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Orignal article

Ixodes ricinus and *Borrelia* prevalence at the Arctic Circle in Norway

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ABSTRACT

The distribution limit of *Ixodes ricinus* ticks in northwestern Europe (Brønnøy, Norway, 1° south of the Arctic Circle), has been known since the 1930s. To reconfirm this finding and extend studies in the areas adjacent to the Arctic Circle (66°33′ N), ticks were collected from dogs and cats in 8 districts in northern Norway from 64°56′ N to 68°48′ N. We detected 549 *I. ricinus*, 244 (44%) of them in Brønnøy district, and 305 (range 6–87 ticks) in 7 districts in the northern part of the study area. The prevalence of *Borrelia* in these ticks was determined by real-time PCR. In the Brønnøy district (65°28′ N, 12°12′ E), 29% of the *I. ricinus* were *Borrelia* spp.-positive, and the species *B. afzelii* was nearly twice as prevalent as *B. garinii* and/or *B. valaisiana*. In the study area north of Brønnøy district, only 12 (4%) of the collected ticks contained *Borrelia* spp. In conclusion, tick occurrence and *Borrelia* prevalence are high in the Brønnøy district. In contrast, *I. ricinus* occurrence and *Borrelia* prevalence are low further north across the Arctic Circle in Norway.

Introduction

The first published observation of ticks in northern Norway was reported from Brønnøy municipality in 1943 (Tambs-Lyche, 1943). These findings were confirmed in 1983 (Mehl, 1983), but since then, no study has been performed in the region to investigate a possible migration of ticks to other parts of northern Norway and to determine the prevalence of *Borrelia* in ticks in those areas. When the annual incidence of 26 cases of Lyme borreliosis per 100,000 population in Brønnøy is not taken into account, national statistics of Lyme borreliosis during 2007–2011 (http://www.msis.no/) report an annual incidence of 0.52 cases per 100,000 population in the 3 northernmost counties. This indicates that *I. ricinus* may have low *Borrelia* prevalence and/or are sparsely distributed north of Brønnøy.

The North Atlantic Drift Current, which is a branch of the Gulf Stream, transports warm waters along the ever ice-free coast of Norway to higher latitudes than any other ocean, thereby contributing to the relatively warmer climate in the northern part of the country (Bischof et al., 2003). Using a multi-source analysis without any field studies involved, Jore et al. (2011) claim that *I. ricinus* propagation has advanced 400 km northward since previous reports and has reached the latitude of 69° N. In northern Sweden inland (approximately 63–69° N), *I. ricinus* occurrence was low with few records of ticks (Tälleklint and Jaenson, 1998), but a report based on a questionnaire suggests that it appears to be increasing (Jaenson et al., 2012). In addition, field studies reveal that in a small coastal area of the Baltic Sea (63°35′ N, 20°47′ E) ticks are relatively more abundant than in northern Sweden inland and with high *Borrelia* prevalence (Jaenson et al., 2009).

In summer 2009, veterinarians in all counties of northern Norway were requested to collect ticks from pets, and this study revealed decreasing tick occurrence northwards (Jenkins et al., 2012). Since *I. ricinus* ticks may be absent in many parts of northern Norway or too sparsely distributed to allow collection by the flagging method, we therefore surmised collecting ticks from pets is a useful (Hovius et al., 1998) and more sensitive method (Baumgarten et al., 1999). If there was any difference in tick occurrence between areas, we assume it may become apparent as geographical differences in the number of collected ticks, and especially, the difference in tick density in geographical areas can

