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Serological Investigation of Granulocytic *Ehrlichia* Infection in Sheep in Norway

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Stuen S, Bergström K: Serological investigation of granulocytic *Ehrlichia* infection in sheep in Norway. Acta vet. scand. 2001, 42, 331-338. – Serum samples of 749 sheep from 75 sheep flocks in Norway, i.e. 361 lambs (6 to 7 months old) and 388 adults (>1.5 year), were analysed for antibodies to *Ehrlichia equi.* Ten animals from each flock were examined. Seropositive animals were found along the coast of southern Norway from Vestfold to Sør-Trøndelag (as far north as 63°38´N). Seropositive sheep were not found in southeast, east or northern Norway. Thirty-two flocks were seropositive, although tick-borne fever had only been diagnosed earlier in half of these. In 78% of the seropositive flocks, more than 80% of the sheep were seropositive. A total of 35.7 % and 36.3 % of lambs and adults were found seropositive, respectively. However, the overall seroprevalence among animals that had been grazing on *Ixodes* pastures were 0.80 for the lambs and 0.84 for the adults. Mean antibody titres (± SD) ($\log_{10}$) in seropositive lambs and adults were 2.59 (± 0.449) and 2.70 (± 0.481), respectively. No significant differences in either seroprevalence or mean antibody titre between sheep of different ages were obtained in this study. Based on antibodies 94% of sheep flocks on *Ixodes* pastures were infected with a granulocytic *Ehrlichia* infection. The association between seropositive flocks and *Ixodes* infested pasture shows a very high degree of agreement ($p<0.00001$). The present study indicates that granulocytic *Ehrlichia* infection in sheep is underdiagnosed in Norway.

***Ehrlichia phagocytophila;* antibodies; lambs; seroprevalence.**

Introduction

The most common tick-borne disease in domestic animals in Norway is tick-borne fever (TBF), caused by *Ehrlichia phagocytophila,* and transmitted by the tick *Ixodes ricinus* (*Øverås* 1972, *Stuen* 1997). TBF may cause abortion in ewes and temporary infertility in rams (*Woldehiwet & Scott* 1993), but the main consequence of an *E. phagocytophila* infection in sheep is the ensuing immunosuppresion that leads to secondary infections, such as *Staphylococcus aureus* pyaemia and *Pasteurella hemolytica (trehalosi)* septicaemia (*Brodie et al.* 1986, *Stuen* 1996). In the UK, it has been estimated that more than 300 000 lambs develop tick pyaemia annually (*Brodie et al.* 1986).

TBF has for decades been considered as an important disease in lambs in certain areas along the coast of southern Norway (*Stuen* 1998). The purpose of the present study was to investigate the distribution of *E. phagocytophila* infection in sheep in different areas of Norway, especially in areas with a distribution of *I. ricinus.*

Materials and methods

Flocks from each county in Norway were included in this study, such that flocks in *Ixodes*

areas along the coast and areas with a high number of winterfed sheep were preferred. However, representative flocks in each area were chosen and sampled by the local veterinarians.

Serum samples from sheep flocks were obtained in October/November. Samples from 10 sheep were randomly collected in each herd, around half of the samples were from lambs (6 to 7-months-old). A questionaire was filled out by the veterinarian during the visit of each flock, including questions about ectoparasitic treatment, *Ixodes* infested pastures, earlier treatment against TBF, and occurrence of tick-associated infections. Four sheep flocks were chosen from each of the 18 counties in Norway, except from the county of Sør-Trøndelag, where 8 flocks were selected. The reason for this was that the northernmost observation of tick-borne fever so far has been in the county of Sør-Trøndelag (*Stuen* 1997).

An indirect immunofluorescence antibody assay (IFA) was used to determine the antibody titre to *Ehrlichia equi* (*Artursson et al.* 1999). Two-fold dilutions of sera were added to slides precoated with *E. equi* antigen (Protatek International and Organon Teknika). Bound antibodies were visualized by fluorescein-isothiocyanate (FITC)-conjugated rabbit-anti-sheep immunoglobulin (Cappel, Organon Teknika). Sera were screened for antibodies at dilution 1:40. If positive, the serum was further diluted and retested. A titre of 1.6 ($\log_{10}$ reciprocal of 1:40) or more was regarded as positive.

The statistical analysis was done according to *Martin et al.* (1987). The overall seroprevalence and mean antibody titre were estimated and stratified by ectoparasitic treatment and age. Statistical calculations were done by using Statistix®, version 4.0 (Analytical software). Statistical analyses on seroprevalence were performed using a chi-square test and the antibody titres were compared using a Students *t*-test for independent samples. Significance was set at $p<0.05$.

