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The Presence of *Borrelia miyamotoi*, A Relapsing Fever Spirochaete, in Questing *Ixodes ricinus* in Belgium and in The Netherlands

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Impacts • This is the first report of the occurrence of *Borrelia miyamotoi* in Belgium. • *Borrelia miyamotoi* is more widespread than previously expected. • *Borrelia miyamotoi* is a newly discovered human pathogen of importance for Public Health. Keywords: *Borrelia miyamotoi*; relapsing fever; *Ixodes ricinus*; Belgium; the Netherlands Correspondence: C. Cochez. Queen Astrid Military Hospital, Bruynstraat, 1, Brussels, Belgium. Tel.: +32 2 264 40 44; E-mail: christel.cochez@mil.be Received for publication November 14, 2013 doi: 10.1111/zph.12154

Summary *Borrelia miyamotoi* is a tick-borne bacterium that may cause relapsing fever in humans. As this pathogen has been discovered in Europe only recently, only little is known about its local impact on human health and its spatial distribution. In this study, we show the results of PCR screenings for *B. miyamotoi* in flagged *Ixodes ricinus* from Belgium and the Netherlands. *B. miyamotoi* was detected in nine of thirteen, and three of five locations from the Netherlands and Belgium, respectively. These outcomes indicate that *B. miyamotoi* is more spread than previously thought. The mean infection rate *B. miyamotoi* was 1.14% for Belgium and 3.84% for the Netherlands.

Introduction The *Borrelia* genus consists of two groups of pathogenic species, i.e. the Lyme borreliosis (LB) group and the relapsing fever (RF) group. *Borrelia miyamotoi*, a member of the RF group, is transmitted by *Ixodes* ticks and was first discovered in ticks in Eurasia and North America (Fukunaga et al., 1996). *B. miyamotoi*, as other relapsing fever spirochaetes,

is interstadially transmitted (Rollend et al., 2013). The first finding of *B. miyamotoi* was isolated from *Ixodes persulcatus* in Japan in 1995 (Fukunaga et al., 1995). In Europe and Russia, *B. miyamotoi* was detected in *I. persulcatus* and *I. ricinus* as well as in human blood (Platonov et al., 2011). The knowledge on the geographic distribution of *B. miyamotoi* is only fragmentary. It has been detected in *Ixodes* ticks in Eurasia and North America, i.e. Japan, the USA, Canada, Estonia, Slovakia, Poland, Sweden, Czech Republic, France, Germany and Russia (Geller et al., 2012). Human disease caused by *B. miyamotoi* has been described in the USA (Gugliotta et al., 2013), Russia and recently in the Netherlands (Hovius et al., 2013). The presence of *B. miyamotoi* was so far only confirmed in one location in the Netherlands (Hovius et al., 2013) but no information on the presence of *B. miyamotoi* in Belgium was available. Enzootic circulation of *B. miyamotoi* in questing ticks is a prerequisite for a potential public health risk for *B. miyamotoi*.

Materials and Methods To assess the presence of *B. miyamotoi* in Belgian ticks, we selected seven batches of *I. ricinus* from seven geographically well-defined regions and applied a quantitative polymerase chain reaction (qPCR) targeting the *B. miyamotoi* flagellin gene (Hovius et al., 2013), as well as a duplex qPCR targeting the *B. burgdorferi* *sls* *Osp A* and flagellin *B* genes (Heylen et al., 2013). Ticks were collected by flagging and conserved in 100% ethanol in the field. Upon arrival in the laboratory, the ticks were identified using keys (Hillyard, 1995), washed with distilled water, dried and placed at -80°C prior to DNA extraction. DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen, Venlo, The Netherlands), and DNA was stored at -80°C prior to use. *Ixodes ricinus* were captured in Jemelle, Groenendaal, Thielen and Bellem in 2013. Ticks originating from the Flanders and the Wallonia region were captured in 2010, and ticks from the Brasschaat region were captured in 2012 (Table 1, Fig. 1). *Ixodes ricinus* ticks from 13 different locations in the Netherlands, collected in 2011, i.e. 520 in total, were also analysed for the presence of *B. miyamotoi* (Table 1, Fig. 1). The majority of ticks analysed were *I. ricinus* nymphs ($n = 953$), and only 6 were *I. ricinus* adults. Results *B. miyamotoi* was detected in ticks from the

Brasschaat region (1/88), Jemelle (1/48), Thielen (1/48), the Flanders region (1/82) and the Wallonia region (1/77). All ticks from Bellem and Groenendaal were negative for *B. miyamotoi*.

