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Borrelia burgdorferi Sensu Lato and *Ehrlichia* spp. in *Ixodes* Ticks from Southern Norway

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We report the results of a study of the prevalence of *Ehrlichia* and *Borrelia* species in 341 questing *Ixodes ricinus* ticks from two locations in southern Norway. The prevalences of *Borrelia burgdorferi* sensu lato and *Ehrlichia* spp. were, respectively, 16 and 11.5% at site 1 and 17 and 6% at site 2. Prevalence and species composition of *Borrelia* and *Ehrlichia* varied with location and date of collection. The dominant *Borrelia* species at both sites was *Borrelia afzelii*, followed by *Borrelia burgdorferi* sensu stricto. *Borrelia garinii* was found in only a single tick. The dominant member of the *Ehrlichia* group was a recently described *Ehrlichia*-like organism related to the monocytic ehrlichiae. Variants of *Ehrlichia phagocytophila* and the agent of human granulocytic ehrlichiosis were also found. The highest prevalences for *B. afzelii*, *B. burgdorferi* sensu stricto, and the *Ehrlichia*-like organism were observed in May. *B. afzelii* was most prevalent in females, less prevalent in nymphs, and least prevalent in males, while the prevalence of *Ehrlichia* was highest in nymphs, lower in females, and least in males. Double infections with *B. afzelii* and *B. burgdorferi* sensu stricto and with *B. afzelii* and the *Ehrlichia*-like organism were significantly overrepresented. Tick densities were highest in May, when densities of more than 200 ticks/100 m^2 were observed, and declined during the summer months to densities as low as 20 ticks/100 m^2. We conclude that estimates of the prevalence of tick-borne bacteria are sensitive to the choice of date and site for collection of ticks. This is the first study of tick-borne *Borrelia* and *Ehrlichia* in Norway and the lowest reported *B. garinii* prevalence in Northern Europe. The prevalence of the *Ehrlichia*-like organism is described for the first time in questing ticks.

Tick-borne *Borrelia* and *Ehrlichia* species cause disease both in humans and animals (13). In northwest Europe these bacteria are transmitted predominantly by the bite of the hard tick *Ixodes ricinus*. Ticks are infected when they feed on an infected animal, and the bacteria persist in the tissues of the tick through metamorphosis and can be transmitted to a new host when the tick again feeds. Transovarial transmission is not considered to be important for *Borrelia burgdorferi* sensu lato (19). *I. ricinus* feeds widely on terrestrial vertebrates (31), which gives it the potential to support the enzootic cycles of diseases with many different host reservoirs.

Of the 10 *Borrelia* species known collectively as *Borrelia burgdorferi* sensu lato, 4 (*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana*) are known to occur in northwestern Europe. The complex of diseases caused *by B. burgdorferi* sensu lato is known as Lyme borreliosis. Symptoms include arthritis, carditis, dermal symptoms, and neurological symptoms, usually preceded by erythema migrans, a characteristic rash that spreads from the bite site. Arthritis and carditis are preferentially associated with *B. burgdorferi* sensu stricto, *B. garinii* infection predisposes to neuroborreliosis, and the degenerative skin disorder acrodermatitis chronica et atrophicans (ACA) is specifically associated with *B. afzelii*. The implied tissue tropisms are not absolute, and the clinical symptoms of infection with the different *B. burgdorferi* sensu lato species overlap. *B. valaisiana*'s status as a pathogen has yet to be confirmed (38). Symptoms of Lyme borreliosis have been recognized in Europe since the early 1900s, and *B. burgdorferi* sensu lato has been detected in archival ticks dating back more than 100 years (15, 22). The bacterial etiology of Lyme disease was first elucidated in the United States in 1982 by Burgdorfer et al. (5).

