SEROPREVALENCE OF *BORRELIA BURGDORFERI* SENSU LATO IN HORSES IN FLANDERS

Seroprevalentie van Borrelia burgdorferi *sensu lato bij paarden in Vlaanderen*

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ABSTRACT

Serum samples from 100 horses in Flanders with no clinical evidence of Lyme disease were examined for the presence of IgG antibodies against *Borrelia burgdorferi* sensu lato using an indirect ELISA. In 36 of the samples antibodies against this bacterium were detected, indicating that infections with *B. burgdorferi* sl regularly occur in horses in Flanders. These findings also indicate the potential exposure of humans to this spirochete in this region.

SAMENVATTING

Serumstalen van 100 paarden in Vlaanderen die geen klinische symptomen vertoonden van de ziekte van Lyme, werden onderzocht op de aanwezigheid van IgG-antistoffen tegenover *Borrelia burgdorferi* sensu lato met behulp van een indirecte ELISA. In 36 stalen werden antistoffen tegenover deze kiem aangetoond, wat erop wijst dat infecties met *B. burgdorferi* sl geregeld voorkomen bij paarden in Vlaanderen. Dit wijst ook op een mogelijke blootstelling van de mens in deze streek aan de spirocheet.

INTRODUCTION

Borrelia burgdorferi sensu lato, a spirochete which is transmitted by ticks, is responsible for Lyme borreliosis in humans and animals (Johnson *et al.*, 1984; Steere *et al.*, 2004). *B. burgdorferi* sl comprises several genospecies, but hitherto only *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* have been isolated from human patients suffering from Lyme borreliosis (Piesman and Gern, 2004). In horses, Lyme borreliosis is a multisystemic disease with diverse manifestations (Parker and White, 1992).

Lyme borreliosis or serological evidence of *B. burgdorferi* sl infections in horses have been reported mainly in the United States (Marcus *et al.*, 1985; Magnarelli and Anderson, 1989; Bernard *et al.*, 1990; Magnarelli *et al.*, 2000), Sweden (Egenvall *et al.*, 2001), Germany (Käsbohrer and Schönberg, 1990; Gerhards and Wollanke, 1996; Liebisch *et al.*, 1999), France (Trap, 1990) and the United Kingdom (Carter *et al.*, 1994). No data are available on the occurrence of these infections in Belgium. Therefore, in the present study, sera of horses living in Flanders were tested for the presence of antibodies to *B. burgdorferi* sl.

MATERIALS AND METHODS

Serum samples from 100 horses in Flanders (Belgium) with no clinical evidence of Lyme borreliosis were tested for the presence of IgG antibodies against *B. burgdorferi* sl at the Zecklab reference laboratory for tick-born infections (Zecklab, Burgwedel, Germany). The ages of the horses varied between one day and 30 years. Fifty-six horses were five years or older, 40 horses were younger than five years, and for four of the sampled horses the age was unknown. Fifty-four mares, 16 stallions

and 24 geldings were included in the survey. For six of the sampled horses, the sex was not registered. Twenty-five samples were collected during January, February or March, 45 samples during April, May or June, 19 samples during July, August or September, and 11 samples during October, November or December. A modified commercial indirect ELISA for the diagnosis of Lyme borreliosis in dogs was used (recomWell IgG Canis, Mikrogen GmbH, Neuried, Germany). Samples had been randomly selected from a collection of 286 sera from horses that visited the Faculty of Veterinary Medicine between December 2002 and September 2005. ELISA plates were coated with a mixture of antigens p100, OspC, VlsE and p18 from *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii* produced in recombinant *Eschericha coli*. As secondary antibody, peroxidase labelled anti-horse IgG produced in goats (Kirkgaard and Perry Laboratories, Gaithersburg, Maryland USA) was used. H_2O_2 and tetramethylbenzidin (Mikrogen) were used as substrate and chromogen, respectively. The absorbance was measured at 450 nm. Serum samples from non-infected and *B. burgdorferi* sl-infected horses (Liebisch *et al.*, 1999) were used as negative and positive controls, respectively. According to Zecklab, the sensitivity and specificity of this test assay is 100%.

