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Seroepidemiological Studies of *Borrelia burgdorferi* Infection in Sheep in Norway

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An enzyme-linked immunosorbent assay for antibodies to *Borrelia burgdorferi* in sheep was established. The assay was used in a seroepidemiological survey in which serum samples from 327 Norwegian rams were screened for antibodies to *B. burgdorferi*. These rams were randomly chosen, clinically healthy animals from all parts of Norway. They came both from areas where *Ixodes ricinus* is common and from areas where the tick has not been found. The age of the animals varied from 0.5 to 7.5 years. Sera from 10% of the animals tested were seropositive by the enzyme-linked immunosorbent assay, with the percentage of positive animals varying from 0 to 20% between counties. The distribution of seropositive animals was generally in good agreement with the known distribution of *I. ricinus*, with the highest proportion of seropositive animals being in southern coastal areas of Norway. There were some exceptions, however, because seropositive animals were also found in areas where the tick has not been recorded. The majority of animals appeared to become infected during the first 2 years of life, with 12% of animals that were 1.5 years old being seropositive. The animals were all healthy at the time of serum sampling, and the clinical significance of *B. burgdorferi* in sheep is still uncertain.

Lyme borreliosis (Lyme disease) is a zoonosis caused by the spirochete *Borrelia burgdorferi* (11, 56). The disease is transmitted primarily by infected ticks, the most common vector in Europe being the tick *Ixodes ricinus* and those in the United States being the ticks *Ixodes dammini* and *Ixodes pacificus* (1). In humans, Lyme borreliosis in its early stages is characterized by erythema migrans or erythema chronicum migrans, a skin lesion that spreads outward from around the site of a tick bite. Such local signs are often accompanied by systemic influenzalike symptoms, and if it is left untreated, the disease may proceed to a second or a third stage in which neurological symptoms and arthritis are common (48, 56).

Much less is known about Lyme borreliosis in animals than is known about the disease in humans. *B. burgdorferi* infection, or serologic evidence of infection, has been reported in dogs, horses, and cows in the United States (12, 15–17, 21, 32, 33, 37, 38, 40, 50). In Europe, few reports exist on Lyme borreliosis in animals. In the United Kingdom, Denmark, and Sweden, antibodies to *B. burgdorferi* and clinical symptoms of Lyme borreliosis have been found in dogs (23, 41, 47). A serosurvey in Sweden revealed antibodies to *B. burgdorferi* in sheep and cattle in areas where *I. ricinus* is present and where arthritis occurs endemically in lambs (28).

I. ricinus is widely distributed in Norway (44). As early as 1955, a case of human infection was described in which the patient developed a migrating erythema and meningopolyradiculitis after a tick bite and was successfully treated with penicillin (6). A few years later, cases of meningoencephalitis were described; these cases were thought to be caused by a tick-borne virus and, therefore, were not treated with penicillin (9, 31). Retrospectively, these cases are now thought to have been Lyme borreliosis (7). Every year, new cases of human Lyme borreliosis are diagnosed (7, 58). The exact number of cases in Norway is not known, because Lyme borreliosis is not a notifiable disease in Norway.

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In 1966, *Borrelia*-like organisms were found in the urine of sheep from a tick-infested area in Norway (26). Special media for culturing *Borrelia* species were not available at that time, and attempts to cultivate the organisms on laboratory media and to infect guinea pigs, mice, and sheep with it were not successful. The spirochetes could thus not be identified any further.

In Norway, sheep farming is practiced in areas that are heavily infested with ticks as well as in areas where the tick has not been found, and sheep in tick-infested areas graze freely on vegetation in which the ticks are abundant. Tick-borne diseases in sheep such as tick-borne fever caused by *Ehrlichia phagocytophilia* (10, 53) and louping-ill (tick-borne encephalitis) caused by louping-ill virus (genus *Flavivirus*) (49) are common in many tick-infested areas and, together with secondary infections after tick-borne fever, have been a problem in sheep farming in some areas (60). Greater losses of lambs grazing in *I. ricinus*-infested pastures than of lambs grazing in tick-free pastures have been reported (46).

This study involved the establishment of an enzyme-linked immunosorbent assay (ELISA) for *B. burgdorferi* antibodies in sheep. Serological testing was then used to ascertain the exposure of sheep to the spirochete. Furthermore, the data on the prevalence of seropositive animals were correlated with the known geographic distribution of *I. ricinus* in Norway.

MATERIALS AND METHODS

Animals. Sera were collected from a total of 327 clinically healthy rams of various breeds. The ages of 322 of these rams were known and ranged from 0.5 to 7.5 years, with a mean age of 2 years and 4 months. The rams were registered in controlled breeding groups in 15 of 20 Norwegian counties. The number of rams tested from different counties varied from 5 to 60, depending on the overall number of sheep in the county in question (see Table 1). Some of the animals were from areas where *I. ricinus* is regularly found, whereas others originated from districts in which the tick has not been recorded. The blood samples were taken during the

period from October 1988 to January 1989. Blood was drawn from the jugular vein, with sera being collected and kept at −20°C until they were tested.