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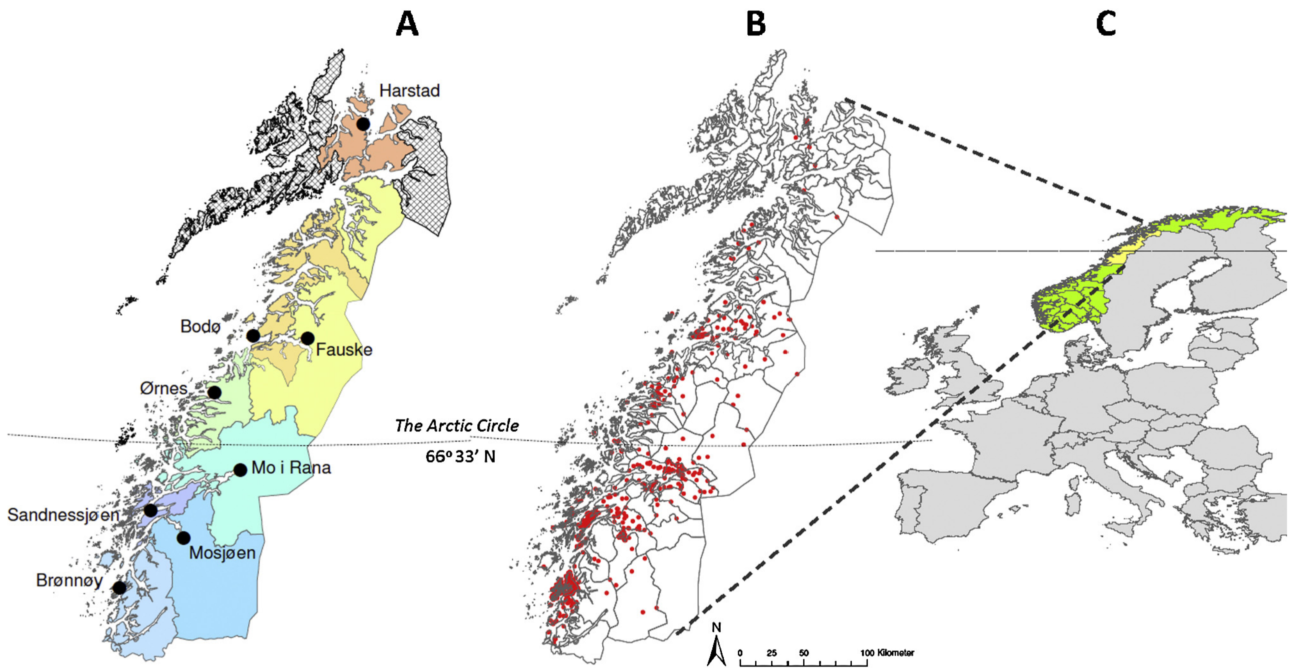

Fig. 1. (A) The study area covers 8 districts in northern Norway (from 64°56′ N to 68°48′ N) across the Arctic Circle (66°33′ N). The Vesterålen and Lofoten archipelago (hatched area, left) and Narvik city (hatched area, right) were not included in the study. (B) Scatter diagram of the origin of ticks in the study area (red dot = one single tick). In the southernmost district (Brønnøy), 244 ticks, and in the northernmost district (Harstad), 6 ticks were collected. (C) Sketch map showing the study area in relation to western Europe.

be estimated by the number of ticks per pet (Tälleklint and Jaenson, 1998).

We aimed to extend previous studies at the tick's distribution limit and to investigate the occurrence of *I. ricinus* and the *Borrelia* prevalence in the region adjacent to the Arctic Circle in Norway (66°33′ N).

Materials and methods

Study area

Six veterinary clinics in cities, from Brønnøy (65°28′ N, 12°12′ E) northwards via Mosjøen, Mo i Rana, Fauske, Bodø to Harstad (68°48′ N, 16°32′ E), were requested to acquire ticks from all kinds of sources from July to October 2010. From June to October 2011, the veterinary clinics immediately north and south of the Arctic Circle (i.e., Ørnes and Sandnessjøen) participated. The study area covered the western coast (4 districts), which has warmer climate than the eastern fjords, inland, and mountains (3 districts) and the northern coast (one district) (Fig. 1A). Only in the 3 southernmost coastal districts roe deer have been hunted (i.e., Brønnøy, Sandnessjøen, and Ørnes districts) (http://www.hjortevilt.no/).

