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Distribution of *Neoehrlichia mikurensis* in *Ixodes ricinus* ticks along the coast of Norway: The western seaboard is a low-prevalence region

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Abstract

Neoehrlichia mikurensis is a tick-borne pathogen widespread among ticks and rodents in Europe and Asia. A previous study on *Ixodes ricinus* ticks in Norway suggested that *N. mikurensis* was scarce or absent on the south-west coast of Norway, but abundant elsewhere. The aim of this study was to further investigate the prevalence and distribution of *N. mikurensis* along the western seaboard of Norway in comparison with more eastern and northern areas. The second aim of the study was to examine seasonal variation of the bacterium in one specific location in the south-eastern part of Norway. Questing *I. ricinus* were collected from 13 locations along the coast of Norway, from Brønnøysund in Nordland County to Spjærøy in Østfold County. In total, 11,113 nymphs in 1,113 pools and 718 individual adult ticks were analysed for *N. mikurensis* by real-time PCR. The mean prevalence of *N. mikurensis* in adult ticks was 7.9% while the estimated pooled prevalence in nymphs was 3.5%. The prevalence ranged from 0% to 25.5%, with the highest prevalence in the southernmost and the northernmost locations. The pathogen was absent, or present only at low prevalence (<5%), at eight locations, all located in the west, from 58.9°N to 64.9°N. The prevalence of *N. mikurensis* was significantly different between counties ($p < .0001$). No significant seasonal variation of *N. mikurensis* prevalence was observed in the period May to October 2015. Our results confirm earlier findings of a low prevalence of *N. mikurensis* in the western seaboard of Norway.

KEYWORDS

Ixodes ricinus, *Neoehrlichia mikurensis*, pooled samples, real-time PCR, sequencing

Arnulf Soleng and Åshild K. Andreassen contributed equally to this study and manuscript.

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1 | INTRODUCTION

Neoehrlichia mikurensis is an emerging tick-borne pathogen. The bacterium's DNA was first discovered in 1999 in the Netherlands and was inferred to belong to an *Ehrlichia*-like species (Schouls, Van De Pol, Rijpkema, & Schot, 1999). In 2004, the bacterium was classified as a member of the *Anaplasmataceae* family and named *Candidatus Neoehrlichia mikurensis* (Kawahara et al., 2004). Isolation of the bacterium in pure culture has recently been reported, and the prefix "Candidatus" is no longer necessary (Wass et al., 2019). *Neoehrlichia mikurensis* has been found widespread in *Ixodes ricinus* ticks and rodents in Europe and Asia (Burri, Schumann, Schumann, & Gern, 2014; Li et al., 2013; Michelet et al., 2014; Palomar, Garcia-Alvarez, Santibanez, Portillo, & Oteo, 2014; Silaghi, Beck, Oteo, Pfeffer, & Sprong, 2016; Szekeres et al., 2015; Tabara et al., 2007; Wass et al., 2019). Although *I. ricinus* is the bacterium's main vector, questing *Ixodes persulcatus* and other tick species collected from their hosts have also been found infected (Blanarova et al., 2016; Kamani et al., 2013; Krucken et al., 2013; Rar et al., 2010; Silaghi, Woll, Mahling, Pfister, & Pfeffer, 2012). Rodents, such as bank voles (*Myodes glareolus*), other voles (*Microtus* spp.) and field mice (*Apodemus* spp.), are considered to be reservoirs for *N. mikurensis* and play an important role in the maintenance of the bacterium (Andersson & Raberg, 2011; Burri et al., 2014; Obiegala et al., 2014).

Neoehrlichia mikurensis may cause neoehrlichiosis in humans, primarily in immunocompromised individuals, although immunocompetent individuals may be infected, with milder symptoms (Quarsten et al., 2017; Wennerås, 2015). Symptoms of neoehrlichiosis include high and long-lasting fever, severe muscle and joint pain and a risk of thromboembolic events (Wennerås, 2015). Cases of neoehrlichiosis have been reported in several European countries, including Sweden, Germany, Czech Republic, Switzerland and Norway (Dadgar, Grankvist, Wernbro, & Wennerås, 2017; Frivik, Noraas, Grankvist, Wennerås, & Quarsten, 2017; von Loewenich et al., 2010; Maurer et al., 2013; Pekova et al., 2011). Although only one case of neoehrlichiosis has been so far reported in Norway (Frivik et al., 2017), *N. mikurensis* is the second most frequent pathogen in *I. ricinus* after *Borrelia afzelii* (Jenkins et al., 2019; Kjelland et al., 2018).

Norway is a long country, covering several climatic zones, and therefore has great variation in vegetation and animal life (Moen, Lillethun, & Odland, 1999; Peel, Finlayson, & McMahon, 2007). *Ixodes ricinus* is found in coastal regions from Østfold County in south-eastern Norway to the southern part of Nordland County in the north (Mehl, 1983; Soleng et al., 2018). *Neoehrlichia mikurensis* was first found in south-eastern Norway in ticks collected in 1999 and 2000 (Jenkins & Kristiansen, 2013). Recently the bacterium was detected in southern, eastern and northern Norway, but not in the south-western part of Norway (Jenkins et al., 2019; Kjelland et al., 2018; Larsson, Hvidsten, Stuen, Henningsson, & Wilhelmsson, 2018). This raises the question of whether there is a cold spot for *N. mikurensis* on the west coast of the country. The aim of this study was to further investigate the prevalence and distribution of

Impacts

- The western seaboard of Norway is a low-prevalence area of *Neoehrlichia mikurensis* bordered by high-prevalence areas to the North and South.
- Northern and Southern Norway are high-prevalence areas and are expected to be risk areas for neoehrlichiosis.
- Investigating the cause of this prevalence variation may cast light on the bacterium's infectious cycle.