On the basis of 16S rRNA gene sequences, the ehrlichiae appear to fall into three clades: the monocytic *Ehrlichia* species, including *E. canis*, *E. chaffeensis*, *E. ewingii*, and *E. muris*; the granulocytic *Ehrlichia* species, including *E. bovis*, *E. platys*, *E. phagocytophila*, *E. equi*, and the human granulocytic ehrlichiosis (HGE) agent; and the *E. risticii-E. sennetsu* group. In addition, several species not traditionally classified as *Ehrlichia* fall within this clade. *Cowdria ruminantium* clusters with the monocytic ehrlichiae, *Anaplasma marginale* clusters with the granulocytic ehrlichiae, and *Neorickettsia helminthoeca* clusters with *E. risticii* and *E. sennetsu* (30). A review of the DNA sequence databases reveals a number of other *Ehrlichia* 16S rRNA gene sequences which have yet to be classified taxonomically. Among these are two 16S rRNA variants of the *E. phagocytophila* group and an *Ehrlichia*-like organism related to the monocytic group (30). *Ehrlichia* infection in humans characteristically causes an acute fever, often accompanied by myalgia, headache, rigors, and gastrointestinal problems, but without symptoms of upper respiratory infection, while leukopenia, thrombocytopenia, and elevated serum transaminases

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are typical laboratory findings (37); veterinary *Ehrlichia* infections are apparently similar. Leukocytes are the primary targets of infection, and these may become severely depleted, facilitating secondary infection (13). *Ehrlichia* infections may be quite severe, and fatalities occur both in humans and in animals.

Prevalence data for *Borrelia* and *Ehrlichia* in ticks provide a guide to the health risk associated with a tick bite and are therefore of public health interest. This question has been addressed for *Borrelia*, and to a lesser extent for *Ehrlichia*, in the United States, Japan, and a number of European countries (3, 6, 17, 18, 21, 25, 27, 36). Both prevalence and species distribution are subject to geographical variation.

In Norway, Lyme arthritis, neuroborreliosis, and ACA are endemic. HGE has recently been reported (4), and symptoms of tick-borne fever caused by *E. phagocytophila* have been recorded in sheep, goats, and cattle since 1780 (33, 34). *I. ricinus* reaches its northern limit about the 66th parallel on the northwest coast of Norway. North of this limit, Lyme borreliosis is a very uncommon import disease in humans and tick-borne fever in livestock is not reported (33). In 1999 there were 146 notified cases of serious Lyme borreliosis in Norway, an incidence rate of 3.4/100,000. Erythema migrans is not included as this is not a notifiable disease. Of the reported cases, 62% were from the southern counties of Telemark, Aust-Agder, and Vest-Agder; 49% of the reported cases were neuroborreliosis, 33% were Lyme arthritis, and 6% were ACA; the remaining 12% were unclassified (10). The prevalence of *Borrelia* and *Ehrlichia* in ticks in Norway has not hitherto been studied.

In order to establish an epidemiological context for the prevalence of tick-borne diseases in Norway, we have surveyed the prevalence of *Ehrlichia* and *Borrelia* in ticks from two locations in southeastern Norway—one chosen for its very high tick density and the other for its association with a case of *E. phagocytophila* infection in a moose (16). In order to determine to what extent the results of prevalence surveys may be extrapolated beyond the date and vicinity of collection and also to provide data on the epizootology of tick-borne diseases, we report the differences in *Ehrlichia* and *Borrelia* prevalence between the two localities and their variation through a season. In addition, we investigate the association of *Ehrlichia* and *Borrelia* infection with developmental stage and the correlation between species in multiple infections.

MATERIALS AND METHODS

Localities. Ticks were collected from two sites. Site 1, Langøya, is a limestone island (9°47′E, 59°0′N) close to the mainland coast of southeastern Norway. Although previously used as pasturage for cattle and sheep, the island is no longer used for agriculture and is uninhabited. Local residents report that ticks became troublesome on the island after a series of dry summers in the mid-1980s. The island supports a flock of roe deer as well as a range of smaller mammals. Vegetation is mixed oak woodland with varied undergrowth interspersed with areas of open grassland. The collection area is one such area, an overgrown meadow. Site 2, Marka (9°48′E, 59°8′N), is a mainland area of mixed woodland on alkaline igneous rock in the vicinity of sheep pasture. A moose suffering from an *E. phagocytophila* infection was found in the area in July 1999 and tick-borne fever is endemic in local sheep flocks (16).

