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Short communication

New foci of *Haemaphysalis punctata* and *Dermacentor reticulatus* in the Netherlands

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ABSTRACT

In 2014 *Haemaphysalis punctata* was found in several locations on the mainland of the Netherlands for the first time since 1897. In the same areas *Dermacentor reticulatus* and *Ixodes ricinus* were found. *Haemaphysalis punctata* and *D. reticulatus* were tested for presence of *Babesia* spp. and *Rickettsia* spp. by PCR. *Babesia* spp. and spotted fever *Rickettsiae* were not detected in any of the collected *H. punctata*, while several *D. reticulatus* (6%) collected from the same areas were found to be positive for *R. raoultii*, a causative agent of tick-borne lymphadenopathy. We discuss the role of free-ranging domestic animals in maintaining *H. punctata* and *D. reticulatus* populations in dune areas in the Netherlands.

1. Introduction

The tick species *Haemaphysalis punctata* is generally assumed to be a species with a Mediterranean distribution (Estrada-Peña et al., 2013). However, the species is occasionally found in more temperate regions, often along the coast, such as populations on islands in the Baltic sea and the Wadden sea and in the United Kingdom (Liebisch and Rahman, 1976; Tälleklint, 1996; Tijsse-Klasen et al., 2013). In the Netherlands, the species was most recently found on the island of Texel in 1978 and 1979 (Uilenberg et al., 1980; Garben et al., 1981). After these publications, the tick species was not recorded in the Netherlands until June 2014, when two adult females were collected by blanket dragging in a forest edge on the mainland coast of the Netherlands (Pettemerduinen, Table 1). The only previous records of *H. punctata* on the mainland of the Netherlands were from Utrecht and Arnhem in 1884 and 1897, respectively (Garben et al., 1981). The new finding of *H. punctata* was close to a location where a population of *Dermacentor reticulatus* was present (St. Maartenszee; Nijhof et al., 2007; Jongejan et al., 2015).

Dermacentor reticulatus has increased its range in the Netherlands in recent decades, where it is predominantly found along the coast (Nijhof et al., 2007; Jongejan et al., 2015). In the Netherlands, *D. reticulatus* and *H. punctata* both seem to occur mainly in open dune areas and salt marshes which are grazed by cattle, horses or sheep (Garben et al., 1981; Nijhof et al., 2007; Jongejan et al., 2015). This is consistent with where the species are found in the United Kingdom (Tijsse-Klasen et al., 2013). Both species transmit pathogens which are of veterinary and medical importance such as several *Babesia* and *Rickettsia* species (Uilenberg et al., 1980; Nijhof et al., 2007; Tijsse-Klasen et al., 2013; Jongejan et al., 2015). As both tick species occur in similar habitats and share vertebrate hosts in the Netherlands, there is a potential overlap in pathogens between these species, which might have implications for pathogen transmission to domestic animals and to humans.

2. Materials and methods

To better understand the distribution of *H. punctata* and *D. reticulatus*, and to explore their role in tick-borne disease transmission, ticks were collected by blanket dragging in protected nature areas in the dunes surrounding the old and new records of both species in the northern part of the Netherlands (Table 1). Fieldwork was performed from July to October 2014. In each area ticks were dragged with a cotton blanket of 1 m^2 for 200–1400 m in intervals of 10 m

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Table 1
Locations of tick sampling areas in the Netherlands, the number of *H. punctata* and *D. reticulatus* examined by PCR per location, with the total number caught between brackets if different, and the number of infected ticks with *Rickettsia raoultii*.