N. mikurensis along the western coast of Norway in comparison to more eastern and northern areas. Furthermore, we wanted to examine seasonal variation in prevalence of the bacteria at one specific location in the south-eastern part of Norway.

2 | METHODS

2.1 | Study area and tick collection

Questing *I. ricinus* were collected by flagging (Hillyard, 1996) from 13 locations along the coast of Norway, from Brønnøysund in Nordland County to the island of Spjærøy in Østfold County (Figure 1). Flagging was mainly conducted in moist deciduous forests with rich undergrowth, where traces of rodents and cervids were often observed (Table 1). Each collection site was sampled once during May or June in 2014, 2015 or 2016. From the location in Spjærøy, ticks were collected at 3- to 5-week intervals from May to October 2015. Nymphs and adult ticks were included in the study. In total, 11,130 nymphs and 718 adult ticks were investigated. Nymphs were analysed in pools of ten, while adults were analysed individually. Collection and storage of ticks, extraction of total RNA from nymphs and total nucleic acid from adults and preparation of cDNA have been previously described by Andreassen et al. (2012) and Paulsen et al. (2015).

2.2 | Detection of *Neoehrlichia mikurensis*

Reverse-transcribed total nucleic acid from individual adult ticks and reverse-transcribed RNA from nymphs in pools of ten were analysed with a *N. mikurensis* specific real-time PCR (Jenkins et al., 2019) using SYBR Green PCR Master Mix on the StepOne PCR system (Applied Biosystems). Samples from Spjærøy were analysed using PerfeCTa SYBR Green FastMix (Quantabio) on the Rotor-Gene Q (QIAGEN). A synthetic plasmid containing the target sequence cloned in vector pUC57 (GenScript) was used as positive control and nuclease-free water as negative control. Controls were included in each real-time PCR run.

SYBR Green gives stronger signals compared to probe, but may bind unspecifically. Hence, all positive samples were reanalysed, using a specific probe targeting the *groEL* gene (Jenkins et al., 2019). Only samples positive with both tests were considered true positives. Due to low sample volume, all samples were diluted 1:2 in both PCR tests and two samples from Lote and one

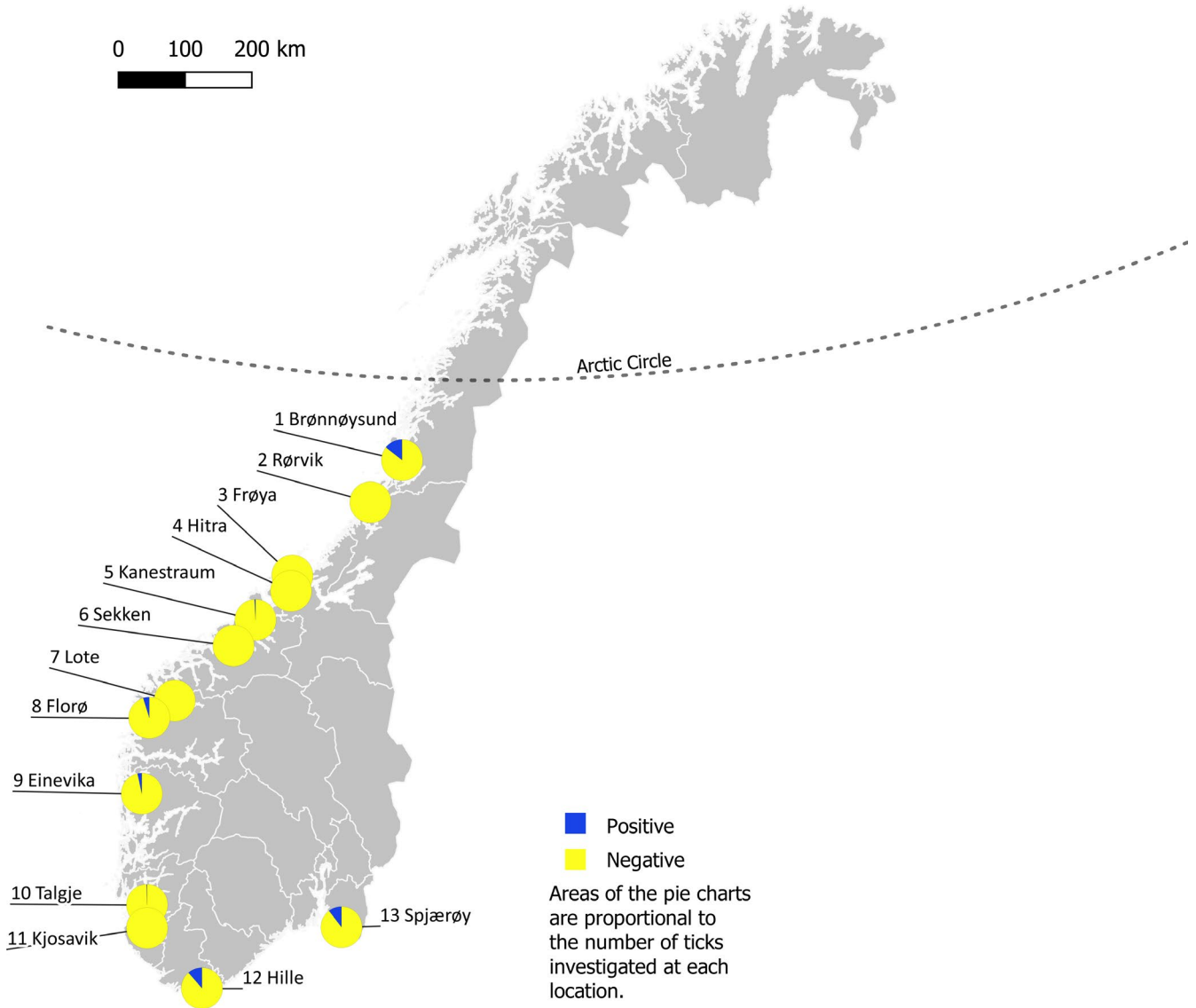


FIGURE 1 Map of Norway showing the 13 collection sites of *Ixodes ricinus* ticks from Spjørøy in south-east to Brønnøysund in north. Ticks were analysed for *Neohhrlichia mikurensis*. The blue area of the pie charts indicates the proportion of positives at the collection site and is the weighted mean of the prevalence in nymphs and adult ticks. Location number corresponds to location numbers in Tables 1 and 2. At Spjørøy, ticks were collected with 3–5 week intervals from May to October 2015