Collection of ticks. Questing ticks were collected by flagging undergrowth with a 70-by-120-cm white towel (40). Ticks attaching themselves to the towel were picked with tweezers and immersed in 70% ethanol. Collection at site 1 was carried out at approximately monthly intervals during the summer months of 1998 and 1999. To measure the tick density, collection was carried out in the same 10-by-10-m square marked area. For *Borrelia* and *Ehrlichia* prevalence studies, we aimed to collect 25 adult males, 25 adult females, and 50 nymphs. Larvae were not included in the study. If an insufficient number of ticks was found in the 100-m^2 collection area, supplementary ticks were collected by flagging randomly around the collection area, concentrating on paths and animal tracks. Collection at site 2 was conducted in July 1999 solely by flagging along paths and tracks.

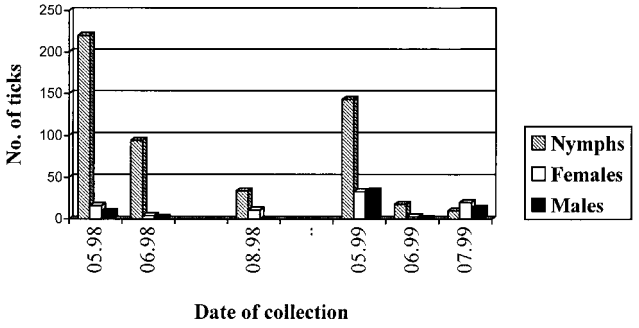

FIG. 1. Density and stage of ticks per 100 m^2 at site 1, May 1998 to July 1999.

For site 1, PCR analysis was conducted on 25 adult females, 24 to 26 adult males, and 47 to 50 nymphs for each collection date. For site 2, all 46 ticks collected were analyzed. Only ticks collected in 1999 were subjected to PCR analysis.

Extraction of DNA from ticks. After removal of excess ethanol, ticks were cut in half longitudinally with a flame-sterilized scalpel blade and transferred to a pellet-pestle tube (Kontes Scientific Glassware/Instruments, Vineland, N.J.) containing 60 μl of proteinase K (40 μg/ml)–1 mM Tris-HCl (pH 8.0)–2 mM EDTA–0.01% Tween 20 and crushed using a pellet pestle until release of abdominal contents was visible. After incubation at 65°C for 1 h and 100°C for 10 min, tick extracts were stored at −20°C until use for PCR.

Detection of *Borrelia* and *Ehrlichia* by PCR. Ten-microliter aliquots of the tick extract were amplified in 100-μl PCRs using granulocytic *Ehrlichia*-specific primers EHR521-EHR747 (24). Five-microliter aliquots of tick extract were amplified in 50-μl multiplex PCRs using species-specific *Borrelia* primers GI-R–GI-L (*B. burgdorferi* sensu stricto), GII-R–GII-L (*B. garinii*), and GIII-R–GIII-L (*B. afzelii*) (8) as previously described (16). PCR products were detected on 2% agarose gels stained with SYBR-gold (Molecular Probes, Eugene, Oreg.) and photographed under 302-nm UV transillumination.

Samples positive using the EHR521-EHR747 primer set were reamplified using the primer set 16S8FE–B-GA1B, and *Ehrlichia* species were determined using the reverse line blot assay as previously described (30). Where this assay was negative, the sequence of the EHR521-EHR747 amplicon was determined (MWG Biotech, Ebersburg, Germany) and compared with public-domain databases using the BLAST software (Swiss Institute for Bioinformatics [http://www.ch.embnet.org]).

Statistical methods. Statistical significance was calculated using the χ^2 test.

RESULTS

Density and population structure of ticks. The density of ticks at site 1 was monitored over a period of 2 years (Fig. 1). Densities varied during the season, reaching a maximum of 245 ticks/100 m^2 in May 1998 and showing a general tendency to decline over summer, reaching a minimum of 20 ticks/100 m^2 in June 1999.

Nymphs were generally the most abundant stage found. The ratio of nymphal to adult ticks varied between 15.6:1 in June 1998 and 0.3:1 in July 1999 and was higher in 1998 (8.3:1) than in 1999 (1.6:1). Female ticks were more abundant than males in both years (3:1 in 1998, 1.2:1 in 1999).