Antigens. In the ELISA, a Swedish *B. burgdorferi* strain, ACA-I, was used as an antigen. The strain was originally isolated from the skin of a patient with acrodermatitis chronica athrophicans (3) and was kindly provided by A. Hovmark, Södersjukhuset, Stockholm, Sweden. The antigen was prepared by a method described by Craft et al. (18), with some modifications. Briefly, the spirochetes were grown for 5 to 7 days at 35°C in BSK-II medium (modified Barbour-Stoenner-Kelly medium) (4), with 0.7 M L-glutamine (Flow Laboratories, Lugano, Switzerland) added prior to use. Cultures from 50-ml flasks were pooled and centrifuged (20,000 × *g*, 30 min, 22°C). The pellets were washed twice in phosphate-buffered saline (PBS; pH 7.2) with 0.005 M $MgCl_2$ (20,000 × *g*, 25 min, 4°C), resuspended in PBS, and sonicated four times for 5 min each time on an ice water bath in a W-220 sonicator-ultrasonic processor (Heat Systems-Ultrasonics Inc., Farmingdale, New York) at the maximum microtip setting. The sonic extract was centrifuged (20,000 × *g*, 30 min, 4°C), and the protein content of the supernatant was then estimated (55). The supernatant was divided into aliquots and was kept at −70°C.

ELISA procedure. The sera were tested in an ELISA (24), with some modifications. Briefly, immunoplates (Maxisorp F96; Nunc A/S, Roskilde, Denmark) were coated with 100 μl of sonicated antigen (1 μg/ml) that was diluted in 0.06 M carbonate buffer (pH 9.6) (42 h, 4°C). Nonspecific binding was blocked with 150 μl of 0.1% Tween 20 in PBS (1 h, 22°C). Test and control sera were diluted 1:2,000 in PBS–Tween 20 and were tested in duplicate (100 μl per well). After incubation overnight at 4°C, a 100-μl volume of peroxidase-conjugated rabbit anti-sheep immunoglobulin G (Organon Teknika Corp., West Chester, United Kingdom) was added in dilutions of 1:1,500 (in PBS–Tween 20), and the solutions were incubated at 37°C for 45 min. Between all steps, the plates were washed in a Microwash II (Skatron A/S, Lier, Norway) on a three-wash cycle with PBS–Tween 20 used as a washing buffer.

As a substrate, 100 μl of 2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (0.4 mg/ml; Sigma, St. Louis, Mo.) was used in 0.07 M citrate-phosphate buffer (pH 4.0) with 0.00144% H_2O_2. The optical density (OD) at 405 nm was read in a Titertek Multiskan Plus MKII apparatus (Flow Laboratories). The time of the substrate reaction was adjusted to the OD of a positive control serum sample that was included on each plate and was approximately 10 min.

Control sera. (i) Positive sera from immunized sheep. Sera from two sheep immunized with sonicated *B. burgdorferi* antigen were used as positive controls. These sheep were first immunized subcutaneously with antigen in Freund's complete adjuvant, and subsequent immunizations 3 and 15 weeks later were performed with antigen in Freund's incomplete adjuvant. The antibody levels were tested weekly. One week after the last immunization, blood was collected from the animals and the serum was stored at −70°C. The immunized animals had immunoglobulin G antibody titers of 32,000 (reciprocal dilution) in the ELISA, but they were negative with respect to immunoglobulin M antibodies. When sera were tested for background reaction in uncoated control wells which were blocked with 4% rabbit serum, 1% bovine serum albumin, 10% dry skim milk, or 0.1% Tween 20, they gave no background reaction. Sera taken both before and after immunization were tested for antibodies to *Leptospira interrogans* serovar hardjo, *L. interrogans* serovar icterohaemorrhagiae, and *Treponema pallidum* antibodies; and all sera were found to be negative.

(ii) Positive sera from sheep in *I. ricinus*-infested areas. Positive high-titer sera were obtained from animals in *I. ricinus*-infested areas and were negative for *Leptospira* and *Treponema* antibodies.

(iii) Negative control sera. Negative control sera originated from the two immunized sheep, prior to immunization, and from two lambs living in an area where *I. ricinus* has not been found.

(iv) Negative sera for cutoff determination. Twenty-two negative serum samples were used to determine the cutoff for a positive reaction. These were from rams in the two most northerly counties of Norway, Troms and Finnmark, where *I. ricinus* has not been found. A mean OD ratio and standard deviation (SD) of the mean were calculated for the 22 negative serum samples and were used to determine the cutoff value, which was 0.20.

Standardization of the test. (i) Controls. To standardize the test, the same positive sera and a negative control serum sample from immunized and nonimmunized animals were tested on each plate. The time of substrate reaction was adjusted to the OD reading for the controls on every plate to eliminate day-to-day variation (57). A mean OD value was calculated for each duplicate serum sample, and if the duplicates differed by more than 10% from the mean, they were retested (24, 57). On each test day, one randomly chosen serum sample from each plate was tested additionally on a separate plate. All positive sera were tested in two uncoated wells, and if the OD for the uncoated wells exceeded 25% of the value for the coated wells, the samples were retested (57).

(ii) OD ratio. To eliminate plate-to-plate variation, the ELISA result was expressed as an OD ratio (51), i.e., the ratio of the mean OD of a test serum sample to the mean OD of a positive control serum sample on the same plate.

