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SHORT COMMUNICATION

# Foci report on indigenous *Dermacentor reticulatus* populations in Belgium and a preliminary study of associated babesiosis pathogens

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**Abstract.** The occurrence of autochthonous clinical cases of canine and equine babesiosis in Belgium during the last two decades suggests that the vector of the pathogens responsible for these diseases, *Dermacentor reticulatus* (Ixodida: Ixodidae), may be present in this country. Consequently, evidence for the presence of this tick species in different locations within Belgium was investigated. Four different locations were monitored by flagging in 2010; these included the locations at which *D. reticulatus* was previously found on a dog in 2009 and on two red deer in 2007. Two different species of tick were identified, *Ixodes ricinus* (Ixodida: Ixodidae) and *D. reticulatus*. A total of 282 *D. reticulatus* adult ticks (98 males, 184 females) were collected from the four sites. Ticks were found mainly from early March until the end of May and a peak in activity was apparent in March. A *Babesia* spp. (Piroplasmida: Babesiidae) genus-specific polymerase chain reaction test based on the amplification of a fragment of the 18S rRNA gene was used to investigate the potential presence of *Babesia* spp. All DNA extracts isolated from the total tick samples yielded negative results. Additional studies to accurately determine the distribution and vectorial capacity of this important tick species in Belgium are warranted.

**Key words.** *Dermacentor reticulatus*, *Babesia* spp., tick, Belgium.

*Ixodes ricinus* (Linnaeus 1758), the most abundant tick species in northern Europe, is widely distributed and is mainly found in habitats with a high level of humidity (Hillyard, 1996). *Dermacentor reticulatus* (Fabricius 1794) in northwestern Europe is more scarce and localized (Hillyard, 1996; Gray *et al*., 2009). The geographic range of this species extends from

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**Fig. 1.** Map showing the four locations investigated in Belgium. Beveren, location 1; Moen, location 2; Mons, location 3; Martilly, location 4.

France and the southwest of the U.K. in the west through to central Asia in the east. Until recently, the French–Belgian border was considered to represent the northern boundary of *D. reticulatus* in Western Europe (Heile *et al*., 2006). Recently, several reports have indicated that the geographic distribution of *D. reticulatus* is expanding. Indeed, over the last decade, *D. reticulatus* ticks have been collected from the environment in the Netherlands (Nijhof *et al*., 2007) and in different German states (*Länder*) (Dautel *et al*., 2006). Evidence of a change in the distribution of *D. reticulatus* is supported by the identification of canine babesiosis in regions of Germany (Heile *et al*., 2006; Barutzki *et al*., 2007), Hungary (Sréter *et al*., 2005), Switzerland (Porchet *et al*., 2007) and the Netherlands (Nijhof *et al*., 2007) not previously associated with this disease. In Belgium, adult *D. reticulatus* were found on a dog and on an unspecified animal species in the south of Luxembourg province, close to the French border, in 1945 and 1950, respectively (Fain, 1989). However, these historical observations did not provide any information on the origin of the tick or the travel history of the dog. More recently, low numbers of *D. reticulatus* were reported on dogs from one location in Belgium (Losson *et al*., 2003). In France, *D. reticulatus* is the most important vector of canine babesiosis (*Babesia canis*) (Martinod & Gilot, 1991), but it can also transmit other protozoans, viruses and bacteria. The occurrence of autochthonous cases of canine (Losson *et al*., 1999) and equine (Mantran *et al*., 2004) babesiosis in Belgium during the last two decades suggests that this tick species may be present in this country.

Four locations in Belgium where *D. reticulatus* ticks were suspected to be present were identified: Beveren (referred to as location 1) and Moen (location 2) in northern Belgium, and Mons (location 3) and Martilly (location 4) in southern Belgium (Fig. 1).

The Beveren site (location 1, in the province of East Flanders) was identified in 2009 when *D. reticulatus* ticks were found repeatedly on a domestic dog that had no history of foreign travel. The site is a suburban marshland with a shallow artificial pond at its centre. It is situated between a railway track and a provincial road and is often used as recreational area for dog walking. The marshy area consists of a mixture of grasses and the dryer patches are occupied mainly by hornbeam (*Carpinus betulus*) and blackberry (*Rubus fruticosus*).