While the overall prevalence of *B. burgdorferi* was 7.74%, the total *B. miyamotoi* prevalence was 1.14% for ticks collected in Belgium. In the ticks originating from the Netherlands, only the series from Dronten, Nijverdal, Schiermonnikoog and Twiske were negative. In the remaining nine locations, *B. miyamotoi* was demonstrated in varying percentages (Table 1). The mean overall infection rate was 3.84%. None of the six adult *I. ricinus* tested positive. Conclusion This is the first time that the presence of *B. miyamotoi* was confirmed in Belgian ticks; no human cases are known so far. The data from Belgium and the Netherlands suggest that *B. miyamotoi* is probably widespread in the region. We found in addition that the prevalence in Belgium is rather low (0 to 2.1%) compared to the *B. burgdorferi* prevalence (Gassner et al., 2011). In view of the already established risk for humans for infection with *B. miyamotoi* in the Netherlands and various other countries (Hovius et al., 2013, and references therein), it is prudent to alert the Belgian and the Dutch Public Health authorities and the medical community for possible *B. miyamotoi* infection after tick bite. References Fukunaga, M., Y. Takahashi, Y. Tsuruta, O. Matsushita, D. Ralph, M. McClelland, and M. Nakao. 1995: Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *Int. J. Syst. Bacteriol.* 45, 804–810. Fukunaga, M., K. Okada, M. Nakao, T. Konishi, and Y. Sato. 1996: Phylogenetic analysis of *Borrelia* species based on flagellin gene sequences and its application for molecular typing of Lyme disease borreliae. *Int. J. Syst. Bacteriol.* 46, 898–905. Gassner, F., A. J. van Vliet, S. L. Burgers, F. Jacobs, P. Hovius, E. K. Hovius, S. Mulder, N. O. Verhulst, van Overbeek L. S., and W. Takken. 2011: Geographic and temporal variations in population dynamics of *Ixodes ricinus* and associated *Borrelia* infections in The Netherlands. *Vector Borne Zoonotic Dis.* 11, 523–532. Geller, J., L. Nazarova, O. Katargina, L. Jarvekulg, N. Fomenko, and I. Golovljova, 2012: Detection and genetic characterization of relapsing fever spirochete *Borrelia miyamotoi* in Estonian ticks. *PLoS One* 7, 1–7. Gugliotta, J. L., H. K. Goethert, V. P. Berardi, and S. R. 3rd Telford. 2013: Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N. Engl. J. Med.* 368, 240–245.

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Location	Year	N	<i>B. miyamotoi</i> (%)	<i>B. burgdorferi</i> (%)
Belgium				
Bellem	2013	48	0 (0.0)	5 (10.4)
Groenendaal	2013	48	0 (0.0)	4 (8.3)
Jemelle	2013	48	1 (2.1)	9 (18.8)
Thielen	2013	48	1 (2.1)	6 (12.5)
Braschaat	2012	88	1 (1.1)	10 (11.4)
Flanders	2010	82	1 (2.1)	ND
Wallonia	2010	77	1 (1.3)	ND
Total		439	5	34
% positive			1.14%	7.74%
The Netherlands				
Bilthoven	2011	49	2 (4.1)	ND
Dronten	2011	31	0 (0.0)	ND
Ede	2011	35	1 (2.9)	ND
Eijsden	2011	32	3 (9.4)	ND
Gieten	2011	32	3 (9.4)	ND
Hoog Baarlo	2011	55	1 (1.8)	ND
Montferland	2011	64	4 (6.3)	ND
Nijverdal	2011	10	0 (0.0)	ND
Schiermonnikoog	2011	20	0 (0.0)	ND
Twiske	2011	5	0 (0.0)	ND
Vaals	2011	55	3 (5.5)	ND
Veldhoven	2011	65	1 (1.5)	ND
Wassenaar	2011	67	2 (3.0)	ND
Total		520	20	—
% positive			3.84%	—

Table 1. Location, region and number of Ixodes ticks trapped in Belgium and The Netherlands, examined for the presence of *Borrelia miyamotoi* and *B. burgdorferi*. In the three regions, ticks were captured from several different locations within that region

Fig. 1. Aggregated presence/absence map of questing *Ixodes ricinus* infected with *Borrelia miyamotoi*. Circles denote locations, and stars denote regions.