(iii) Cutoff. In *B. burgdorferi* ELISAs, it is common to use the numbers of SDs above the mean for a group of negative controls as a cutoff (39, 51). The numbers of SDs used vary from one laboratory to another, but it is most common to use either 2 SDs (18, 51, 54) or 3 SDs (5, 22, 34). To determine the cutoff levels for a positive test result and to eliminate day-to-day variation, 22 negative serum samples were tested on each test day. These were from rams in the most northerly counties of Norway, Troms and Finnmark, where *I. ricinus* has not been found. The chance of these rams having been exposed to the tick-borne spirochete should thus be very low. A mean OD ratio and SD of the mean were calculated for the 22 negative serum samples which had the same serum dilution as the test sera.

To assess the reproducibility of the test, all the sera included in the serosurvey were retested 9 months later with a new batch of antigen. The best reproducibility was obtained when 3 SDs were used as a cutoff, because 97% of the serum samples were consistently either positive or negative in both tests. If, on the other hand, a 2-SD cutoff was used, only 79% of the serum samples were repeatedly positive or negative. Of the sera which additionally became positive when the cutoff was lowered, 75% were borderline sera; i.e., when the same sera were tested repeatedly, they gave various results, either positive or negative. This led to a much poorer reproducibility of the test. On the basis of these results, the cutoff between a positive and a negative test result was set at 3 SDs above the normal mean, and a positive animal was defined as one that had an OD ratio above the cutoff of 0.20.

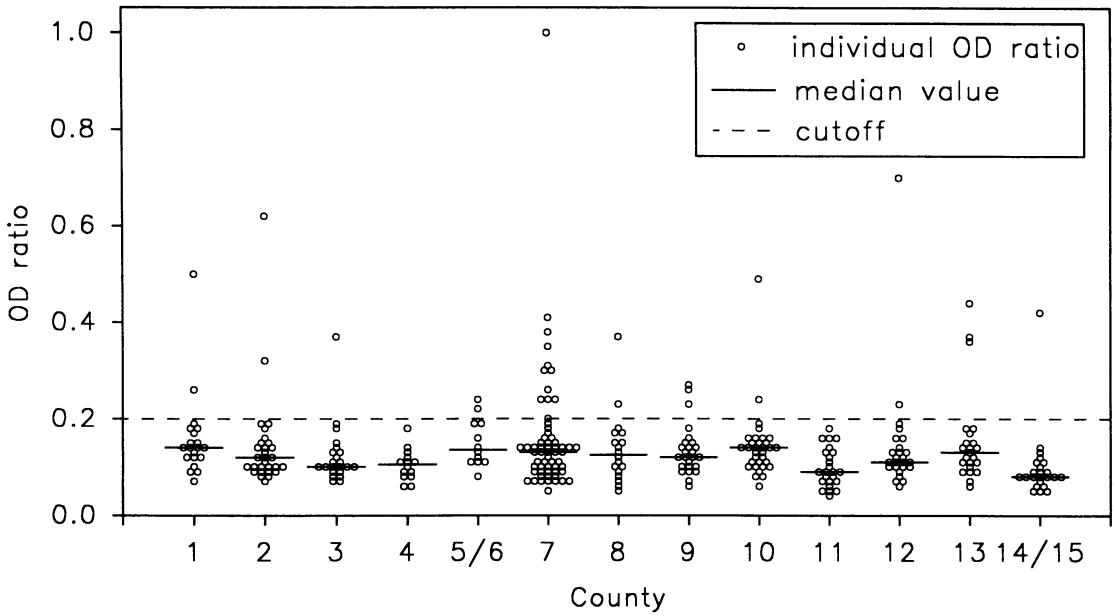

FIG. 1. *B. burgdorferi* antibodies in Norwegian sheep given as the distribution of OD ratios in different counties.

Titers. A total of 65 serum samples, including all the positive sera, were tested in double dilutions from 1:250 to 1:32,000, and the titers were related to the OD ratios. The cutoff titer corresponding to the 3-SD cutoff was 2,000 (reciprocal dilution); i.e., titers greater than or equal to 2,000 were considered positive.

In the serosurvey, OD measurements at one fixed serum dilution rather than titers were used, because this proved to be a more sensitive and accurate way of measuring the antibody response (52).

Specificity testing. All sera that were positive in the *B. burgdorferi* ELISA were tested for a background reaction in uncoated wells which were blocked with 4% rabbit serum, 1% bovine serum albumin, 10% dry skim milk, or 0.1% Tween 20. All *B. burgdorferi*-positive sera were also tested for antibodies to *L. interrogans* serovar hardjo and *L. interrogans* serovar icterohaemorrhagiae in a microagglutination and lysis test (at dilutions of 1:10, 1:30, 1:100, and 1:300), which was performed at the National Veterinary Institute, Oslo. Furthermore, the sera were tested for antibodies to *T. pallidum* in a microhemagglutination assay, which was performed at the National Institute of Health, Oslo.

RESULTS

The serosurvey. Upon testing of all 327 serum samples from different parts of Norway, a total of 32 (10%) proved to be seropositive. The sera of animals that tested positive had titers that ranged from 2,000 to 16,000. Sera from the majority of the animals had titers of 4,000, sera from two of the animals had a titer of 8,000, while serum from one animal had a titer of 16,000.

OD ratios of from 0.04 to 1.0 were found, and the distribution of OD ratios in different counties, together with the medium OD ratio found in each county, is plotted in Fig. 1. The medium OD ratio varied from 0.08 in counties 14 and 15 to 0.14 in counties 1 and 10. Animals showing strong positive reactions (high OD ratio) were found in all parts of the country.