For every respondent delivering ticks, the following data were collected: postal code of home address, telephone number, number of ticks, and kind of source (e.g., dog, cat, human being, vegetation). The respondents were requested to indicate the most likely geographical origin of the ticks. If the pet had stayed outside the study area in the past 10 days before collection, or if it was unclear from where in the study area the ticks had originated, the ticks were excluded. Only few ticks originated from Lofoten, Vesterålen, and Narvik during the study in 2009, thus, ticks from these areas were not included (Fig. 1A).

Tick collection

During the project period, we advertised for ticks 5 times in the main local newspapers in all 8 districts. In addition, there were articles about the study in all these newspapers and features in the regional broadcasting radio and television programme. We also informed about the study and ticks in general on the web (http://www.arctick.no/). Thus, we tried to distribute the information equally in the study area.

Each tick was placed into a 2.5-ml tube containing 1 ml 70% ethanol and kept at 4–8 °C until transportation to the clinical microbiology laboratory in Jönköping, Sweden. Using light stereo microscopy, the tick species and instars were determined according to authoritative books in the field (Arthur, 1963; Filippova, 1977; Hillyard, 1996). The feeding status was estimated (i.e., fed/unfed). Only *I. ricinus* ticks were included in the study. Mutilated ticks that made it impossible to determine the tick species were excluded.

Ticks can be infected with *Borrelia* by systemic spread on a reservoir host or local skin infection during feeding (i.e., co-feeding) (Randolph et al., 1996), which might contribute to an overestimation of *Borrelia* prevalence. To avoid this bias, the *Borrelia* result of ticks from animals harbouring 2 or more ticks was not included except when the ticks were unfed, if only one of the pet ticks was *Borrelia*-positive, or when all *Borrelia* species were different.

Molecular analysis of Borrelia prevalence and species distribution in I. ricinus

Reverse transcribed total nucleic acid was prepared from ticks as described previously (Jenkins et al., 2012). Reverse transcription increases the sensitivity of the LUX 16S rRNA real-time PCR test (Wilhelmsson et al., 2010) 10- to 100-fold, resulting in a sensitivity of less than 1 cell per PCR reaction (Wilhelmsson et al., 2013). This test was used for detection of *Borrelia* spp. Then, ticks containing *Borrelia* spp. were characterized to species level by nested PCR amplification and sequencing of the 5S-23S rRNA as well as 16S-23S intergenic spacer regions according to Postic et al. (1994) and Bunikis et al. (2004). Sequencing was performed by GATC Biotech (GATC Biotech AG, Konstanz, Germany). Chromatograms were analyzed using the RipSeq web application (iSentio, Bergen, Norway)

Table 1
I. ricinus ticks delivered to veterinary clinics in 8 districts in northern Norway ($n = 549$).

District centre[a]	Population	Latitude[b]	Longitude	No. of ticks on cats	No. of ticks on dogs	No. of ticks (F/M/N)[c]	No. of dogs[e]	Ratio (ticks/dog) (‰)[f]
Harstad (C)	23,300	68°48′ N	16°32′ E	4	2	6/0/0	1586	1
Bodø (C)	47,300	67°16′ N	14°24′ E	13	28	39/1/1	3321	8
Fauske (F)	9600	67°15′ N	15°23′ E	8	19	26/0/0/1[d]	768	25
Ørnes (C)	6600	66°52′ N	13°42′ E	22	16	36/1/0/1[d]	459	35
Mo i Rana (F)	25,300	66°18′ N	14°08′ E	40	47	85/2/0	2388	20
Sandnessjøen (C)	7200	66°01′ N	12°36′ E	26	30	55/1/0	482	62
Mosjøen (F)	13,400	65°50′ N	13°11′ E	30	20	48/2/0	1188	17
Brønnøysund (C)	7700	65°28′ N	12°12′ E	139	105	179/62/3	509	206
Total	140,400			320	229	549	10,701	