sample from Brønnøysund were only analysed using the probe test. Unfortunately, adult ticks collected from Spjørøy in early June, as part of the seasonal study, were unavailable for analysis and were not included in the study.

Nineteen samples were sequenced. The samples were randomly chosen from samples positive by SYBR Green, before confirmation by probe-based PCR. Sequencing on 3130xl Genetic Analyzer (Applied Biosystems) was performed as previously described by Jenkins et al. (2019).

2.3 | Statistics

The estimated pooled prevalence (EPP) with confidence intervals for pooled nymphs was estimated using EpiTools epidemiological calculator (Sergeant, 2019). The 95% confidence intervals for

the prevalence in adult ticks were calculated using the following formulae:

$$P_L = \frac{(2np + z_{\alpha/2}^2 - 1) - z_{\alpha/2} \cdot \sqrt{z_{\alpha/2}^2 - \{2 + (1/n)\} + 4p(nq + 1)}}{2(n + z_{\alpha/2}^2)}$$

$$P_U = \frac{(2np + z_{\alpha/2}^2 + 1) + z_{\alpha/2} \cdot \sqrt{z_{\alpha/2}^2 + \{2 - (1/n)\} + 4p(nq - 1)}}{2(n + z_{\alpha/2}^2)}$$

P_L and P_U are the lower and upper confidence limits, respectively, n is the number of samples, p and q are the proportions of positive and negative samples, and $z_{\alpha/2}$ is the critical value of the normal

TABLE 1 Description of collection sites of *Ixodes ricinus*

Collection number	Location name	County	Coordinates	Date of sampling	Description of collection site
1	Brønnøysund	Nordland	65.4°N 12.1°E	June 2015	Small deciduous trees, grass, ferns and heather. Numerous rodent burrows, bedding sites and tracks from roe deer
2	Rørвик	Trøndelag	64.9°N 11.1°E	June 2015	Small deciduous trees, grass, ferns and heather. Numerous rodent burrows, bedding sites and tracks from roe deer and moose
3	Frøya	Trøndelag	63.8°N 8.8°E	June 2014	Field with small bushes and grass. A combination of a planted pine forest and some deciduous trees with an undergrowth of grass. Many tracks from red deer
4	Hitra	Trøndelag	63.6°N 8.9°E	June 2014	Birch forest, heather, grass, numerous rodent burrows and tracks from red deer
5	Kanestraum	Møre og Romsdal	63.1°N 8.1°E	May 2014	Moist deciduous forest, mostly birch and alder. Grass, ferns and heather
6	Sekken	Møre og Romsdal	62.7°N 7.3°E	May 2014	Birch forest at the edge of a field. Undergrowth consisting of grass. Bedding sites and tracks from roe deer
7	Lote	Sogn og Fjordane	61.9°N 6.1°E	June 2016	Steep hillside with deciduous trees, grass, ferns and heather. Numerous tracks from red deer
8	Florø	Sogn og Fjordane	61.6°N 5.3°E	June 2016	Deciduous trees with grass, ferns and heather. Some rodent burrows and some tracks from cervids
9	Einevika	Hordaland	60.7°N 5.6°E	June 2016	Deciduous forest and undergrowth consisting of grass. Traces of cervids
10	Talgje	Rogaland	59.1°N 5.8°E	June 2016	Deciduous forest and undergrowth consisting of grass and ferns. Close to a grazing area for livestock
11	Kjosavik	Rogaland	58.9°N 5.9°E	June 2015	Deciduous forest and undergrowth consisting of grass
12	Hille	Vest-Agder	58.0°N 7.4°E	May 2015	Deciduous trees, grass, herbs and shrubs. Numerous rodent burrows and tracks from roe deer
13	Spjærøy	Østfold	59.1°N 10.9°E	May–Oct 2015	Mixed forest, grass, ferns and heather. Some rodent burrows and tracks from roe deer

distribution for $\alpha/2$, in this case 1.96. If p or $q \leq 5/n$, the confidence limits are not valid and were not reported (Fleiss, 1981; Jenkins et al., 2019).

The chi-square test was performed to test for statistical monthly variation of *N. mikurensis* at Spjærøy and differences in prevalence between locations.

The weighted mean of the prevalence in nymphs and adult ticks was calculated to indicate the proportion of positives used in Figure 1.

3 | RESULTS

In total, 57 of 718 adult ticks (7.9%) and 333 of 1,113 nymph pools (EPP 3.5%) were positive for *N. mikurensis* (Table 2). Further, five adults and 17 nymph pools were positive by real-time PCR using SYBR Green, but could not be confirmed by real-time PCR using probe (data not shown). These samples were considered false positives.

Seventeen of 19 samples were confirmed as *N. mikurensis* by sequencing. The 72 base pair long sequence between the primers showed no sequence differences between sampling locations and

shared 100% identity to several sequences submitted to GenBank (e.g. MN151367). Samples negative by sequencing were also negative by real-time PCR using probe (false positives; see above).