The proportion of positive animals in different counties varied from 0 to 20% (Table 1). The results were combined for counties 5 and 6 and counties 14 and 15, because these counties are geographically very close and each county was represented by only a small number of animals. The highest percentage of positive animals (20%) was found in the southern county of Rogaland, and the percentage of positive animals in the other southern coastal counties varied from 6 to 17% (Table 1). No seropositive animals were found in the

TABLE 1. Names and numbers of Norwegian counties included in the study, number and mean age of animals tested, and number and percentage of *B. burgdorferi* seropositive animals found in each county

County		No. of animals	Mean age (yr, mo)	No. (%) of positive animals
Number	Name			
1	Hedmark	20	2, 5	2 (10)
2	Oppland	29	2, 3	2 (7)
3	Buskerud	22	2, 5	1 (4)
4	Telemark	14	2, 5	0 (0)
5/6	Øst-Agder/Vest-Agder[a]	12	2, 7	2 (17)
7	Rogaland	60	2, 5	12 (20)
8	Hordaland	18	1, 8	2 (11)
9	Sogn og Fjordane	25	2, 11	3 (12)
10	Møre og Romsdal	32	2, 4	2 (6)
11	Sør-Trøndelag	24	2, 1	0 (0)
12	Nord-Trøndelag	25	2, 11	2 (8)
13	Nordland	23	1, 11	3 (13)
14/15	Troms/Finnmark[b]	23	1, 11	1 (4)
Total or average		327	2, 4	32 (10)

[a] Øst-Agder, 7 animals; Vest-Agder, 5 animals.
[b] Troms, 16 animals; Finnmark, 7 animals.

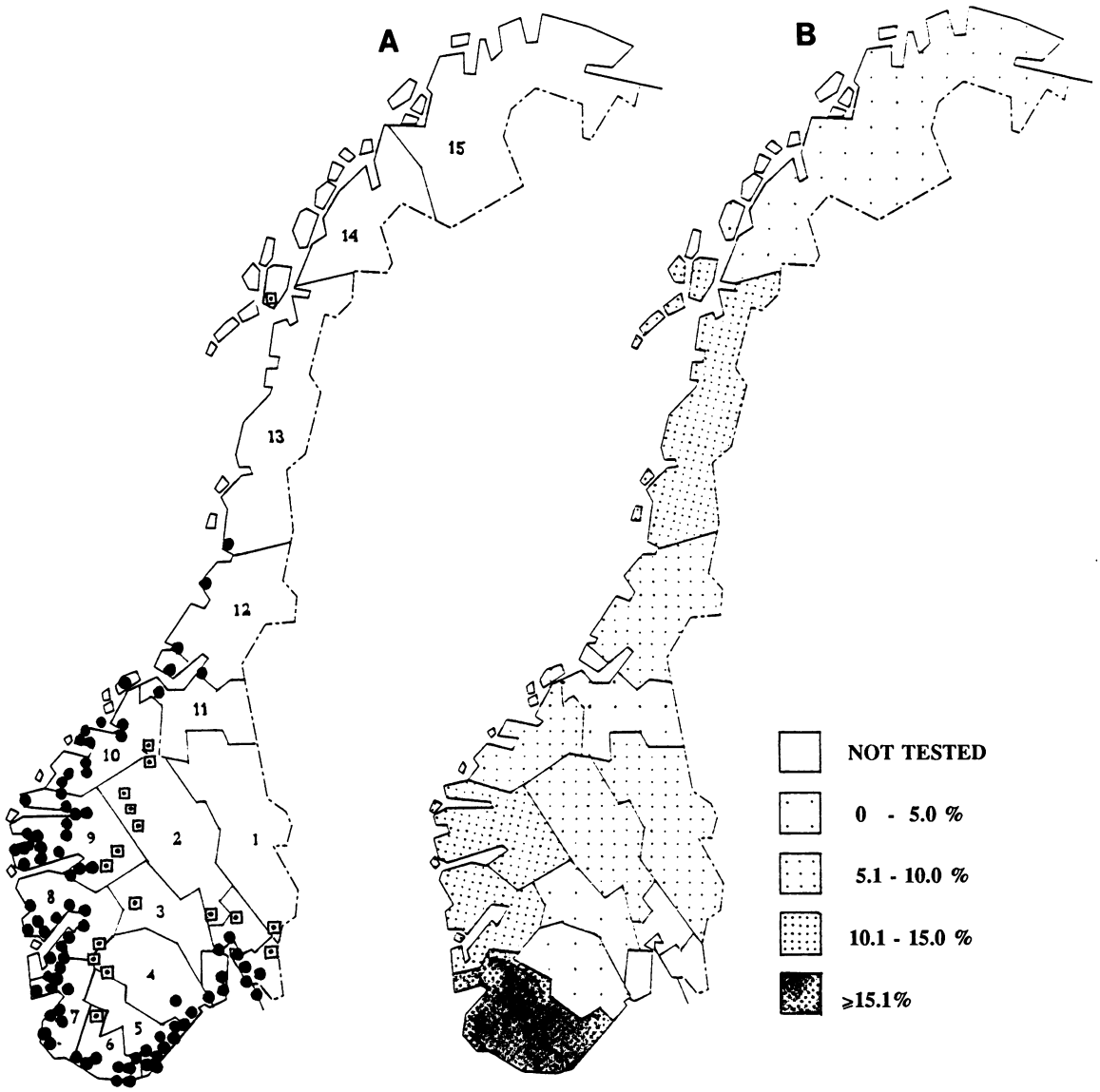

FIG. 2. (A) Map of Norway showing the counties (numbered) and the recorded occurrences of *I. ricinus* ticks (from reference 44). ●, recorded occurrences of *I. ricinus*; ⊡, *I. ricinus* found at locations outside the areas where *I. ricinus* ticks normally exist. (B) Recorded prevalence of sheep with antibodies to *B. burgdorferi* in Norway.

counties of Telemark and Sør-Trøndelag. In the northern county of Nordland, three (13%) positive animals were found. In the two most northern counties, Troms and Finnmark, one positive animal was found (in Troms).