C, located at the coast; F, located at the head of a fjord.
[a] The districts are named after the largest municipality (city) in the region.
[b] The latitude of the Arctic Circle is 66°33′ N.
[c] F/M/N, the number of females, males, and nymphs of *I. ricinus*, respectively.
[d] No instar date, in the Fauske and Ørnes districts.
[e] The estimated number of dogs in the municipalities was based on the register of the Norwegian kennel club.
[f] The ratio of ticks collected on dogs to all dogs in the municipality was negatively correlated with the latitude of the districts: $r = -0.69$ (Spearman rank correlation; $P = 0.03$; one-tailed).

(Kommedal et al., 2009), which allows species determination in samples containing different *Borrelia* spp. simultaneously (Jenkins et al., 2012; Wilhelmsson et al., 2013).

Statistics

The number of ticks per dog and cat was expressed as mean and 95% confidence interval (CI), and the difference between Brønnøy and the 7 northern districts was tested with 2-sample *t* test. Spearman's rank correlation (non-parametric) was used to test the correlation between the latitude of the veterinary centres and the ratio of ticks collected on dogs to all dogs in the municipality. The number of dogs in a municipality was estimated as follows: number in the Norwegian kennel club's register for the municipality in 7 years (2006–2012) plus 20%, which is the kennel club's estimated percentage of unregistered dogs (Anne Hofmo Bjølgerud, pers. communication). The average dog life span is 10.0 years (Proschowsky et al., 2003), and thus, the estimated number of dogs in the study years was found by extrapolation. Differences in *Borrelia* prevalence between the Brønnøy district and the 7 northern districts and the *Borrelia* prevalence between fed female ticks and unfed female/male ticks, were calculated with chi-square table. Differences of *P* value < 0.05 (two-tailed if not otherwise stated) were considered significant.

Results

Ticks

Altogether, 719 ticks were collected from 215 dogs, 167 cats, 4 cows, one rodent, and 15 ticks were collected from vegetation. In addition, 44 ticks (30 females, 1 male, 12 nymphs, no instar data from $n = 1$) were collected from 26 humans, and 7 (16%) of these ticks (5 females, 1 male, and 1 nymph) were *Borrelia*-positive. The ticks from humans (except 2) came from the 2 southernmost coastal districts, Brønnøy and Sandnessjøen.

Nine (1%) non-*I. ricinus* ticks were excluded: a male of *Hyalomma marginatum marginatum* that had attached firmly to the thigh of a man in Narvik (68°28′ N, 17°30′ E) in July 2010; a *Rhipicephalus sanguineus*, most probably imported on a dog from Portugal; and 7 engorged adult female *Ixodes trianguliceps*, 3 of which came from cats, 3 from a rodent, and one from a dog. The characteristics of *Ixodes persulcatus* were sought for, but not found (Dubinina et al., 2007). There were relatively few ticks from other sources than pets, therefore, in order to uniform the material, only the 549 ticks from the dogs and cats were further studied. The *I. ricinus* (hereafter called ticks) were categorized in dog/cat source, geographical origin, instar, and feeding status; there were 474 female ticks, 69 males, and 4 nymphs (no instar data from $n = 2$).

There was a drop in the number of delivered ticks from the Brønnøy district northwards to Harstad (Table 1; Fig. 1B). The ratios of the number of ticks collected from dogs to all dogs in a district could be calculated, and these were significantly negatively correlated with the latitude of the 8 districts ($r = -0.69$, $P = 0.03$; one-tailed) (Table 1). The majority of male ticks ($n = 62$) and nymphs ($n = 3$) came from the Brønnøy district. The mean number (CI) of ticks infesting dogs was 2.8 (1.8–3.7) in the Brønnøy district and 1.1 (1.0–1.1) in the 7 northern districts. In cats, the mean number (CI) was 3.1 (2.3–3.9) and 1.1 (1.1–1.2), respectively. The mean number of ticks from Brønnøy was significantly higher when compared with those from the 7 northern districts for both dogs ($P = 0.001$) and cats ($P < 0.001$).