Location	Name	Coordinates (*latitude, longitude*)	*H. punctata*	*D. reticulatus*	Infection with *Rickettsia* spp.
1	Slufter, Texel	53°8′23.5”, 4°49′29.3”			
2	Muy, Texel	53°7′25.3”, 4°48′11.2”	1 nymph		
3	Ecomare, Texel	53°4′26.8”, 4°45′4.5”			
4	Jan Ayeslag, Texel	53°1′58.1”, 4°43′4.1”			
5	Mokbaai, Texel	53°0′41.6”, 4°44′48.0”	4 nymphs		
6	Grafelijkheidsduinen	52°56′33.3”, 4°43′9.4”	6 (59) larvae, 3 nymphs	6 males, 6 females	1 female *D. reticulatus*
7	Zwanenwater	52°49′37.6”, 4°41′34.6”			
8a	Pettemerduinen	52°46′46.9”, 4°40′17.6”	15 (22) larvae, 6 nymphs, 2 females	5 nymphs, 19 males, 16 female	1 female *D. reticulatus*
8b	Korfwater	52°46′21.1”, 4°39′56.2”	23 (287) larvae		
9	Camperduin Schoorl	52°42′13.1”, 4°38′43.1”		2 females	1 female *D. reticulatus*

on a single day with suitable weather for dragging, which meant dry days with a temperature above 10 °C, and wind speeds below 4 Beaufort scale (<20 km/h). A total of 10,900 m was dragged in suitable habitat in 9 locations in four months. After each 10 m section the blanket was checked for ticks. If an individual of *H. punctata* or *D. reticulatus* was found in an area, a more thorough sampling was performed in the area to determine the importance of livestock and habitat type (forested dunes vs open dunes) on the presence and density of both species, by dragging 200 m in habitat of four categories: (1) open areas without livestock, (2) open areas with livestock, (3) forested areas without livestock and (4) forested areas with livestock.

All adults and nymphs, and several larvae found by dragging were collected, identified to species using morphological keys (Arthur, 1963; Hillyard, 1996) and stored at −20 °C until analysis for the presence of *Babesia* spp. and *Rickettsia* spp. Total DNA was extracted from these ticks by DNA alkaline lysis. The presence of the DNA of different *Babesia* spp. was determined by a generic polymerase chain reaction (PCR) targeting the 18S rRNA gene of *Babesia* and closely related organisms such as *Theileria* using the methods described by Wielinga et al. (2009). For the detection of spotted fever *Rickettsia* spp., a duplex qPCR was designed consisting of a specific qPCR for *Rickettsia helvetica* and an already existing qPCR for spotted fever and typhus group *Rickettsia* spp. fragments of the citrate synthase (Stenos et al., 2005). Briefly, we used 5′-TCGCAAATGTTCACGGTACTTT-3′ and 5′-ATGATCCGTTTAGGTTAATAGGCTTCGGTC-3′ as forward primers, 5′-TCGTGCATTTCTTTCCATTGTG-3′ and 5′-TTGTAAGAGCGGATTGTTTTCTAGCTGTC-3′ as reverse primers and 5′-Atto520-TGC AAT AGC AAG AAC CGT AGG CTG GAT G-BHQ1-3′ and 5′-Atto425-CGATCCACGTGCCGCAGT-BHQ1-3′ probes. qPCR was performed using the iQ Multiplex Powermix PCR reagent kit, which contains iTaq DNA polymerase (Bio-Rad Laboratories, Hercules, USA), in a LightCycler 480 Real-Time PCR System (F. Hoffmann-La Roche, Basel, Switzerland). Optimal reaction conditions in a final volume of 20 μl were iQ multiplex Powermix, primers 400 nM each, probes at 400 nM each, and 3 μl of template DNA. Cycling conditions included an initial activation of the iTaq DNA polymerase at 95 °C for 5 min, followed by 60 cycles of a 5 s denaturation at 95 °C followed by a 35 s annealing-extension step at 60 °C (Ramp rate 2.2 °C/s and a single point measurement at 60 °C) and a cooling-cycle of 37 °C for 20 s. Analysis was performed using the second derivative calculations for cp (crossing point) values. Amplification curves were assessed visually. Specificity of the positive signals was confirmed using a conventional PCR as described by Sprong et al. (2009). For all positive samples, the amplicon was sequenced to determine the species.

