity.

In analysing the ecology of *B. burgdorferi* in the particular biotope examined in this study, the authors suggest that the circulation of *B. burgdorferi* is possible because of the high density of competent reservoir hosts (small mammals) and, moreover, because of the high density of large animals which act as food resources for the ticks. Thus, the levels of seroprevalence detected in this study indicate that the animals under investigation have had contact with infected ticks.

This study demonstrates the significance of small mammals in the ecology of *B. burgdorferi*, not only because they are present but also because of the intensity of their infestation by *I. ricinus*. Moreover, the presence of large competent, as well as non-competent, reservoir hosts is equally significant as these hosts feed the ticks during their various life stages.

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## Preuve de la présence d'immunoglobulines G spécifiques de *Borrelia burgdorferi* dans le sérum du gibier et des petits mammifères de l'Est de la Slovaquie

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### Résumé

Le sérum de 185 animaux a été analysé dans le cadre d'une enquête sur le gibier et les petits mammifères, destinée à dépister la présence éventuelle d'anticorps spécifiques constitués d'immunoglobulines G et dirigés contre le spirochète *Borrelia burgdorferi*, *sensu lato*. Ces animaux comprenaient 59 daims (*Dama dama*), 56 mouflons (*Ovis musimon*) et 70 petits mammifères de six espèces différentes. Alors que le sérum prélevé sur les daims et les mouflons a été analysé par hémagglutination indirecte, celui des petits mammifères a été soumis à un dosage immuno-enzymatique par une méthode modifiée, réalisable avec une trousse commerciale. Les sérums des 59 daims possédaient des anticorps spécifiques dans 40,77 % des cas (titres de 1:40 à 1:80) et ceux des 56 mouflons dans 17,8 % des cas (1:40 à 1:80). Les sérums des petits mammifères possédaient des anticorps dans une proportion particulièrement élevée avec des valeurs de 42,1 % (titres de 1:200 à 1:1 600) pour le mulot à collier (*Apodemus flavicollis*), 14,3 % (1:400-1:800) pour le campagnol roussâtre (*Clethrionomys glareolus*), 12,5 % (1:200) pour le campagnol des champs (*Microtus arvalis*) et 10,0 % (1:200-1:400-800) pour le mulot rayé (*A. agrarius*).

En outre, les auteurs précisent le taux d'infestation de ces petits mammifères par la tique vectrice *Ixodes ricinus*, dans la mesure où cette dernière peut utiliser ces espèces mammifères comme hôtes réservoirs. L'étude est consacrée plus spécifiquement à la relation existant entre l'éventualité d'une infestation par *I. ricinus* et l'aptitude des espèces concernées à servir d'hôtes réservoirs, ainsi qu'aux possibilités de propagation de la maladie. Les taux de prévalence des anticorps observés indiquent que tous les animaux de l'étude ont été en contact avec des tiques infectées.

### Mots-clés

*Borrelia burgdorferi* – Daim – *Ixodes ricinus* – Méthode immuno-enzymatique – Mouflon – Petit mammifère – Test d'hémagglutination indirecte.



## Pruebas serológicas de la presencia de inmunoglobulinas G contra *Borrelia burgdorferi* en caza menor y pequeños mamíferos de Eslovaquia oriental

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### Resumen

Los autores describen un estudio en el que se analizaron muestras séricas de 185 animales de caza menor y pequeños mamíferos para detectar en ellas la presencia de inmunoglobulinas G específicas de la espiroqueta *Borrelia burgdorferi sensu lato*. Compañían la muestra de animales 59 gamos (*Dama*



dama), 56 muflones (*Ovis musimon*) y 70 pequeños mamíferos de seis especies distintas. El suero de gamo y de muflón se analizó por hemaglutinación indirecta, y el de los pequeños mamíferos con un ensayo inmunoenzimático (ELISA) modificado que está comercializado. Los porcentajes de seropositividad fueron de un 40,77% (títulos 1:40 a 1:80) en las 59 muestras de gamo y de un 17,8% (1:40 a 1:80) en el caso de los 56 muflones. En cuanto a los sueros de pequeños mamíferos, los porcentajes más elevados fueron, por orden decreciente: 42,1% (títulos 1:200 a 1:1.600) en el ratón leonado (*Apodemus flavicollis*); 14,3% (1:400 a 1:800) en el topillo rojo (*Clethrionomys glareolus*); 12,5% (1:200) en el topillo de campo (*Microtus arvalis*); y 10,0% (1:200 a 1:400-800) en el ratón de campo rayado (*A. agrarius*).

Los autores se refieren también a la tasa de infestación por la garrapata *Ixodes ricinus* que se observó en esos roedores, pues todos ellos son reservorios en potencia de dicho vector. El estudio se centra en la relación entre la posibilidad de infestación por *I. ricinus* y la capacidad de esas distintas especies para actuar de reservorio, así como la posibilidad de diseminación de la enfermedad. La tasa de seroprevalencia obtenida pone de manifiesto que todos los animales estudiados habían estado en contacto con garrapatas infectadas.

#### Palabras clave

*Borrelia burgdorferi* – Ensayo inmunoenzimático – Gamo – *Ixodes ricinus* – Muflón – Pequeño mamífero – Prueba de hemaglutinación indirecta.

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