Prevalence of *Borrelia* in ticks. The prevalence of *Borrelia* species is shown in Table 1. The overall prevalence of *Borrelia*

TABLE 1. Results of *Borrelia* and *Ehrlichia* species determination for ticks from site 1 and site 2

Location and date	Instar (no.) of ticks	No. of ticks infected with[a]:											Total no. of ticks that were[b]:			
		Borrelia				*Ehrlichia*				*Borrelia* + *Ehrlichia*						
		Afz	SS	Gar	Afz + SS	ELO	HGE	Ph	Esp	ELO + Afz	ELO + Afz + SS	Esp + Afz + SS	Borr Pos	Ehr Pos	Borr + Ehr Pos	Neg
Site 1																
May 1999	Nymph (47)	4	1		3				1	5	2	1	8	1	8	30
	Male (24)	1	1		1						1		3		1	20
	Female (25)	7	2			2		1		1	1		9	3	2	11
	Total (96)	12	4	0	4	2	0	1	1	6	4	1	20	4	11	61
June 1999	Nymph (47)	3				1		2	1				3	4		40
	Male (26)	1					1						1	1		24
	Female (25)	3				1		1	1				3	3		19
	Total (98)	7	0	0	0	2	1	3	2	0	0	0	7	8	0	83
July 1999	Nymph (50)	2			1	2	1	1	1				3	5		42
	Male (25)															25
	Female (25)	4		1		1			1				5	2		18
	Total (100)	6		1	1	3	1	1	2	0	0	0	8	7	0	85
	Total for site 1 (294)	25	4	1	5	7	2	5	5	6	4	1	35	19	11	229
Site 2 (July 1999)	Nymph (41)	6				1				2			6	1	2	32
	Male (6)															6
	Female (0)															
	Total (47)	6	0	0	0	1	0	0	0	2	0	0	6	1	2	38

[a] Abbreviations: Afz, *B. afzelii*; SS, *B. burgdorferi* sensu stricto; Gar, *B. garinii*; ELO, *Ehrlichia*-like organism; HGE, HGE agent variant; Ph, *E. phagocytophila* variant; Esp, *Ehrlichia*, species not determined.

[b] Borr Pos, positive for *B. burgdorferi* sensu lato; Ehr Pos, positive for *Ehrlichia*; Borr+Ehr Pos, positive for both *B. burgdorferi* sensu lato and *Ehrlichia*; Neg, negative for both *Borrelia* and *Ehrlichia*.

in ticks at site 1 (May to July 1999) was 16% (46 of 294 ticks) and was significantly higher ($P < 0.0005$) in May (32% [31 of 96 ticks]) than in June (7% [7 of 98 ticks]) or July (8% [8 of 98 ticks]). The prevalence of *Borrelia* at site 2 in July was 17% (8 of 47 ticks).

B. afzelii was the dominant species at both sites and at all time points. Its prevalence at site 1 varied between 29% (27 of 96 ticks) in May and 7% (7 of 98 ticks) in June and July. The prevalence of *B. burgdorferi* sensu stricto at site 1 was 14% (13 of 96 ticks) in May; in subsequent months only a single *B. burgdorferi* sensu stricto-positive tick was detected. *B. garinii* was observed only in a single tick collected at site 1 in July. The prevalence of *B. afzelii* at site 2 in July was 17% (8 of 47 ticks); this was the only *Borrelia* species detected at this site.

***Ehrlichia* prevalence in ticks.** Screening of ticks by PCR with primers EHR521-EHR747 gave positive results with 37 of 341 ticks. Results of species determination by reverse line blot and DNA sequencing are shown in Table 1. Four positive results could be attributed to sequences with 98% homology to species of *Wolbachia* (12, 28, 29). The remaining 33 positive results are attributed to the presence of *Ehrlichia*, although in six cases species determination could not be completed. A total of 30 of 294 ticks (11.5%) collected at site 1 in the period May to July 1999 and 3 of 44 ticks (7%) collected at site 2 in July 1999 contained *Ehrlichia*. The predominant *Ehrlichia* species was an *Ehrlichia*-like organism previously described in ticks from Holland (20 of 33 ticks); variants of *E. phagocytophila* (5 of 33 ticks) and HGE agent (2 of 33 ticks) were also detected.

The prevalence of the *Ehrlichia*-like organism at site 1 declined from 13% (12 of 96 ticks) in May to 2% (2 of 98 ticks) in June and 3% (3 of 98 ticks) in July ($\chi^2 = 11.73$; $P = 0.003$). The prevalence of other *Ehrlichia* species was too low to allow assessment of monthly variation.