The site in Moen (location 2, in the province of West Flanders) was selected on the basis of photographs posted on an Internet site that collects fauna and flora observations (www.waarnemingen.be) showing what appeared to be questing *D. reticulatus* ticks that had been misidentified as *I. ricinus*. The site is a natural reserve of about 26 ha and is located next to a canal that is accessible for recreational purposes. Location 3 is situated in Mons (in the province of Hainaut) and consists of fallow land flanked by a road. It is close to a leisure area that includes an artificial lake surrounded by a path that is extensively used for walking and exercising dogs. The vegetation consists of grasses, hawthorn, blackthorn (*Prunus spinosa*), brambles (*R. fruticosus*) and birch (*Betula pendula*). According to local veterinarians, this area is a focus of canine babesiosis (Losson *et al*., 1999).

Location 4 is situated in Martilly (in the province of Luxembourg). Two areas, referred to as 'zone 1' and 'zone 2', were monitored at this site. These zones are approximately 600 m apart and are located in a rural environment consisting of woodland (mainly *Picea abies*) and pastures used for cattle grazing. In zone 1, which is situated close to a small stream, the trees were felled some years ago and the vegetation now comprises brambles (*R. fruticosus*), ferns (*Pteridium aquilinum*), blackthorn (*P. spinosa*) and jennets (*Genista scorpius*). Zone 2 comprises woodland consisting mainly of birch (*B. pendula*) and oak (*Quercus robur*), with brambles (*R. fruticosus*) and ferns (*Pt. aquilinum*) also present. In both zones roe deer (*Capreolus capreolus*) and red deer (*Cervus elaphus*) are present, as evidenced by the presence of numerous faecal deposits. Both zones were selected because *D. reticulatus* were found attached to two red deer hunted at these sites in February 2007.

Ticks were trapped by flagging with flannel cloth (Bram, 1978) in dry or sufficiently dry conditions.

Location 1 was monitored on a weekly basis, from the end of February 2010 to the third week of November 2010. Location 2 was visited on only a few occasions. Locations 3 and 4 were sampled on a weekly basis during March–August 2010. Ticks were stored in 100% ethanol immediately after trapping and morphologically identified using a standard key (Arthur, 1963). Tick DNA extraction was performed according to the proteinase K protocol (20 mg/mL) of Boom *et al*. (1990). To discount potential false negative results caused by polymerase chain reaction (PCR) inhibition and to validate the efficiency of the DNA extraction, an initial PCR test targeting the tick 16S rRNA gene was performed using the 16S + 1/16S − 2 primer set (Baumgarten *et al*., 1999). Only tick DNA-positive samples were further analysed for the presence of *Babesia* spp. The *Babesia* spp. genus-specific PCR was developed according to Casati *et al*. (2006) and was designed to amplify a fragment of the 18S rRNA gene (Lempereur *et al*., 2011).

A total of 282 *D. reticulatus* adult ticks (98 males and 184 females) were collected from the four sites. The majority