The highest *N. mikurensis* prevalences were found in adults from Hille in Vest-Agder County (location 12; 58.0°N) and Brønnøysund in Nordland County (location 1; 65.4°N). At Hille, the prevalence of *N. mikurensis* was 25.5% in adult ticks and 9.9% (EPP) in nymphs. In Brønnøysund, the prevalence was 23.8% in adult ticks and 7.8% (EPP) in nymphs. In the intervening region, ten localities, along the coast from Kjosavik in Rogaland County (location 11; 58.9°N) to Rørвик in Trøndelag County (location 2; 64.5°N), the prevalence in adult ticks was <5%, with the exception of two locations, Florø (location 8; 61.6°N; 6.5%) and Einevika (location 9; 60.7°N; 15.4%). The EPP in nymphs was <5% at all 10 locations. At five of these locations, the observed prevalence was zero in both adult ticks and nymphs (Figure 1; Table 2).

In order to obtain more robust statistics for geographical comparison, results from the 13 locations were combined on the basis of county ($N = 8$) before performing the chi-square test. The prevalence of *N. mikurensis* varied significantly between counties, both in pooled nymphs ($\chi^2 = 468.0$; $df = 7$; $p < .0001$) and individual adults ($\chi^2 = 82.4$; $df = 7$; $p < .0001$).

TABLE 2 Prevalence of *Neoehrlichia mikurensis* in *Ixodes ricinus* ticks

Location number	Location name	<i>Neoehrlichia mikurensis</i> in adult ticks		<i>Neoehrlichia mikurensis</i> in nymphs	
		Positive ticks/total adult ticks analysed	Prevalence % ^a	Positive pools of nymphs/total pools analysed ^b	EPP % ^a
1	Brønnøysund	15/63	23.8 (14.6–37.0)	5/9	7.8 (2.4–18.0)
2	Rørvik	0/104	0	0/74	0
3	Frøya	0/47	0	0/74	0
4	Hitra	0/46	0	0/74	0
5	Kanestraum	2/61	3.3 ^c	4/74	0.6 (0.2–1.4)
6	Sekken	0/19	0	0/74	0
7	Lote	0/43	0	0/74	0
8	Florø	3/46	6.5 ^c	22/58	4.7 (2.9–7.0)
9	Einevika	2/13	15.4 ^c	15/56	3.1 (1.7–5.0)
10	Talgje	0/40	0	2/48	0.4 (0.1–1.5)
11	Kjosavik	0/34	0	0/64	0
12	Hille	13/51	25.5 (14.8–39.9)	31/48	9.9 (6.6–14.0)
13	Spjærøy ^d	22/151	14.6 (9.6–21.4)	254/386	10.2 (8.9–11.5)
	Total	57/718	7.9 (6.1–10.2)	333/1113	3.5 (3.1–3.9)

Abbreviation: EPP, estimated pooled prevalence.

^a95% confidence interval in parentheses.

^bEach pool consists of 10 nymphs.

^cThe proportion of positive samples is $<5/n$, and the confidence interval could not be calculated.

^dAt Spjærøy, ticks were collected with 3–5 week intervals from May to October 2015.

3.1 | Seasonal variation of *Neoehrlichia mikurensis* at Spjærøy

Seasonal variation of *N. mikurensis* prevalence was studied at Spjærøy in Østfold County (location 13; 59.1°N) between May and October. The mean prevalence in adult ticks was 14.6%, and the mean EPP in nymphs was 10.2% (Table 3). The prevalence varied between 6.7% and 28.0% in adult ticks, and between 8.6% and 12.9% (EPP) in nymph pools, but this was not statistically significant, neither in pooled nymphs ($\chi^2 = 3.76$; $df = 5$; $p = .59$) nor in individual adults ($\chi^2 = 6.77$; $df = 4$; $p = .15$).

4 | DISCUSSION

This study confirms a previous report of low prevalence of *N. mikurensis* on the south-west coast of Norway (Jenkins et al., 2019). Our results indicate that the low-prevalence region extends along the coast from 64.9°N (Rørvik) to 58.9°N (Kjosavik) and, on the basis of the data of Jenkins et al. (2019), it may extend as far south as 58.2°N. Beyond this region, prevalence rises sharply both northward (Brønnøysund, 65.4°N; 7.8%) and southward (Hille, 58.0°N; 9.9%). Within the low-prevalence region, there seems to be a pocket of higher prevalence between Florø (61.6°N; 4.7%) and Einevika (60.7°N; 3.1%). These prevalences are for nymphs, but the same pattern is observed for adults. Although the prevalence of other tick-borne pathogens in Norway is known to vary from place to

place (Kjelland et al., 2018; Paulsen et al., 2015; Soleng et al., 2018; Soleng & Kjelland, 2013; Tveten, 2014a, 2014b), we are not aware of any study showing such a clear and sharply delineated area of reduced prevalence. *Borrelia afzelii* and *N. mikurensis* have been found co-infecting ticks with a higher prevalence than is expected by random chance (Andersson, Bartkova, Lindstad, & Raberg, 2013; Andersson, Scherman, & Raberg, 2014; Kjelland et al., 2018). Because of this association, it would be particularly interesting to investigate whether *B. afzelii* shows a similar distribution. The low prevalence of *N. mikurensis* in western regions cannot at present be compared with the incidence of neoehrlichiosis in humans, as only one case has so far been reported in Norway and the disease is neither notifiable nor routinely diagnosed (Frivik et al., 2017). The low incidence of neoehrlichiosis may be due to lack of diagnosing the disease or low pathogenicity of the bacterium circulating in Norway.