To verify whether there was a correlation between seroprevalence, on the one hand, and age or sex of the sampled horses or period of sample collection, on the other hand, data were compared using the chi-square test. Differences with p-value below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Of the 100 serum samples tested, 36 were positive. *B. burgdorferi* sl shares several antigens with other bacteria. Therefore, whole-cell *B. burgdorferi* sl ELISA's are less suitable in sero-surveys since cross-reactivity can occur with spirochetal flagellin antibodies (e.g. from *Leptospira* spp.) or even with antibodies to other antigens shared among unrelated bacteria such as *E. coli* (Hansen *et al.*, 1988; Magnarelli *et al.*, 2000). In the present study, the ELISA plates were coated with *B. burgdorferi* sl specific p100, OspC, VlsE and p18 antigens produced in recombinant *E. coli,* which considerably enhances the specificity of the test assay (Hunfeld *et al.*, 2002; Wilske, 2005).

An analysis of seroprevalence by age showed that the seroprevalence of *B. burgdorferi* sl was significantly ($p = 0.01$) higher in older horses (age > 5 years) compared to younger ones, which corresponds to previously reported results in dogs (Pejchalová et al., 2006). No correlation was found between the sex of the horses or the period of sampling, on the one hand, and the seroprevalence, on the other.

Although the number of samples tested in the present study is rather low and a seroprevalence of 36% may be an overestimation, the results indicate that infections with *B. burgdorferi* sl regularly occur in horses in Flanders. It also indicates the potential exposure of humans to this spirochete in Flanders. Most probably, horses are at greater risk than humans. Prompt removal of any feeding ticks reduces the risk of infection because, in most cases, between 24 and 48 hours is required for the transfer of *B. burgdorferi* sl (Kahl, 1998). The detection of ticks on horses, however, is more difficult than on humans (Trap, 1990).

The seroprevalence found in the present study is in the range of previously determined prevalences in horses in Germany (Käsbohrer and Schönberg, 1990; Gerhards and Wollanke, 1996; Liebisch *et al.*, 1999), France (Trap, 1990) and the UK (Carter *et al.*, 1994) which were nearly 50%, 16.7-36.7% and 3-37%, respectively. Lower seroprevalence was reported from Sweden, where Egenvall and colleagues (2001) detected antibodies to *B. burgdorferi* sl in 6.8% of sera from horses.

Although many infections with *B. burgdorferi* sl most probably pass subclinically, this agent has been associated with diverse disease manifestations in horses, including chronic weight loss, arthritis, laminitis, lameness, low grade fever, muscle tenderness and anterior uveitis (Parker and White, 1992). Clinicians should consider Lyme borreliosis in their differential diagnosis if horses are presented with these disease signs, especially when there is a history of tick exposure. Demonstration of *B. burgdorferi*-specific antibodies in sera of suspected horses represents the easiest method for confirming a *B. burgdorferi* sl infection. One must, however, be aware of the risk of overdiagnosis of Lyme disease. IgG antibodies remain detectable for several months or even years after infection (Liebisch *et al.*, 1999; Steere, 2001). Seropositivity from a single sample, therefore, does not allow differentiation between past and present exposure, and the examination of paired serum samples may be more appropriate. The desired collection interval depends on the stage of the infection. Preferably, the first sample should be collected as early as possible in the course of the disease, and the second sample three to four weeks later. It has indeed been shown that IgG antibodies are detectable from three weeks onwards after infection of horses with *B. burgdorferi* sl (Liebisch *et al.*, 1999). However, since the initial clinical symptoms are often aspecific, true seroconversion is rarely demonstrated because of delayed sampling.

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Uit het verleden

KATTENJACHT IN DE STRATEN VAN GENT

Volgens de 'Gazette van Gend' werden er in de winter van 1837 – 1838 drieduizend katten gestroopt te Gent. Dit getal is gebaseerd op de verklaringen van vellenverkopers die verder beweerden dat de huid van katten van betere kwaliteit was in de winter. Hun vlees werd als lekker bestempeld.

Uit: 'Ghendtsche Tydinghen', jaargang 28 (1999), p. 107

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