Surveys for *I. ricinus* ticks have been conducted in all parts of Norway, and Fig. 2A shows the recorded distribution of *I. ricinus* in Norway. Figure 2B shows the counties that were included in this serosurvey and the percentage of seropositive animals in each county. The highest percentage of seropositive animals was found in counties where *I. ricinus* is widespread, but positive animals were also found in areas where the ticks are not known to exist.

Age of the animals correlated with seropositivity. The age of the animals tested ranged from 0.5 to 7.5 years, with a mean age of 2 years and 4 months, the largest proportion (49%) being 1.5 years old. None of the 0.5-year-old animals were seropositive, but in the 1.5-year-old group, 12% of the animals were positive, and a similar percentage of positive animals was found in animals in the older age groups. The three animals with the highest titers were all 1.5 years old.

Specificity testing. None of the sera which were positive in the *B. burgdorferi* ELISA gave high background reactions in uncoated wells. All sera tested were negative for antibodies to *L. interrogans* serovars hardjo and *L. interrogans* serovar icterohaemorrhagiae, and only two of the *B. burgdorferi*-positive serum samples were borderline or weakly positive in the *T. pallidum* antibody test.

DISCUSSION

Upon testing of a representative sample of sheep from all parts of Norway, 10% of the 327 animals tested proved to harbor antibodies to *B. burgdorferi*, indicating exposure to the agent. All were clinically healthy at the time of serum

sampling. The distribution of seropositive animals was generally in good agreement with the known distribution of *I. ricinus* in Norway (44), with the prevalence of seropositive animals being highest in the more southern coastal areas. However, a complete correlation between the geographical distribution of seropositive animals and the reported distribution of *I. ricinus* was not found, because some seropositive animals were also found further inland, where *I. ricinus* has not been recorded.

The highest percentage of positive animals was found in the county of Rogaland, where *I. ricinus* is very common. The finding of one positive animal in the northern county of Troms was unexpected, because *I. ricinus* has not been recorded that far north. The positive animal in Troms had remained in the county all its life. Furthermore, we did not expect to find so many animals to be seropositive with a high titer in the county of Nordland, where *I. ricinus* is rarely found, and surprisingly, many positive animals were found in the county of Oppland, where the tick is not widespread.

Serosurveys done in the United States and Scandinavia have shown various degrees of correlation between the number of *B. burgdorferi*-seropositive animals and the distribution of *Ixodes* ticks (12, 20, 23, 38, 40).

One possible explanation for the discrepancies that we found between the distribution of *B. burgdorferi*-positive animals and the recorded *I. ricinus* distribution could be that the ticks were nevertheless actually present in those areas, regardless of the previously recorded tick distribution patterns. In Sweden, a population of *I. ricinus* infected with the spirochete was recently found farther north than the ticks had previously been recorded (30). The spread of infected ticks by migratory birds has been suggested as an explanation for the appearance of new foci of infection (2, 27, 61). In Norway, 95% of the ticks found on migratory birds were *I. ricinus* (43).

Another explanation might be that vectors other than ticks carry the spirochete. *B. burgdorferi* has been found in deer flies, horse flies, and mosquitoes in the United States (35); and one case of erythema chronicum migrans after a mosquito bite has been reported in Sweden (25). In Norway, *B. burgdorferi*-like spirochetes have been found in the tabanid fly (*Haematopata pluvialis*), in addition to *I. ricinus* (42). In the United States, in an area where *I. dammini* ticks were not reported, Lyme borreliosis was diagnosed in a cow (16). The affected animal and 60% of the other cows in the herd had antibodies to *B. burgdorferi*, and the authors speculated whether the animals could have been infected via other arthropod vectors or possibly by direct contact. The transmission of *B. burgdorferi* infection by direct contact has been reported in mice (*Peromyscus* spp.) (14). Furthermore, live *B. burgdorferi* have been isolated from the urine of mice (*Peromyscus leucopus*) (8), and *B. burgdorferi* antigens have been found in the urine of infected mice and humans (29). Experiments with mallard ducks (*Anas platyrhynchos platyrhynchos*) have shown that they can be infected with *B. burgdorferi* orally and that they can shed the organism in their droppings (13). They may thus have the capacity to spread spirochetes over long distances without an arthropod vector.