Western Norway receives considerably more rain than the rest of the country (Moen et al., 1999) and climate factors seem a plausible explanation for the low prevalence of *N. mikurensis*. Microclimatic conditions, such as temperature, saturation deficit and relative humidity, are important for the tick activity and behaviour and may also affect the transmission of tick-borne pathogens (Andreassen et al., 2012; Burri, Bastic, Maeder, Patalas, & Gern, 2011; Ostfeld, Levi, Keesing, Oggenfuss, & Canham, 2018). A high relative humidity may cause the ticks to quest higher in the vegetation and lead to their parasitizing different hosts (Randolph & Storey, 1999). Small rodents are an important reservoir for *N. mikurensis*, and if ticks quest higher in the vegetation, they may

TABLE 3 Prevalence of *Neoehrlichia mikurensis* in *Ixodes ricinus* ticks at Spjærøy (Østfold County), 2015

Date of sampling	<i>Neoehrlichia mikurensis</i> in adult ticks		<i>Neoehrlichia mikurensis</i> in nymphs	
	Positive ticks/ total adult ticks analysed	Prevalence ^a	Positive pools of nymphs/total pools analysed ^b	% EPP ^a
8th May	2/30	6.7 ^c	46/74	9.3 (6.7–12.3)
5th June	—	—	51/74	11.0 (8.1–14.5)
29th June	4/22	18.2 ^c	44/74	8.6 (6.2–11.6)
6th August	2/29	6.9 ^c	24/32	12.9 (8.0–19.5)
7th September	7/45	15.5 (8.5–32.6)	47/72	10.0 (7.3–13.3)
8th October	7/25	28.0 (11.7–52.3)	42/60	11.3 (8.0–15.4)
Total	22/151	14.5 (9.6–21.4)	254/386	10.2 (8.9–11.5)

Abbreviation: EPP, estimated pooled prevalence.

^a95% confidence interval in parentheses.

^bEach pool consists of 10 nymphs.

^cThe proportion of positive samples are $<5/n$, and the confidence interval could not be calculated.

parasitize larger hosts that are not reservoirs for the bacterium. Whether larger mammals are suitable reservoir hosts for *N. mikurensis* is not at present known. For *Borrelia burgdorferi*, it is shown that some tick hosts' immune systems kill the bacterium in the tick gut (Belperron & Bockenstedt, 2001), but whether corresponding mechanisms apply for *N. mikurensis* is not known. Alternatively, the low prevalence observed might be due to a lack of reservoir-competent small rodent hosts. Detailed information on the distribution of small rodents in Norway is lacking and, in the light of our findings, it would merit more study. Lastly, at the present stage, we cannot entirely exclude the possibility that the observed low *N. mikurensis* prevalence is the chance result of patchy distribution and year-to-year variation (Grzeszczuk & Stanczak, 2006; Zeman, 1997). Hence, further studies, investigating climatically comparable locations as well as the reproducibility of our results, are needed.

The prevalence of *N. mikurensis* in adults at Hille (25.5%) and in Brønnøysund (23.8%) was comparable to the highest prevalences ever reported in Europe (Derdakova et al., 2014; Silaghi et al., 2016, 2012). The high prevalence in Brønnøysund is supported by findings in Brønnøy area in Northern Norway by Larsson et al. (2018), where the prevalence in questing nymphs and adults was 18%. Jenkins et al. (2019) found no difference in prevalence of *N. mikurensis* between nymphs and adults and inferred this to imply that *N. mikurensis* is acquired during the first blood meal. We find a higher prevalence in adults (7.9%) than in nymphs (3.5%), which calls that conclusion into question. However, the difference we observed is not amenable to statistical testing as the adult ticks were analysed individually and the nymphs in pools. Because the precision of EPP declines at high prevalence, pooled sampling at the high-prevalence areas, Brønnøysund, Hille and Spjærøy, is not ideal (Ebert, Brlansky, & Rogers, 2010). Hence, further studies of *N. mikurensis*, particularly when nymphs and adult ticks are compared, should study individual nymphs.

This study also investigated seasonal variation of *N. mikurensis* prevalence in ticks at one of the sites (Spjærøy, Østfold County). A previous study from Norway found a significantly higher

prevalence of the bacterium in May than in June or July (Jenkins et al., 2001), while a study from the Netherlands reported a peak of *N. mikurensis* in ticks in October (Coipan et al., 2013). We collected ticks with 3–5 weeks interval from May to October at Spjærøy, and could also see a peak in October in adults, but the seasonal variation was not significant. However, the number of adults collected at each date of collection is low, resulting in low statistical power. In addition, this study only investigated prevalence variation in 2015, and the seasonal variation might vary from year to year. Further studies should look for seasonal variations at different locations and year-to-year variations, considering changes in climatic conditions and variations in population densities in host animals.

Our data confirm that Norway is a high-prevalence area for *N. mikurensis*, but that it includes a semi-continuous area of low prevalence along the western seaboard from 58.9°N to 64.9°N. Investigating the cause of this may cast light on the infectious cycle of *N. mikurensis*.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

