

SEROPREVALENCE OF *BORRELIA BURGdorFERI* SENSU LATO IN WILD RABBITS IN FLANDERS

*Seroprevalentie van Borrelia burgdorferi sensu lato
bij wilde konijnen in Vlaanderen*

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ABSTRACT

The number of cases of Lyme disease in humans in Belgium has been steadily increasing. Rabbits might represent a source of human borreliosis due to the fact that they are often infested with ticks. It was therefore the aim of the present study to estimate the seroprevalence of *Borrelia* infections in rabbits in Belgium. For this purpose blood samples from 103 wild rabbits were examined for the presence of antibodies using ELISA. Antibodies against *Borrelia* were detected in 8.7% of the samples, yielding an estimated prevalence of 2.3%. The low prevalence suggests that rabbits play a minor role in the epidemiology and transmission of Lyme disease from animals to man in Flanders.

SAMENVATTING

Het aantal gevallen van de ziekte van Lyme in België neemt de laatste jaren gestaag toe. Omdat konijnen zeer frequent teken dragen, kunnen ze een bron van infectie van *Borrelia burgdorferi* sl voor mensen zijn. Het was daarom het doel van deze studie om de seroprevalentie van *Borrelia*-infecties bij konijnen te schatten. Hiertoe werden bloedstalen van 103 wilde konijnen onderzocht op de aanwezigheid van antistoffen met behulp van ELISA. Antistoffen tegen *Borrelia* werden in 8,7% van de stalen teruggevonden, wat een geschatte prevalentie van 2,3% oplevert. Deze lage prevalentie suggereert dat konijnen slechts een beperkte rol spelen in de epidemiologie en de overdracht van de ziekte van Lyme van dieren naar de mens in Vlaanderen.

Lyme disease is the most prevalent human tick-borne disease in Europe (White, 1998). The number of cases of Lyme disease has been steadily increasing in Belgium from 42 diagnosed cases in 1991 to 975 cases in 2002 (Scientific Institute of Public Health, Brussels). The disease is caused by the spirochete *Borrelia burgdorferi sensu lato* (sl). These bacteria are transmitted by ticks belonging to the genus *Ixodes* (White, 1998). Medium-sized mammals such as rabbits are important for the maintenance of *Ixodes* populations (Juntilla *et al.*, 1999). Rabbit-feeding *Ixodes* in turn account for the enzootic presence of *Borrelia* in the rabbit population (Telford and Spielman, 1989). Due to the fact that they are often infested with

ticks and their potential role as *Borrelia* reservoir, rabbits could represent a source of human borreliosis. The aim of the present study was to estimate the seroprevalence of *Borrelia* infections in rabbits in order to evaluate the public health risk caused by *B. burgdorferi* sl. infected rabbits.

In the winter of 2002-2003, blood samples of 103 wild rabbits belonging to both sexes were collected on heparin in the Belgian provinces of Brabant, East Flanders, West Flanders and Antwerp. Blood samples derived from SPF dogs and from ten SPF rabbits and fifty domestic rabbits were used as negative controls. Serum from a rabbit immunized with the *Borrelia* an-

tigens (flagel and p41 antigen) used in the ELISA test was used as positive control. After centrifugation, the sera were stored at -70°C until examination for the presence of antibodies against *B. burgdorferi* sl using an indirect ELISA at the European Veterinary Laboratory (EVL, Woerden, The Netherlands). For the ELISA a mixture of three antigens, two European and one American strain, was used. Antibody titers were determined using a twofold dilution series. The cut-off value was determined by taking the mean of the negative controls plus three times the standard deviation. Specificity was determined by comparing sera from dogs surviving different infections (leishmaniosis, leptospirosis and viral infections) with sera from well vaccinated dogs protected from any possible contact with ticks. The prevalence was calculated on an estimated rabbit population of 50,000 animals in Belgium (Casear *et al.*, 2002).

Antibodies against *B. burgdorferi* sl were detected in 9 out of 103 serum samples from wild rabbits, yielding an apparent prevalence of 8.7% (confidence interval: 3.26 – 14.4). Correction for sensitivity (97%) and specificity (93.4% due to possible cross reactions with *Leptospira* sp.) yielded a true prevalence of 2.3% (0.0 – 5.2). The antibody titers were: 20 (n = 1), 30 (n = 4), 50 (n = 1), 75 (n = 1), 150 (n = 1), > 250 (n = 1).

Assuming that the sensitivity and specificity values, which were partly derived from determinations on dog sera, are valid for rabbits as well, the seroprevalence of *B. burgdorferi* sl in wild rabbits in Belgium can be estimated to be 2.3%. This prevalence was low compared to that in wild animals in neighboring countries. In the Netherlands, 13% of the roe deer examined were seropositive (Rijpkema *et al.*, 1996) and up to 47% of the rodents were infected with tick-transmitted spirochetes (De Boer *et al.*, 1993). In France, spirochetes were detected in the skin of 28% of the cervids examined (Pichon *et al.*, 2000). To estimate the relative importance of rabbits in the prevalence of tick-transmitted spirochete infections in wildlife in Flanders, further research in wild ungulates and rodents is needed.

Matuschka and others (2000) have demonstrated that ticks that feed on *B. burgdorferi* infected rabbits rarely pick up the infection. Only rabbits that build up an immune response to this spirochete could possibly pass the infection on to ticks. By way of conclusion, the relatively low seroprevalence of *B. burgdorferi* sl in the Belgian rabbit population combined with the limited risk of ticks becoming infected after a rabbit blood meal, suggest that rabbits play a minor role in the epidemiology and transmission of Lyme disease from animals to man in Belgium.

LITERATURE

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