Another explanation for the detection of seropositive animals outside *I. ricinus*-infested areas could be the presence of cross-reacting antibodies against other microorganisms such as other *Borrelia* spp. and *Treponema* and *Leptospira* spp. None of the seropositive animals tested in this study had antibodies to *L. interrogans* serovar hardjo or *L. interrogans* serovar icterohaemorrhagiae, and only two animals were borderline or weakly positive in the *T. pallidum* antibody test. The *B. burgdorferi*-seropositive animal found in the county of Troms was negative by both the *Treponema* and the *Leptospira* antibody tests. In humans with Lyme borreliosis, it has been reported that antibodies to *B. burgdorferi* cross-react in tests for *T. pallidum* but do not cross-react with serovars of *L. interrogans* (36). Also, in the United Kingdom, vaccination of cows against *L. interrogans* did not cause false-positive results in the *B. burgdorferi* test (19). *Leptospira* spp. have not been found in domestic ruminants in Norway (45), and there are no reports on *T. pallidum* infections in sheep in Norway. It thus seems unlikely that the seropositive animals unexpectedly found in this study could be explained by the fact that they had antibodies that cross-reacted with other spirochetes.

When studying the ages of the animals included in the serosurvey described here, it appears that the percentage of seropositive animals did not increase significantly with increasing age. By the time they had reached the age of 1.5 years, about 10% of the animals had become seropositive, which indicates that most animals are exposed to the spirochetes early in life. The animals with the highest antibody titers were in the 1.5-year-old group, and these high titers may reflect a recent infection. None of the 0.5-year-old animals were seropositive, but it should be kept in mind that most of them came from non-tick-infested areas.

When establishing a serological test, the determination of cutoff levels is of importance. In the work described here, the use of 3 SDs as a cutoff gave much better reproducibility and considerably lowered the number of reactions that were doubtful or borderline in the test. It was thus found to be advisable to use the higher specificity provided by the 3-SD cutoff. This reduces the number of false-positive sera, increasing the specificity of the test, but at the cost of a lower sensitivity and probably more false-negative sera (59).

All the animals which had high concentrations of antibodies to *B. burgdorferi* in the present study were clinically healthy at the time of blood sampling. Lyme borreliosis in sheep has not been described, whereas the signs of disease in dogs and horses are lameness, arthritis, loss of appetite, fever, and fatigue (17, 33, 37). The only previous study that included an examination of *B. burgdorferi* in sheep was from Sweden, where lambs showed a rise in antibody titer to *B. burgdorferi* after grazing in an *I. ricinus*-infested area. Arthritis occurs endemically in lambs in the area in question, and it was speculated whether *B. burgdorferi* could be the causal agent. Lambs (and also cows) from this area had significantly higher antibody titers than did animals from a different habitat where *I. ricinus* ticks have not been found (28).

It is not known whether the 32 (10%) healthy Norwegian sheep which were found to have antibodies to *B. burgdorferi* in this serosurvey were healthy carriers of *B. burgdorferi*, had subclinical infection, or had been clinically ill and recovered. The clinical significance of Lyme borreliosis in sheep is still uncertain. However, if sheep are carriers of *B. burgdorferi*, they represent a potential source of infection for humans. This would especially be the case in areas heavily infested with ticks and where sheep farming is common.