- Andersson, M., Bartkova, S., Lindestad, O., & Raberg, L. (2013). Co-infection with 'Candidatus Neoehrlichia mikurensis' and *Borrelia afzelii* in *Ixodes ricinus* ticks in southern Sweden. *Vector-Borne and Zoonotic Diseases*, 13(7), 438–442. <https://doi.org/10.1089/vbz.2012.1118>
- Andersson, M., & Raberg, L. (2011). Wild rodents and novel human pathogen candidatus *Neoehrlichia mikurensis*, Southern Sweden. *Emerging Infectious Diseases*, 17(9), 1716–1718. <https://doi.org/10.3201/eid1709.101058>
- Andersson, M., Scherman, K., & Raberg, L. (2014). Infection dynamics of the tick-borne pathogen "Candidatus *Neoehrlichia mikurensis*" and coinfections with *Borrelia afzelii* in bank voles in Southern Sweden. *Applied and Environment Microbiology*, 80(5), 1645–1649. <https://doi.org/10.1128/aem.03469-13>
- Andreassen, A., Jore, S., Cuber, P., Dudman, S., Tengs, T., Isaksen, K., ... Vainio, K. (2012). Prevalence of tick borne encephalitis virus in tick nymphs in relation to climatic factors on the southern coast of Norway. *Parasit Vectors*, 5, 177. <https://doi.org/10.1186/1756-3305-5-177>
- Belperron, A. A., & Bockenstedt, L. K. (2001). Natural antibody affects survival of the spirochete *Borrelia burgdorferi* within feeding ticks. *Infection and Immunity*, 69(10), 6456–6462. <https://doi.org/10.1128/iai.69.10.6456-6462.2001>
- Blanarova, L., Stanko, M., Miklisova, D., Vichova, B., Mosansky, L., Kraljik, J., ... Derdakova, M. (2016). Presence of *Candidatus Neoehrlichia mikurensis* and *Babesia microti* in rodents and two tick species (*Ixodes ricinus* and *Ixodes trianguliceps*) in Slovakia. *Ticks and Tick-borne Diseases*, 7(2), 319–326. <https://doi.org/10.1016/j.ttbdis.2015.11.008>
- Burri, C., Bastic, V., Maeder, G., Patalas, E., & Gern, L. (2011). Microclimate and the zoonotic cycle of tick-borne encephalitis virus in Switzerland. *Journal of Medical Entomology*, 48(3), 615–627. <https://doi.org/10.1603/ME10180>
- Burri, C., Schumann, O., Schumann, C., & Gern, L. (2014). Are *Apodemus* spp. mice and *Myodes glareolus* reservoirs for *Borrelia miyamotoi*, *Candidatus Neoehrlichia mikurensis*, *Rickettsia helvetica*, *R. monacensis* and *Anaplasma phagocytophilum*? *Ticks and Tick-borne Diseases*, 5(3), 245–251. <https://doi.org/10.1016/j.ttbdis.2013.11.007>
- Coipan, E. C., Jahfari, S., Fonville, M., Maassen, C. B., van der Giessen, J., Takken, W., ... Sprong, H. (2013). Spatiotemporal dynamics of emerging pathogens in questing *Ixodes ricinus*. *Frontiers in Cellular and Infection Microbiology*, 3, 36. <https://doi.org/10.3389/fcimb.2013.00036>
- Dadgar, A., Grankvist, A., Wernbro, L., & Wennerås, C. (2017). Oklar feber hos patient med MS och rituximab-behandling var neoehrlichios - Ny fastingburen infektion som ar svar att diagnostisera. *Lakartidningen*, 114, 1–4.
- Derdakova, M., Vaclav, R., Pangracova-Blanarova, L., Selyemova, D., Koci, J., Walder, G., & Spitalska, E. (2014). *Candidatus Neoehrlichia mikurensis* and its co-circulation with *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks across ecologically different habitats of Central Europe. *Parasit Vectors*, 7, 160. <https://doi.org/10.1186/1756-3305-7-160>
- Ebert, T. A., Brlansky, R., & Rogers, M. (2010). Reexamining the pooled sampling approach for estimating prevalence of infected insect vectors. *Annals of the Entomological Society of America*, 103(6), 827–837. <https://doi.org/10.1603/ANO9158>
- Fleiss, J. L. (1981). *Statistical methods for rates and proportions*. New York, NY: Wiley.
- Frivik, J. O., Noraas, S., Grankvist, A., Wennerås, C., & Quarsten, H. (2017). A man in his sixties from Southern Norway with intermittent fever. *Tidsskrift for Den Norske Laegeforening*, 137(23–24), 1900–1902. <https://doi.org/10.4045/tidsskr.17.0353>