3. Results

Questing *H. punctata* of all stages were found in July and August in five locations, and all nymphs and adults, and part of the larvae were collected (see Table 1 and Fig. 1). The species had been recorded previously in only one of these locations (Mokbaai, Texel; Garben et al., 1981), which might indicate a stable population, as sampling in intermediate years is lacking. Interestingly, *H. punctata* was not collected at the Slufter, Texel, where it was found by Garben et al. (1981). In all five locations, *H. punctata* was found in areas which were grazed by free-ranging cattle, sheep or horses, both in open and forested dunes, but not in areas without livestock. In the open dune areas *H. punctata* was collected both in dry dunes, and in moist dune valleys. Questing *D. reticulatus* adults and nymphs were collected in September and October in three locations (see Table 1 and Fig. 1). The species had been recorded previously in only one of these locations (St. Maartenszee, close to Pettemerduinen; Jongejan et al., 2015). Interestingly, *D. reticulatus* was not found on the island of Texel. In all three locations, *D. reticulatus* was found in open dune areas which were grazed by free-ranging cattle or horses, and not in forested areas or areas without livestock. The species was collected in both dry dunes and moist dune valleys. *Ixodes ricinus* was present at all locations, in all habitat types.

In total, 44 larvae, 13 nymphs and 2 females *H. punctata* and 5 nymphs, 24 females and 25 males *D. reticulatus* were collected and tested for the presence of *Babesia* spp. and *Rickettsia* spp. Of these, 3 female *D. reticulatus* (6%) were infected with *Rickettsia* spp., of which one amplicon was successfully sequenced. The sequence (737 bp) of the infected female from Camperduin Schoorl showed 100% similarity with the *Rickettsia raoultii* sequences in GenBank (http://www.ncbi.nlm.nih.gov/).

4. Discussion

The locations where *H. punctata* and *D. reticulatus* were found, were all situated along the coast in dune areas grazed by free-ranging livestock. This suggests that the mild climate along the coast in combination with the presence of large herbivores results in suitable conditions for the survival of these species outside their natural range (Estrada-Peña et al., 2013). Remarkably, none of the studied locations harbour a local population of wild ungulates, suggesting that in these areas livestock are required as hosts for the adult ticks. This was supported by the fact that we did not find any questing ticks of either species outside the areas grazed by livestock.

The presence of ticks does not imply an established population. Passerine birds are known to carry both *H. punctata* and