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REFERENCES

1. **Anderson, J. F.** 1989. Epizootiology of *Borrelia* in *Ixodes* tick vectors and reservoir hosts. Rev. Infect. Dis. **11:**1451–1459.
2. **Anderson, J. F., R. C. Johnson, L. A. Magnarelli, and F. W. Hyde.** 1986. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. Infect. Immun. **51:** 394–396.
3. **Åsbrink, E., A. Hovmark, and B. Hederstedt.** 1984. The spirochetal etiology of acrodermatitis chronica atrophicans Herxheimer. Acta Dermato-Venereol. **64:**506–512.
4. **Barbour, A. G.** 1984. Isolation and cultivation of Lyme disease spirochetes. Yale J. Biol. Med. **57:**521–525.
5. **Barbour, A. G.** 1988. Laboratory aspects of Lyme borreliosis. Clin. Microbiol. Rev. **1:**399–414.
6. **Bjørnstad, R. T., and K. Mossige.** 1955. Erythema chronicum migrans with meningopolyradiculitis. Tidsskr. Nor. Laegeforen. **75:**264–265.
7. **Bogsrud, T. V., and B. Ødegaard.** 1987. Flåttbåren borreliose—et tilfelle med kronisk meningoencefalitt forårsaket av *Borrelia burgdorferi*. Tidsskr. Nor. Laegeforen. **107:**25–27.
8. **Bosler, E. M., and T. L. Schulze.** 1986. The prevalence and significance of *Borrelia burgdorferi* in the urine of feral reservoir hosts. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **263:**40–44.
9. **Brennaas, O., and S. Ræder.** 1962. Meningoencefalomyeloradiculitt i Sunnhordaland—endemisk virusbetinget sykdom overført av skogflått. Tidsskr. Nor. Laegeforen. **82:**739–743.
10. **Brodie, T. A., P. H. Holmes, and G. M. Urquhart.** 1986. Some aspects of tick-borne diseases of British sheep. Vet. Rec. **118:**415–418.
11. **Burgdorfer, W., A. G. Barbour, S. F. Hayes, J. L. Benach, E. Grunwaldt, and J. P. Davis.** 1982. Lyme disease—a tick-borne spirochetosis? Science **216:**1317–1319.
12. **Burgess, E. C.** 1986. Natural exposure of Wisconsin dogs to the Lyme disease spirochaete (*Borrelia burgdorferi*). Lab. Anim. Sci. **36:**288–290.
13. **Burgess, E. C.** 1989. Experimental inoculation of mallard ducks (*Anas platyrhynchos platyrhynchos*) with *B. burgdorferi*. J. Wildl. Dis. **25:**99–102.
14. **Burgess, E. C., T. E. Amundson, J. P. Davis, R. A. Kaslow, and R. Edelman.** 1986. Experimental inoculation of *Peromyscus* spp. with *Borrelia burgdorferi*: evidence of contact transmission. Am. J. Trop. Med. Hyg. **35:**355–359.
15. **Burgess, E. C., A. Gendron-Fitzpatrick, and M. Mattison.** 1988. Foal mortality associated with natural infection of pregnant mares with *Borrelia burgdorferi*, p. 217–220. *In* D. G. Powell (ed.), Equine infectious diseases V. Proceedings of the Fifth International Conference. University Press of Kentucky, Lexington.
16. **Burgess, E. C., A. Gendron-Fitzpatrick, and W. O. Wright.** 1987. Arthritis and systemic disease caused by *Borrelia burgdorferi* infection in a cow. J. Am. Vet. Med. Assoc. **191:**1468–1470.
17. **Burgess, E. C., D. Gillette, and J. P. Pickett.** 1986. Arthritis and panuveitis as manifestation of *Borrelia burgdorferi* infection in a Wisconsin pony. J. Am. Vet. Med. Assoc. **189:**1340–1342.
18. **Craft, J. E., R. L. Grodzicki, and A. C. Steere.** 1984. Antibody response in Lyme disease: evaluation of diagnostic tests. J. Infect. Dis. **149:**789–795.
19. **Donoghue, A., and T. W. Schillhorn van Veen.** 1989. Investigating cross-reactions between *Leptospira* and *Borrelia*. J. Am. Vet. Med. Assoc. **195:**1460–1462.
20. **Frank, J. C.** 1989. Taking a hard look at *Borrelia burgdorferi*. J. Am. Vet. Med. Assoc. **194:**1521.
21. **Greene, R. T., J. F. Levine, E. B. Breitschwerdt, and H. A. Berkhoff.** 1988. Antibodies to *Borrelia burgdorferi* in dogs in North Carolina. Am. J. Vet. Res. **49:**473–476.
22. **Grodzicki, R. L., and A. C. Steere.** 1988. Comparison of immunoblotting and indirect enzyme-linked immunosorbent assay using different antigen preparations for diagnosing early Lyme disease. J. Infect. Dis. **157:**790–797.
23. **Hansen, K., and H. H. Dietz.** 1989. Serosurvey for antibodies to *Borrelia burgdorferi* in Danish dogs. Acta Pathol. Microbiol. Immunol. Scand. **97:**281–285.
24. **Hansen, K., P. Hindersson, and N. S. Pedersen.** 1988. Measurement of antibodies to the *Borrelia burgdorferi* flagellum improves serodiagnosis in Lyme disease. J. Clin. Microbiol. **26:**338–346.
25. **Hård, S.** 1966. Erythema chronicum migrans (Afzelii) associated with mosquito bite. Acta Dermato-Venereol. **46:**473–476.
26. **Hjelle, A.** 1966. *Borrelia*-like organisms in the urine of lambs suffering from eczema facialis. Nature (London) **212:**856–857.
27. **Hoogstraal, H., M. N. Kaiser, M. A. Traylor, E. Guindy, and S. Gaber.** 1963. Ticks (*Ixodidae*) on birds migrating from Europe and Asia to Africa, 1959–61. Bull. W.H.O. **28:**235–262.
28. **Hovmark, A., E. Åsbrink, O. Schwan, B. Hederstedt, and D. Christensson.** 1986. Antibodies to Borrelia spirochetes in sera from Swedish cattle and sheep. Acta Vet. Scand. **27:**479–485.
29. **Hyde, F. W., R. C. Johnson, T. J. White, and C. E. Shelburne.** 1989. Detection of antigens in urine of mice and humans infected with *Borrelia burgdorferi*, etiologic agent of Lyme disease. J. Clin. Microbiol. **27:**58–61.
30. **Jaenson, T. G., S. Bergström, N. Burman, M. Jonsson, and J. Chrico.** 1989. Smitto-bärande fästingar (*Ixodes ricinus*)—risk för angrepp även i Norrland. Sven. Veterinärtidsskr. **41:**577–578.
31. **Krogh, H. K.** 1958. Meningoencephalitis transmitted by the bite of the wood tick. Tidsskr. Nor. Laegeforen. **78:**472–473.
32. **Lindenmayer, J., M. Weber, A. Onderdonk, and J. Plain.** 1989. *Borrelia burgdorferi* infection in horses. J. Am. Vet. Med. Assoc. **194:**1384.
33. **Lissman, B. A., E. M. Bosler, B. G. Ormiston, and J. L. Benach.** 1984. Spirochete-associated arthritis (Lyme disease) in a dog. J. Am. Vet. Med. Assoc. **185:**219–220.
34. **Magnarelli, L. A., and J. F. Anderson.** 1989. Class-specific and polyvalent enzyme-linked immunosorbent assays for detection of antibodies to *Borrelia burgdorferi* in equids. J. Am. Vet. Med. Assoc. **195:**1365–1368.
35. **Magnarelli, L. A., J. F. Anderson, and A. G. Barbour.** 1986. The etiologic agent of Lyme disease in deer flies, horse flies, and mosquitoes. J. Infect. Dis. **154:**355–358.
36. **Magnarelli, L. A., J. F. Anderson, and R. C. Johnson.** 1987. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. J. Infect. Dis. **156:**183–188.
37. **Magnarelli, L. A., J. F. Anderson, A. B. Schreier, and C. M. Ficke.** 1987. Clinical and serologic studies of canine borreliosis. J. Am. Vet. Med. Assoc. **191:**1089–1094.
38. **Magnarelli, L. A., J. F. Anderson, E. Shaw, J. E. Post, and F. C. Palka.** 1988. Borreliosis in equids in northeastern United States. Am. J. Vet. Res. **49:**359–362.
39. **Magnarelli, L. A., J. M. Meegan, J. F. Anderson, and W. A. Chappell.** 1984. Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. J. Clin. Microbiol. **20:**181–184.
40. **Marcus, L. C., M. M. Patterson, R. E. Gilfillan, and P. H. Urband.** 1985. Antibodies to *Borrelia burgdorferi* in New England horses: serologic survey. Am. J. Vet. Res. **46:**2570–2571.
41. **May, C., D. Bennett, and S. D. Carter.** 1990. Lyme disease in the dog. Vet. Rec. **126:**293.
42. **Mehl, R., T. Bjerknes, P. Sandven, and B. Vandvik.** 1989. *Borrelia burgdorferi* in the tick *Ixodes ricinus* in Norway. Fauna Norv. Ser. B **36:**87.
43. **Mehl, R., J. Michaelsen, and G. Lid.** 1984. Ticks (*Acari, Ixodides*) on migratory birds in Norway. Fauna Norv. Ser. B **31:**46–58.
44. **Mehl, R., P. Sandven, and L. R. Braathen.** 1987. The sheep tick *Ixodes ricinus*. Vector of spirochaetoses. Tidsskr. Nor. Laegeforen. **107:**1642–1644.
45. **Ødegaard, Ø. (National Veterinary Institute, Oslo, Norway).** 1991. Personal communication.
46. **Øverås, J., M. J. Ulvund, H. Waldeland, K. Kummeneje, H. S. Norberg, O. Sparboe, M. Binde, H. Grønstøl, M. Fjølstad, J. Bjørkås, and A. L. Heyeraas.** 1985. Tap og tapsårsaker i utvalgte saueflokker. Nor. Veterinaertidsskr. **97:**469–475.
47. **Pedersen, A. E., and P. Olson.** 1989. *Borrelia burgdorferi*—ett