Interstadial variation in *Borrelia* and *Ehrlichia* prevalence. Figure 2 shows interstadial variation in the prevalence of *Borrelia* and *Ehrlichia* species (see also Table 1). The rates of prevalence of *Borrelia* in nymphs, females, and males were, respectively, 15% (22 of 144 ticks), 25% (19 of 75 ticks), and 7% (5 of 75 ticks), these differences being significant ($\chi^2=9.93$; $P = 0.007$) and largely due to differences in the prevalence of *B. afzelii*. *Ehrlichia* was most prevalent in nymphs (13% [18 of 144 ticks]) and females (13% [10 of 75 ticks]) and least prevalent in males (3% [2 of 75 ticks]). This pattern is seen both for the *Ehrlichia*-like organism and for other *Ehrlichia* species. The reduced prevalence of *Ehrlichia* in male ticks is statistically significant ($\chi^2 = 6.28$; $P = 0.043$).

Double infections. Eleven ticks from site 1 (11 of 294 ticks [3.7%]) contained both *Borrelia* and *Ehrlichia* (Table 1). All these ticks contained *B. afzelii*, and, with a single exception, where the *Ehrlichia* species was not identified, all contained the

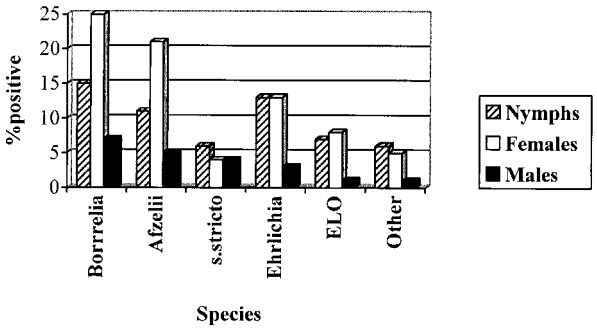

FIG. 2. Interstadial variation in prevalence of *B. burgdorferi* sensu lato (Borrelia), *B. afzelii* (Afzelii), *B. burgdorferi* sensu stricto (s.stricto), *Ehrlichia* species (Ehrlichia), the *Ehrlichia*-like organism (ELO), and other *Ehrlichia* species. Data are for site 1, May to July 1999, and include 144 nymphs, 75 females, and 75 males divided equally between three collection dates. All occurrences of ticks containing the species indicated, including multiply positive ticks, are counted.

Ehrlichia-like organism. All of these doubly infected ticks were collected in May. This is an approximately fourfold excess of double infections over that expected from a random association of these two organisms (0.8%) and is statistically significant both for the entire sample ($\chi^2 = 27.66$; $P < 0.005$) and for May ($\chi^2 = 20.68$; $P < 0.005$). Of these 10 double infections, 7 were in nymphal ticks.

Ten ticks from site 1 contained both *B. afzelii* and *B. burgdorferi* sensu stricto. This represents 71% of ticks (10 of 14) positive for *B. burgdorferi* sensu stricto and 3.4% of all ticks collected at site 1. Nine of the ten doubly positive ticks were collected in May. This is an approximately fivefold excess over that expected by chance association (0.7%) and is significant for the entire sample ($\chi^2 = 40.47$; $P < 0.05$) and for May ($\chi^2 = 12.57$; $P < 0.05$). Eight of the doubly infected ticks were nymphs.

Comparison of the two geographical locations. Comparison of *Borrelia* and *Ehrlichia* prevalence data for site 2 on 13 July 1999 with those for site 1 at the two bracketing dates, 23 June and 27 July (Table 1), suggests marked differences in the burden of tick-borne microflora. In nymphal ticks, the prevalence of *B. afzelii* was 15% (6 of 41 ticks) at site 2 and 6% (6 of 97 ticks) at site 1, while the prevalence of *Ehrlichia* was 6% (3 of 41 ticks) at site 2, including only the *Ehrlichia*-like organism, and 9% (9 of 97 ticks) at site 1, including a wider range of *Ehrlichia* species, of which the *Ehrlichia*-like organism comprised only one-third. However, these differences are not statistically significant.