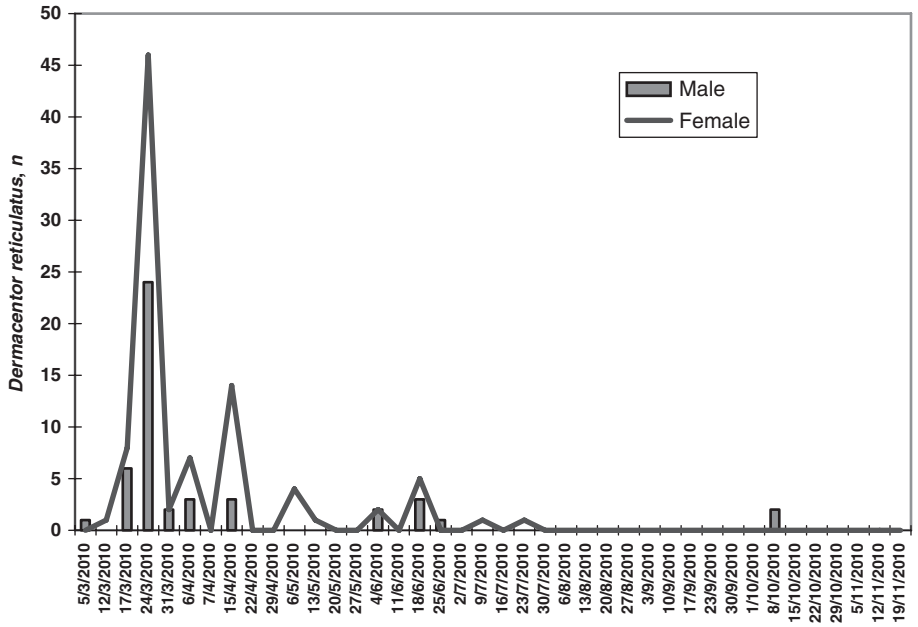


**Fig. 2.** Numbers of *Dermacentor reticulatus* (male and female) flagged at location 1 (total $n = 139$)

of the *D. reticulatus* ticks were found between early March and June, with a peak in abundance in March, although a few specimens were also found in July, August and October. Although *I. ricinus* is the most prevalent tick species in Belgium (Fain, 1990), only *D. reticulatus* was obtained in location 1 (Beveren), where a total of 139 (47 males and 92 females) adult ticks were collected. Weekly numbers of ticks collected indicated that questing activity was highest between March and April (Fig. 2). Four of the 234 available tick DNA extracts remained negative for the tick 16S rRNA gene PCR test, despite the template DNA being diluted to 1 : 10 and 1 : 100, and consequently these samples were removed from the analysis. The remaining 230 DNA extracts were all found to be negative for *Babesia* spp. using the *Babesia*-specific PCR assay.

Although *D. reticulatus* is known to occur in Europe, it is considered to be scarcely distributed in the northwest of the continent (Hillyard, 1996) and has not been accurately recorded there. Recently, questing populations of this tick species were discovered by flagging in the Netherlands (Nijhof *et al*., 2007). *Dermacentor reticulatus* has also been documented in Germany (Dautel *et al*., 2006), Poland (Zygner *et al*., 2009), Austria (Sixl *et al*., 2003) and western Switzerland (Porchet *et al*., 2007). In this survey, indigenous questing populations of *D. reticulatus* were found in Belgium, supporting the findings of a previous survey indicating the presence of low numbers of feeding adults of *D. reticulatus* on dogs from one location in the country (Losson *et al*., 1999). As Belgium is situated between France and the Netherlands, this study and others indicate that the species has extended its distribution area in recent years, moving from northern France to the Netherlands and populating Belgium in the process. Various factors such as landscape use, climate change, altered human activity and increases in host population density (such as in cervids) may explain this dissemination, as Dautel *et al*. (2006) and Lindgren & Gustafson (2001) have summarized. Movements of people with animals carrying ticks and tick-borne diseases across borders may also explain this phenomenon.

Given its vectorial capacity, the spread of *D. reticulatus* may result in the occurrence of certain diseases in areas in which they have not previously been recorded. A similar observation was made in the Netherlands, where autochthonous canine babesiosis foci are known to exist (Matjila *et al*., 2005). No evidence of *Babesia* spp. was found in any of the tick samples analysed. However, the possibility that *D. reticulatus* can act as a vector for *B. canis* cannot be ruled out completely given the low prevalence of tick infection expected (Duh *et al*., 2004; Rar *et al*., 2005) and the relatively low number of ticks analysed in our study. In conclusion, the presence of indigenous populations of *D. reticulatus* was demonstrated for the first time in several distinct areas of Belgium. A more detailed widespread monitoring of *D. reticulatus* populations and screening for *B. canis* and other viral, rickettsial and bacterial pathogens are warranted in future studies.

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