infektionsämne på frammarsch. Sven. Veterinärtidsskr. **41:**545–547.

48. **Petri, W. A., Jr., and B. M. Farr.** 1988. Lyme disease. Curr. Opin. Infect. Dis. **1:**188–195.
49. **Reid, H. W.** 1991. Louping-ill, p. 170–174. *In* W. B. Martin (ed.), Diseases of sheep. Blackwell Scientific Publications, Oxford.
50. **Roush, J. K., P. A. Manley, and R. T. Dueland.** 1989. Rheumatoid arthritis subsequent to *Borrelia burgdorferi* infection in two dogs. J. Am. Vet. Med. Assoc. **195:**951–953.
51. **Russell, H., J. S. Sampson, G. P. Schmid, H. W. Wilkinson, and B. Plikaytis.** 1984. Enzyme-linked immunosorbent assay and indirect immunofluorescence assay for Lyme disease. J. Infect. Dis. **149:**465–470.
52. **Schwan, T. G., K. K. Kime, M. E. Schrumpf, J. E. Coe, and W. J. Simpson.** 1989. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). Infect. Immun. **57:** 3445–3451.
53. **Scott, G. R.** 1991. Tick-borne infections, p. 327–336. *In* W. B. Martin (ed.), Diseases of sheep. Blackwell Scientific Publications, Oxford.
54. **Shresta, M., R. L. Grodzicki, and A. C. Steere.** 1985. Diagnosing early Lyme disease. Am. J. Med. **78:**235–240.
55. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. **150:**76–85.
56. **Steere, A. C., S. E. Malawista, D. R. Snydman, R. E. Shope, W. A. Andiman, M. R. Ross, and F. M. Steele.** 1977. Lyme arthritis. An epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. Arthritis Rheum. **20:**7–17.
57. **Stiernstedt, G. T., M. Granström, B. Hederstedt, and B. R. Sköldenberg.** 1985. Diagnosis of spirochetal meningitis by enzyme-linked immunosorbent assay and indirect immunofluorescence assay in serum and cerebrospinal fluid. J. Clin. Microbiol. **21:**819–825.
58. **Tjørstad, K., and B. Vandvik.** 1986. Subakutt flåttbåren spirochætose. Tidsskr. Nor. Laegeforen. **106:**2506–2510.
59. **Tyler, J. W., and J. S. Cullor.** 1989. Titers, tests, and truisms: rational interpretation of diagnostic serologic testing. J. Am. Vet. Med. Assoc. **194:**1550–1558.
60. **Ulvund, M. J., T. Vik, and J. Krogsrud.** 1983. Louping-ill (tick-borne encephalitis) hos sau i Norge. Nor. Veterinaertidsskr. **95:**639–641.
61. **Weisbrod, A. R., and R. C. Johnson.** 1989. Lyme disease and migrating birds in the Saint Croix River Valley. Appl. Environ. Microbiol. **55:**1921–1924.