DISCUSSION

We have investigated the prevalence of *B. burgdorferi* sensu lato and *Ehrlichia* in questing ticks at two sites in southeastern Norway. At site 1, the prevalence of *Borrelia* was 16%, comprising 14% *B. afzelii*, 5% *B. burgdorferi* sensu stricto, <1% *B. garinii*, and 3.4% *B. burgdorferi-B. afzelii* double infections, and the prevalence of *Ehrlichia* was 11.5%, comprising 6% *Ehrlichia*-like organism, 1.7% *E. phagocytophila* variant, and 0.6% HGE agent variant. At site 2, the prevalence of *Borrelia* was 17% and the prevalence of *Ehrlichia* was 6%. Only *B. afzelii* and the *Ehrlichia*-like organism were detected at site 2. The dominant *Ehrlichia* species detected here, the *Ehrlichia*-like organism, was first detected in ticks feeding on deer in Holland (30). The 16S rRNA gene sequence indicates that this species belongs to the monocytic *Ehrlichia* group, but unfortunately nothing is known about its biology.

Aside from the predominant *Ehrlichia*-like organism, we note the presence of variants of *E. phagocytophila* and the HGE agent previously identified in ticks in Holland (30) and Sweden (36). The *E. phagocytophila* variant has also been found in white-tailed deer in Wisconsin (2), and HGE agent variants have been found in connection with human disease in Scandinavia (4). Ticks carrying bacteria with prototype HGE agent or *E. phagocytophila* 16S rRNA sequences were not found, in spite of the facts that prototype *E. phagocytophila* is known to be endemic in southern Norway and that a moose calf with prototype *E. phagocytophila* infection was found at site 2 prior (2 weeks) to the date of collection of ticks (16).

The clinical picture of Lyme borreliosis in Norway, where neuroborreliosis dominates, might lead one to expect that the dominant *Borrelia* species would be *B. garinii*, which is preferentially associated with neurological symptoms. However, our results show *B. garinii* to be very uncommon and *B. afzelii* to be dominant. Indeed, the *B. garinii* prevalence is, as far as we are aware, the lowest observed in Northern Europe. However, our sample of ticks is not necessarily representative for the whole of Norway (see below).

Comparable studies have been performed in Ireland (18), Switzerland (3, 27), Slovenia (25), Sweden (36), Finland (17), Wisconsin (24), Delaware (6), and Holland (30), although only in the last two of these studies, which investigated ticks collected from animals, were both *Ehrlichia* and *Borrelia* detected. The reported prevalences of *Ehrlichia* vary from 1.3% in Switzerland (*E. phagocytophila* genogroup) (27) to 50% in Connecticut (HGE agent) (21), and *B. burgdorferi* sensu lato prevalences varying from 1.3% in Switzerland (3) to 55% in areas of Finland (17) have been reported. Species composition for *Borrelia* varies, with *B. afzelii* being dominant in Holland (30) and Finland (17), as in this study, and *B. valaisiana* (not detected in this study) being dominant in Ireland (18) and Switzerland (3). In Holland, Schouls et al. (30) found members of the *E. phagocytophila* genogroup in more than 60% of *Ehrlichia*-positive ticks and *Ehrlichia*-like organism in 15%, whereas the *E. phagocytophila* genogroup and *Ehrlichia*-like organism were present in, respectively, 25 and 50% of positive ticks in this study. Geographical differences in the proportions of different tick-borne organisms might reflect local variation in the availability of host organisms with differential susceptibilities, self-perpetuating random differences in abundance, or cyclic fluctuations caused by transient population immunity.

At comparable dates, there was no obvious similarity between the two locations in the frequency and species composition for *Borrelia* and *Ehrlichia* species, except that *B. afzelii* was the dominant *Borrelia* species. The two localities are only 15 km apart, although they are separated by open water.

Site 1 was sampled in May, June, and July 1999. We found that the prevalences of *B. afzelii*, *B. burgdorferi* sensu stricto, and the *Ehrlichia*-like organism were much higher in May than in June and July. It is not possible at this point to determine

whether these changes reflect seasonal effects in the prevalence of *Borrelia* and *Ehrlichia* species in ticks or merely random changes over time. A peak of *Borrelia* prevalence in spring and early summer has been reported from Sweden (35), while Stafford et al. (32) found no significant seasonal trend in the prevalence of *B. burgdorferi* in nymphal ticks in Connecticut over a 9-year period. We are currently working on a more complete time series from the same location in 2000 which should cast light on the question of seasonal trends in *Borrelia* and *Ehrlichia* prevalence.