- Grzeszczuk, A., & Stanczak, J. (2006). Highly variable year-to-year prevalence of *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in northeastern Poland: A 4-year follow-up. *Annals of the New York Academy of Sciences*, 1078, 309–311. <https://doi.org/10.1196/annals.1374.057>
- Hillyard, P. D. (1996). *Ticks of North-West Europe (Vol. No. 52, Synopses of the British fauna)*. Shrewsbury, UK: Field Studies Council.
- Jenkins, A., & Kristiansen, B. E. (2013). *Neoehrlichia*—a new tick-borne bacterium. *Tidsskrift for Den Norske Laegeforening*, 133(10), 1058–1059. <https://doi.org/10.4045/tidsskr.13.0314>
- Jenkins, A., Kristiansen, B. E., Allum, A. G., Aakre, R. K., Strand, L., Kleveland, E. J., ... Schouls, L. (2001). *Borrelia burgdorferi* sensu lato and *Ehrlichia* spp. in *Ixodes* ticks from southern Norway. *Journal of Clinical Microbiology*, 39(10), 3666–3671. <https://doi.org/10.1128/jcm.39.10.3666-3671.2001>
- Jenkins, A., Raasok, C., Pedersen, B. N., Jensen, K., Andreassen, Å., Soleng, A., ... Kristiansen, B. E. (2019). Detection of *Candidatus Neoehrlichia mikurensis* in Norway up to the Northern limit of *Ixodes ricinus* distribution using a novel real time PCR test targeting the *groE* gene. *BMC Microbiology*, 19(1), 199. <https://doi.org/10.1186/s12866-019-1502-y>
- Kamani, J., Baneth, G., Mumcuoglu, K. Y., Waziri, N. E., Eyal, O., Guthmann, Y., & Harrus, S. (2013). Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria. *PLoS Neglected Tropical Diseases*, 7(3), e2108. <https://doi.org/10.1371/journal.pntd.0002108>
- Kawahara, M., Rikihisa, Y., Isogai, E., Takahashi, M., Misumi, H., Suto, C., ... Tsuji, M. (2004). Ultrastructure and phylogenetic analysis of 'Candidatus *Neoehrlichia mikurensis*' in the family Anaplasmataceae, isolated from wild rats and found in *Ixodes ovatus* ticks. *International Journal of Systematic and Evolutionary Microbiology*, 54(Pt 5), 1837–1843. <https://doi.org/10.1099/ijs.0.63260-0>
- Kjelland, V., Paulsen, K. M., Rollum, R., Jenkins, A., Stuen, S., Soleng, A., ... Andreassen, Å. K. (2018). Tick-borne encephalitis virus, *Borrelia burgdorferi* sensu lato, *Borrelia miyamotoi*, *Anaplasma phagocytophilum* and *Candidatus Neoehrlichia mikurensis* in *Ixodes ricinus* ticks collected from recreational islands in southern Norway. *Ticks and Tick-borne Diseases*, 9(5), 1098–1102. <https://doi.org/10.1016/j.ttbdis.2018.04.005>
- Krucken, J., Schreiber, C., Maaz, D., Kohn, M., Demeler, J., Beck, S., ... von Samson-Himmelstjerna, G. (2013). A novel high-resolution melt PCR assay discriminates *Anaplasma phagocytophilum* and "Candidatus *Neoehrlichia mikurensis*". *Journal of Clinical Microbiology*, 51(6), 1958–1961. <https://doi.org/10.1128/jcm.00284-13>
- Larsson, C., Hvidsten, D., Stuen, S., Henningsson, A. J., & Wilhelmsson, P. (2018). "Candidatus *Neoehrlichia mikurensis*" in *Ixodes ricinus* ticks collected near the Arctic Circle in Norway. *Parasit Vectors*, 11(1), 620. <https://doi.org/10.1186/s13071-018-3168-y>
- Li, H., Jiang, J., Tang, F., Sun, Y., Li, Z., Zhang, W., ... Cao, W. (2013). Wide distribution and genetic diversity of "Candidatus *Neoehrlichia mikurensis*" in rodents from China. *Applied and Environment Microbiology*, 79(3), 1024–1027. <https://doi.org/10.1128/aem.02917-12>
- Maurer, F. P., Keller, P. M., Beuret, C., Joha, C., Achermann, Y., Gubler, J., ... Bloembergen, G. V. (2013). Close geographic association of human neoehrlichiosis and tick populations carrying "Candidatus *Neoehrlichia mikurensis*" in eastern Switzerland. *Journal of Clinical Microbiology*, 51(1), 169–176. <https://doi.org/10.1128/jcm.01955-12>
- Mehl, R. (1983). The distribution and host relations of Norwegian ticks (Acari, Ixodidae). *Fauna Norwagica Series B*, 30, 46–51.