Borrelia prevalence and genospecies

From 9 cats and one dog, all from the Brønnøy district, 56 ticks (35 females, 21 males) were excluded from the *Borrelia* analysis because of the possibility of co-feeding transmission. Consequently, the *Borrelia* prevalence in all 8 districts was calculated from 493 ticks (439 females, 48 males, 4 nymphs; no instar data from $n = 2$). Of these, 66 (13%) contained *Borrelia* as detected by real-time PCR.

In Brønnøy, 54 of 188 (29%) ticks contained *Borrelia* (Table 2), and the percentages of female, male, and nymphal ticks in Brønnøy containing *Borrelia* were 26% (37/144), 41% (17/41), and 0% (0/3), respectively. In the 7 districts north of Brønnøy, 12 (all female) out of 305 ticks (4%) were *Borrelia*-positive (Table 2). The Brønnøy district had a significantly higher *Borrelia* prevalence than the 7 northern districts ($P < 0.001$).

Borrelia genospecies were determined in 51 out of 66 infected ticks (Table 2). The proportion of ticks with *B. afzelii* to the number of ticks with *B. garinii* and/or *B. valaisiana*, was higher in Brønnøy (28:16) than in the 7 northern districts (2:6). The *Borrelia* prevalence of fed female ticks was significantly lower than in unfed male and female ticks ($P < 0.05$) (Table 3).

Discussion

In this study, we estimated *I. ricinus* occurrence and *Borrelia* prevalence at the tick's northern distribution limit in the vicinity of the Arctic Circle in Norway. In the northern study area, tick

Table 2
Borrelia PCR-positive samples and *Borrelia* genospecies in *I. ricinus* in 8 districts of northern Norway ($n = 493$).

District centre[a]	*Borrelia*-positive[b]	*Borrelia afzelii*	*Borrelia garinii*	*Borrelia valaisiana*	*Borrelia* mixture[c]	Species not determined
Harstad (C)	1	1	–	–	–	–
Bodø (C)	2	1	1	–	–	–
Fauske (F)	1	–	–	–	–	1
Ørnes (C)	1	–	1	–	–	–
Mo i Rana (F)	4	–	2	–	–	2
Sandnessjøen (C)	0	–	–	–	–	–
Mosjøen (F)	3	–	2	–	–	1
Brønnøy (C)	54	28	11	2	2	11
Total	66	30	17	2	2	15

C, located at the coast; F, located at the head of a fjord.
[a] The districts are named after the largest municipality (city) in the region.
[b] Only female ticks ($n = 48$), except in Brønnøy (male ticks $n = 17$); in Ørnes no instar data ($n = 1$).
[c] In the Brønnøy district, there was a mixture of *B. burgdorferi*/*B. valaisiana* in one tick and *B. garinii*/*B. burgdorferi* sensu stricto/*B. valaisiana* in another tick.

occurrence and *Borrelia* prevalence were lower than in the Brønnøy district.

Our findings on tick occurrence in northern Norway are different from older studies. Tambs-Lyche (1943) received and reported on adult ticks from domestic animals in Brønnøy, and Mehl (1983) observed ticks in the vegetation, on host animals, and on birds in Brønnøy, but *I. ricinus* was not reported further north in these articles. However, in 2009, a study was performed where the number of ticks attached to dogs and cats brought to veterinary clinics were counted, and it was shown that the number of ticks per animal was similar in Telemark county (southern Norway) and Brønnøy, but much lower in the region north of Brønnøy (Jenkins et al., 2012).