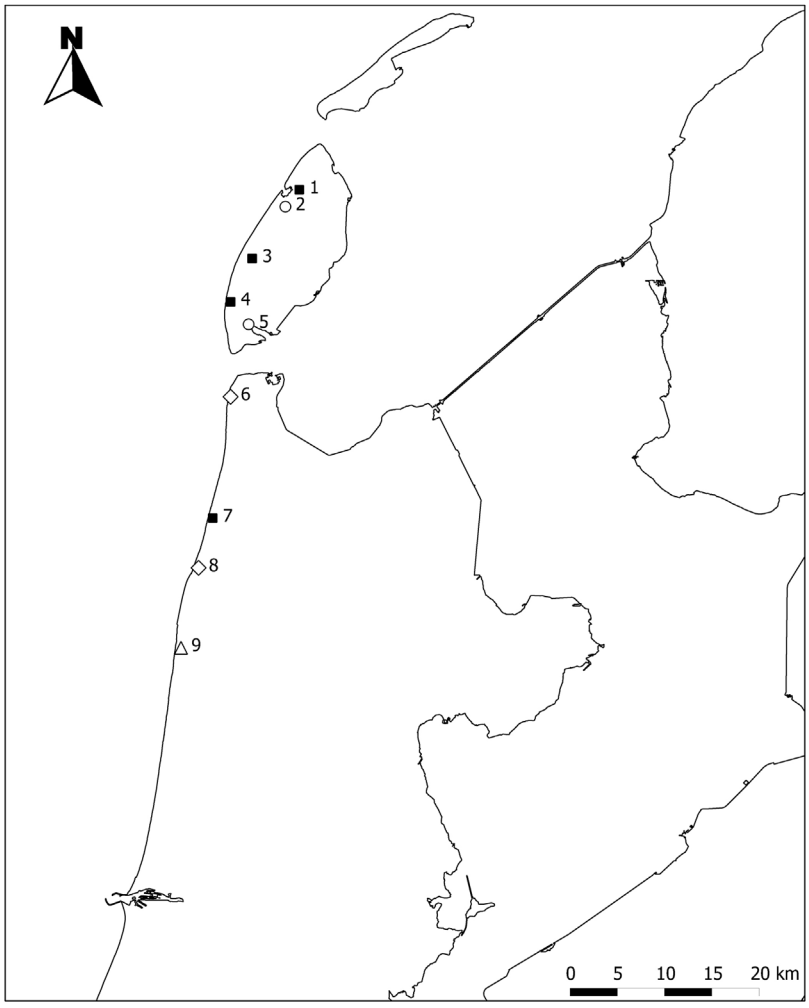

Fig. 1. Locations where ticks were sampled in the Netherlands. ■ locations where no *H. punctata* or *D. reticulatus* were caught, ○ locations where *H. punctata* were caught, but no *D. reticulatus*, △ locations where *D. reticulatus* was caught, but no *H. punctata*, ◊ locations where both *H. punctata* and *D. reticulatus* were caught.

D. reticulatus (Hasle et al., 2009; Palomar et al., 2012) which could indicate that the ticks, found while questing, had engorged on migrating birds and dropped off along the Dutch coast, which is a known flyway for migrating passerines (Van Dobben, 1953). Also the fact that most populations of *H. punctata* in north-western Europe are found on island along the coast, suggests that migrating birds play a large role in introducing this species to this part of Europe (Liebisch and Rahman, 1976; Tälleklint, 1996; Tijsse-Klasen et al., 2013). However, the fact that we found individuals of multiple stages including larvae at the Grafelijkheidsduinen and Pettemerduinen suggests that these areas now harbour established populations of both *H. punctata* and *D. reticulatus*, which were possibly introduced by migrating birds.

Adult *H. punctata* were only found in June, which is consistent with a previous report stating that the activity of *H. punctata* in the Netherlands differs from populations in other European countries as nymphs and adults were only found active in spring, and larvae in late summer and autumn (Garben et al., 1981). However, our finding that nymphs and larvae were active in July and August, is more consistent with findings from other European countries (Arthur, 1963; Nosek, 1971). Three areas on Texel where no *H. punctata* were found during this study, were sampled in September and October, when *H. punctata* was not found in other locations either. This indicates that *H. punctata* in the Netherlands is most probably not active in autumn, in contrast to other European populations (Arthur, 1963; Nosek, 1971). Therefore, more investigations in spring and summer are needed to verify the presence or absence of *H. punctata* in these location. All areas were sampled within the questing season of *D. reticulatus* adults (September/October).

None of the collected *H. punctata* were found to be infected with *Babesia* spp. or *Rickettsia* spp. However, given the relatively low number of ticks tested, the presence of these pathogens cannot be excluded. Several *D. reticulatus* from the same areas were found to be infected with *Rickettsia* spp., most probably *R. raoultii*. *Rickettsia raoultii* was found in 3% of *D. reticulatus* in the UK (Tijsse-Klasen et al., 2013) and 14% of *D. reticulatus* in the Netherlands (Nijhof et al., 2007) and can cause tick-borne lymphadenopathy (TIBOLA) in humans (Parola et al., 2009). All of the areas in which both species were found are regularly visited by recreationists. Therefore, it is important to get a better understanding of *D. reticulatus* and *H. punctata* distributions in coastal areas in north-western Europe, where both species occur in areas that are frequently visited by people (Garben et al., 1981; Jongejan et al., 2015). Furthermore, it is important to test more ticks to better identify the possible risks for animal and public health.

We found several novel foci of *H. punctata* and *D. reticulatus* in the north-western part of the Netherlands, all in grazed, open dune areas which are intensively used for recreation. Part of the *D. reticulatus* (6%) were infected with *Rickettsia* spp., most probably *R. raoultii*. This finding shows that open dune areas with livestock grazing possibly pose a risk to public and animal health and this

calls for a more active surveillance of these areas for tick species other than *I. ricinus*.

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