- Michelet, L., Delannoy, S., Devillers, E., Umhang, G., Aspan, A., Juremalm, M., ... Moutailler, S. (2014). High-throughput screening of tick-borne pathogens in Europe. *Frontiers in Cellular and Infection Microbiology*, 4, 103. <https://doi.org/10.3389/fcimb.2014.00103>
- Moen, A., Lillethun, A., & Odland, A. (1999). In A. Lillethun (Ed.), *Vegetation National Atlas of Norway*. Hønefoss, Norway: Norwegian Mapping Authority.
- Obiegala, A., Pfeffer, M., Pfister, K., Tiedemann, T., Thiel, C., Balling, A., ... Silaghi, C. (2014). *Candidatus Neoehrlichia mikurensis* and *Anaplasma phagocytophilum*: Prevalences and investigations on a new transmission path in small mammals and ixodid ticks. *Parasit Vectors*, 7, 563. <https://doi.org/10.1186/s13071-014-0563-x>
- Ostfeld, R. S., Levi, T., Keesing, F., Oggenfuss, K., & Canham, C. D. (2018). Tick-borne disease risk in a forest food web. *Ecology*, 99(7), 1562–1573. <https://doi.org/10.1002/ecy.2386>
- Palomar, A. M., Garcia-Alvarez, L., Santibanez, S., Portillo, A., & Oteo, J. A. (2014). Detection of tick-borne 'Candidatus *Neoehrlichia mikurensis*' and *Anaplasma phagocytophilum* in Spain in 2013. *Parasit Vectors*, 7, 57. <https://doi.org/10.1186/1756-3305-7-57>
- Paulsen, K. M., Pedersen, B. N., Soleng, A., Okbaldet, Y. B., Petterson, J. H., Dudman, S. G., ... Andreassen, A. (2015). Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* ticks from three islands in north-western Norway. *Apmis*, 123(9), 759–764. <https://doi.org/10.1111/apm.12412>
- Peel, M. C., Finlayson, B. L., & McMahon, T. A. (2007). Updated world map of the Köppen-Geiger climate classification. *Hydrology and Earth System Sciences Discussions*, 4(2), 439–473. <https://doi.org/10.5194/hessd-4-439-2007>
- Pekova, S., Vydra, J., Kabickova, H., Frankova, S., Haugvicova, R., Mazal, O., ... Kozak, T. (2011). *Candidatus Neoehrlichia mikurensis* infection identified in 2 hematologic patients: Benefit of molecular techniques for rare pathogen detection. *Diagnostic Microbiology and Infectious Disease*, 69(3), 266–270. <https://doi.org/10.1016/j.diagmicrobio.2010.10.004>
- Quarsten, H., Grankvist, A., Hoyvoll, L., Myre, I. B., Skarpaas, T., Kjelland, V., ... Noraas, S. (2017). *Candidatus Neoehrlichia mikurensis* and *Borrelia burgdorferi* sensu lato detected in the blood of Norwegian patients with erythema migrans. *Ticks and Tick-borne Diseases*, 8(5), 715–720. <https://doi.org/10.1016/j.ttbdis.2017.05.004>
- Randolph, S. E., & Storey, K. (1999). Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): Implications for parasite transmission. *Journal of Medical Entomology*, 36(6), 741–748. <https://doi.org/10.1093/jmedent/36.6.741>
- Rar, V. A., Livanova, N. N., Panov, V. V., Doroschenko, E. K., Pukhovskaya, N. M., Vysochina, N. P., & Ivanov, L. I. (2010). Genetic diversity of *Anaplasma* and *Ehrlichia* in the Asian part of Russia. *Ticks Tick Borne Dis*, 1(1), 57–65. <https://doi.org/10.1016/j.ttbdis.2010.01.002>
- Schouls, L. M., Van De Pol, I., Rijpkema, S. G., & Schot, C. S. (1999). Detection and identification of *Ehrlichia*, *Borrelia burgdorferi* sensu lato, and *Bartonella* species in Dutch *Ixodes ricinus* ticks. *Journal of Clinical Microbiology*, 37(7), 2215–2222.
- Sergeant, E. S. G. (2019). *Epitools epidemiological calculator AusVet animal health services and Australian Biosecurity Cooperative Research Centre for Emerging Infectious Disease*. Retrieved from <http://epitools.ausvet.com.au>
- Silaghi, C., Beck, R., Oteo, J. A., Pfeffer, M., & Sprong, H. (2016). Neoehrlichiosis: An emerging tick-borne zoonosis caused by *Candidatus Neoehrlichia mikurensis*. *Experimental and Applied Acarology*, 68(3), 279–297. <https://doi.org/10.1007/s10493-015-9935-y>
- Silaghi, C., Woll, D., Mahling, M., Pfister, K., & Pfeffer, M. (2012). *Candidatus Neoehrlichia mikurensis* in rodents in an area with sympatric existence of the hard ticks *Ixodes ricinus* and *Dermacentor reticulatus*, Germany. *Parasit Vectors*, 5, 285. <https://doi.org/10.1186/1756-3305-5-285>
- Soleng, A., Edgar, K. S., Paulsen, K. M., Pedersen, B. N., Okbaldet, Y. B., Skjetne, I. E. B., ... Andreassen, A. K. (2018). Distribution of *Ixodes ricinus* ticks and prevalence of tick-borne encephalitis virus among questing ticks in the Arctic Circle region of northern Norway. *Ticks and Tick-borne Diseases*, 9(1), 97–103. <https://doi.org/10.1016/j.ttbdis.2017.10.002>
- Soleng, A., & Kjelland, V. (2013). *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in *Ixodes ricinus* ticks in Bronnoysund in northern Norway. *Ticks and Tick-borne Diseases*, 4(3), 218–221. <https://doi.org/10.1016/j.ttbdis.2012.11.006>
- Szekeres, S., Claudia Coipan, E., Rigo, K., Majoros, G., Jahfari, S., Sprong, H., & Foldvari, G. (2015). *Candidatus Neoehrlichia mikurensis* and *Anaplasma phagocytophilum* in natural rodent and tick communities in Southern Hungary. *Ticks and Tick-borne Diseases*, 6(2), 111–116. <https://doi.org/10.1016/j.ttbdis.2014.10.004>
- Tabara, K., Arai, S., Kawabuchi, T., Itagaki, A., Ishihara, C., Satoh, H., ... Tsuji, M. (2007). Molecular survey of *Babesia microti*, *Ehrlichia* species and *Candidatus Neoehrlichia mikurensis* in wild rodents from Shimane Prefecture, Japan. *Microbiology and Immunology*, 51(4), 359–367.
- Tveten, A. K. (2014a). Exploring diversity among Norwegian *Borrelia* strains originating from *Ixodes ricinus* Ticks. *International Journal of Medical Microbiology*, 2014, 397143. <https://doi.org/10.1155/2014/397143>
- Tveten, A. -K. (2014b). Prevalence and diversity among *Anaplasma phagocytophilum* strains originating from *Ixodes ricinus* Ticks from Northwest Norway. *Journal of Pathology*, 2014, 8. <https://doi.org/10.1155/2014/824897>
- von Loewenich, F. D., Geissdorfer, W., Disque, C., Matten, J., Schett, G., Sakka, S. G., & Bogdan, C. (2010). Detection of "Candidatus *Neoehrlichia mikurensis*" in two patients with severe febrile illnesses: Evidence for a European sequence variant. *Journal of Clinical Microbiology*, 48(7), 2630–2635. <https://doi.org/10.1128/jcm.00588-10>
- Wass, L., Grankvist, A., Bell-Sakyi, L., Bergstrom, M., Ulfhammer, E., Lingblom, C., & Wennerås, C. (2019). Cultivation of the causative agent of human neoehrlichiosis from clinical isolates identifies vascular endothelium as a target of infection. *Emerging Microbes and Infections*, 8(1), 413–425. <https://doi.org/10.1080/22221751.2019.1584017>
- Wennerås, C. (2015). Infections with the tick-borne bacterium *Candidatus Neoehrlichia mikurensis*. *Clinical Microbiology and Infection*, 21(7), 621–630. <https://doi.org/10.1016/j.cmi.2015.02.030>
- Zeman, P. (1997). Objective assessment of risk maps of tick-borne encephalitis and Lyme borreliosis based on spatial patterns of located cases. *International Journal of Epidemiology*, 26(5), 1121–1129. <https://doi.org/10.1093/ije/26.5.1121>

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