An abrupt change in tick abundance along the North-South axis has been noted in Finland (Öhman, 1961) and in Sweden where it coincides with the *Limes Norrlandicus* ecotone (Tälleklint and Jaenson, 1998). Three decades ago, *I. ricinus* was very infrequent north of the *Limes Norrlandicus* in Sweden, while recent surveys indicate that the tick appears to have extended its range northwards in the subsequent decades (Jaenson et al., 2012). In the present study, there seems to be a boundary some 6° north of that in Sweden, and this may be due to the warming effect of the Gulf Stream (Bischof et al., 2003). Global warming is also expected to facilitate a northward expansion of the range of *I. ricinus*, and it is predicted that changes in vector and disease distribution will be noticed first at the peripheries of the tick's distribution (Gray et al., 2009). In Norway, despite an increase in the mean annual temperature in the past 20 years (http://www.met.no/), ticks remain scarce north of Brønnøy. Nonetheless, even if suitable habitats for *I. ricinus* further north do not exist, yet, it will be interesting to monitor whether they emerge as a result of the predicted temperature increase of 1.5–2.5 °C over the next few decades in northern temperate Europe (Gray et al., 2009).

Although a mountain plateau and a glacier, partly 'covering' the Norwegian part of the Arctic Circle, are natural barriers for northward expansion of terrestrial wildlife, ticks may be transported further northward by migratory birds or cervids. Roe deer live and are hunted in the southern coastal part of the study area, and elks live sparsely in the studied area of northern Norway (http://www.hjortevilt.no/). However, the relationship between tick distribution and cervids needs to be studied in Norway (Medlock et al., 2013).

Although the methods applied in this study do not provide information on whether or not tick populations have established in areas north of the Arctic Circle, several scenarios may be envisaged. In case there are established populations of *I. ricinus* north of 65° N, their very low density or a very patchy distribution might explain why only nymphs and no larvae at all, were found. In addition, the public's unawareness is an important factor; this is underscored by the fact that no tick larvae were collected in Brønnøy in this study, although larvae have been sampled regularly for years in this district (D. Hvidsten, pers. communication). The absence of larvae argues against the presence of established tick populations since the number of questing larvae usually exceeds that of nymphs and adult ticks by a factor of several tens (Randolph et al., 2002).

The *Borrelia* prevalence in ticks from the Brønnøy district (29%) is among the highest reported anywhere in Europe (Rauter and Hartung, 2005). Our findings are close to the highest previously reported prevalence in Norway, which is 31% in questing nymphs and adult ticks in the southernmost part of Norway (Kjelland et al., 2010). However, our estimate is a conservative one, since if 2 or more ticks, carrying the same *Borrelia* species, were found on the same animal, co-feeding transmission could not be ruled out and the ticks were excluded from the study, which will give a slight negative bias to the results. In addition, the majority of ticks in our study were engorged female ticks, and there was a difference between the *Borrelia* prevalence in fed female ticks (i.e., 20%) and unfed female and male ticks (i.e., 42%) in the Brønnøy district. The reason for this difference is not known. In cervids, complement factors in the blood have a negative effect on *Borrelia* (Kurtenbach et al., 2002b). This is in contrast to when ticks feed on competent hosts, then the borreliae in the mid-gut multiply and the bacterial count increases (Kurtenbach et al., 2002a). It is not known whether dogs are competent hosts for all *Borrelia* species occurring in Europe or whether they have complement factors with borreliacidal effects. Due to former *Borrelia* infections, a large proportion of the dogs and cats in Brønnøy may have antibodies, which may have borreliacidal effects and a negative effect on detection by PCR (Hovius et al., 2000). In another study, the authors found a decrease in *Borrelia* prevalence in ticks with an increasing degree of engorgement (Hovius et al., 1998).

Between 42° and 60° N, latitude does not seem to have any effect on the *Borrelia* prevalence of ticks in Europe (Gray et al., 1998), but to the north of 65° N, the observed decline in the number of ticks was accompanied by a low *Borrelia* prevalence in ticks (i.e., 4%). Based on national prevalence data, Gray et al. (1998) found that

Table 3
Borrelia prevalence (%) in fed and unfed ticks[a] from dogs and cats in Brønnøy ($n = 188$).