Our results show that samples taken at different time points and from different locations may have very different prevalences of *Borrelia* and *Ehrlichia*. This would suggest that estimates based on spot studies such as this study may have only local and temporary applicability, which would limit their value in forming public health policy.

Double infections with *B. afzelii* and the *Ehrlichia*-like organism and with *B. afzelii* and *B. burgdorferi* occurred at a level four to five times that expected by chance association. Most occurred in ticks collected in May, when the prevalence of tick-borne bacteria and the density of ticks were highest, and they involved the three most prevalent tick-borne bacteria detected. Most doubly infected ticks were nymphs. As a nymphal tick has taken only one blood meal, these double infections must have been acquired from the same animal. We suggest that the excess of double infections may be the result of very high tick densities in the previous year. Under such conditions, host animals will be highly infested with ticks and thus prone to acquire multiple infections. Doubly infected animals have been shown to transmit double infections to feeding ticks (20). *Borrelia-Ehrlichia* coinfections and two-species *Borrelia* coinfections have been reported by a number of authors (9, 26). A seroepidemiological study in Norway (1) showed that 10% of patients seropositive for *B. burgdorferi* also had antibodies to *Ehrlichia*.

There was evidence of interstadial variation in the prevalence of *B. afzelii*, of the *Ehrlichia*-like organism, and of other *Ehrlichia* species. *B. afzelii* was most prevalent in adult females, less so in nymphs, and least so in males, while for *Ehrlichia*, the prevalence was similar in nymphs and females and low in males. The low prevalence of *Borrelia* and *Ehrlichia* in males might be explained in three ways: differential feeding (male and female immature ticks selecting different hosts), differential survival of the bacteria in males and females, or differential survival of infected ticks. Other studies have reported the prevalence of *Borrelia* to be greater in adults (18), greater in nymphs (22), or equal in both stages (23). We are not aware of any previous reports of interstadial variation in *Ehrlichia* prevalence.

Measurement of tick density over 2 years indicates a seasonal peak in May or earlier. This is consistent with previous findings of maximum nymphal tick activity in spring and early summer (14). This is likely to be a consequence of day length, which affects emergence from diapause, and humidity. The low-humidity conditions which are likely to be encountered in the later summer months inhibit both emergence from diapause and survival of questing ticks (7). The overall predominance of nymphal ticks is probably partly a consequence of the natural tendency of all populations to be dominated by young individuals but may also be a consequence of the fact that our collection period does not extend into autumn, when adults are predicted to be most abundant (11).

The PCR primers (EHR521-EHR747) used in this study were designed to be specific for the *E. phagocytophila* genogroup (24). However, these primers also detect the *Ehrlichia*-like organism, a member of the monocytic *Ehrlichia* group, and sequences related to *Wolbachia*. Thus, although these primers are useful for screening ticks prior to the application of more-precise species-specific methods, they might seriously overestimate the prevalence of the *E. phagocytophila* group if used alone. *Wolbachia persicus* has been isolated from a number of tick species and is known to be pathogenic for the soft tick *Ornithodorus moubata* (39). *Wolbachia* symbionts modulate reproductive function in arthropods, causing, among other effects, cytoplasmic incompatibility and parthenogenesis and distorted sex ratios (12, 28, 29). This might explain the high ratio of females to males (3:1) observed in 1998.

This is the first study of *Ehrlichia* and *Borrelia* in ticks from Norway. We observe the lowest hitherto reported Northern European prevalence of *B. garinii*, though a more widespread survey will be needed to determine if this is related to the fact that *I. ricinus* reaches the northwestern limit of its distribution in Norway. This is also the first study in questing ticks of the prevalence of a recently described *Ehrlichia*-like organism, related to the monocytic ehrlichiae, and shows this organism to be the dominant *Ehrlichia* species in the locality. Time series results are consistent with a peak prevalence of this organism in spring and early summer, though further data are needed to confirm this trend. Temporal variations in the prevalence of *Ehrlichia* species have not to our knowledge been reported previously.

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