Ticks	Fed	Unfed
Females from dogs	19% (9/48)	27% (4/15)
Females from cats	23% (9/40)	71% (5/7)
Females (total)	20% (18/88)[b]	41% (9/22)
Females and males	–	42% (26/62)[b]

[a] Excluded ticks: females with non-distinct state of engorgement (i.e., fed/unfed) and/or missing data ($n = 34$); males with indistinct feeding status ($n = 1$); nymphs ($n = 3$).
[b] $p < 0.05$.

the abundance of adult ticks and the abundance of infected adult ticks are positively correlated. Anyhow, the *Borrelia* prevalence in nymphal and adult ticks in areas with tick densities close to zero per 100 m^2 is difficult to predict (Gray et al., 1998; Randolph, 2001). If the tick population in the northern part of the study area is in fact already established, the *Borrelia* prevalence appears to be low, as observed in newly established tick populations; but the *Borrelia* prevalence may also be mirrored by locations where the ticks come from (Ogden et al., 2006). The low tick occurrence and the low *Borrelia* prevalence in the northern part of the study area were further accompanied by a change in the *Borrelia* species composition. In the Brønnøy district, the dominant *Borrelia* species was *B. afzelii*, a species associated with rodents, while north of Brønnøy, although few *Borrelia*-positive ticks, the dominant species were *B. garinii* and/or *B. valaisiana*, which are associated with birds (Kurtenbach et al., 2002b). This could be explained on the basis that the tick population north of Brønnøy is mainly derived from migratory birds. In Canada, thousands of adult *Ixodes scapularis*, the North American counterpart of *I. ricinus*, were sent to public health organizations over more than a decade. Nearly all of them came from locations beyond the tick's established northern range, implying that these ticks were derived from engorged larvae and nymphs transported by and fed on migratory birds (Ogden et al., 2006), and as in our study, mostly female ticks and only 1% nymphs were collected. However, the role of migratory birds in the spread of *I. ricinus* ticks in northern Norway has to be further elucidated.

In 99% of the cases, the ticks obtained in this study were *I. ricinus*, the same percentage that was found in ticks collected in Sweden (Jaenson et al., 2012; Tälleklint and Jaenson, 1998). This contrasts with a study in veterinary practises in the British Isles where only 52% of ticks were *I. ricinus* (Ogden et al., 2000). In the present study, we found *I. trianguliceps* attached to pets, which, to our knowledge, has not been previously reported (Arthur, 1963; Hillyard, 1996; Jaenson et al., 1994; Ogden et al., 2000). In addition, we have no information that *H. marginatum marginatum*, a tick species from southern Europe and southwestern Asia, has been detected so far north. Being a 2-host species, makes it possible for both the larvae and nymphs to stay for 12–26 days on the same migratory bird (Hillyard, 1996), and thus, to reach northern latitudes.

In this study, we have attempted to assess tick occurrence by exploiting the general public to send ticks via veterinary clinics. Even though we have made efforts to ensure equal and plentiful information through media in all areas, we cannot be sure of equal public response to the survey. Only one veterinary clinic was chosen as the collection centre in each district, and pet owners who were not customers of this clinic may have under-responded. However, this does not apply to small communities where there is often only one veterinary practise. The effect of this skewness on the present results is unknown.

The area of this study covers what is probably one of the northernmost *I. ricinus* populations in the world, including an area of high tick abundance in the archipelago of Brønnøy only 125 km south of the Arctic Circle. Climate, day length, habitat, mixture of hosts, and host abundance seem favourable for *I. ricinus* in this area. The high *Borrelia* prevalence in ticks and tick occurrence in Brønnøy imply a significant risk of Lyme borreliosis. Further studies are needed to unravel if established tick populations exist north of the Arctic Circle.

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