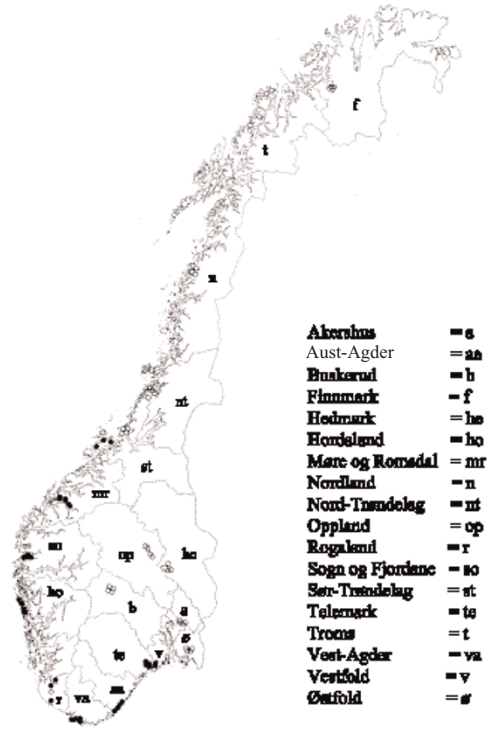

Figure 1. Geographical distribution of sheep flocks examined for antibodies to *Ehrlichia equi* in Norway. A titre less than 1:40 was considered negative. ● - seropositive flock, ○ - seronegative flock

Results

Of a total of 749 sheep from 75 flocks, 71 flocks in 1996 and 4 flocks in 1997, 270 sheep (36%) were found positive for antibodies to granulocytic *Ehrlichia* infection. Seropositive flocks were found in the coastal areas from Vestfold to Sør-Trøndelag. The northernmost seropositive flocks were found south of Trondheimsfjorden on the island of Hitra (63°38´N). The geographical distribution of the flocks is shown in Fig. 1. Thirty-two flocks were found positive, but only 16 of these had a history of previous

Table 1. Serological investigation of sheep sera for antibodies to *Ehrlichia equi* from different counties of Norway.

County	Number of positive flocks / total number of flocks	Number of flocks on tick pasture	Number of flocks treated against ticks	Number of flocks with a history of tick-borne fever (during the year of sampling)
Akershus #	0 / 4	0	0	0 (0)
Aust-Agder	4 / 4	4	3	2 (0)
Buskerud #	0 / 4	0	0	0 (0)
Finnmark #	0 / 4	0	0	0 (0)
Hedmark #	0 / 4	0	0	0 (0)
Hordaland	4 / 4	4	3	0 (0)
Møre og Romsdal	4 / 4	4	1	4 (2)
Nordland	0 / 4	0	0	0 (0)
Nord-Trøndelag	0 / 4	0	0	0 (0)
Oppland #	0 / 4	0	0	0 (0)
Rogaland	3* / 4	3	2	2 (1)
Sogn og Fjordane	4 / 4	4	2	4 (1)
Sør-Trøndelag	3 / 8	4	2	0 (0)
Telemark	4 / 4	4	2	0 (0)
Troms #	0 / 4	0	0	0 (0)
Vest-Agder	4 / 4	4	4	4 (2)
Vestfold	2* / 3	3	1	0 (0)
Østfold	0 / 4	0	0	0 (0)
Total	32 / 75	34	20	16 (6)

No known occurrence of *I. ricinus*
* Only one seropositive lamb in one flock

TBF infection (Table 1). Symptoms of disease were not observed in any sampled animal.
Clinical symptoms indicating a TBF infection, such as arthritis, polyarthritis and sudden death, were observed in only 6 flocks (8%); 4 of these had been prophylatically treated with insecticides. Twenty flocks were given prophylactic treatment against ticks with insecticides / repellents (mainly synthetical pyrethroids); lambs and adults were treated in 15 flocks, while only lambs were treated in 5 flocks.

Table 2. Distribution of *E. equi* antibodies in seropositive sheep flocks in Norway. Ten animals were investigated in each flock.

Percentage of seropositive animals	Seropositive flocks	
	Numbers	(%)
100	18	(56)
80-99	7	(22)
50-79	4	(13)
31-49	1	(3)
<30	2*	(6)

* Only one seropositive lamb in each flock

In 78% of the seropositive flocks, more than 80% of the sheep were seropositive and in 91% of the flocks, more than half of the animals were seropositive (Table 2).
The antibody titres in 361 lambs and 388 adults (>1.5 years) were recorded. A total of 129 of the lambs (35.7%) and 141 of the adults (36.3%) were found seropositive (Table 3). However, among animals that had been grazing on tick infested pasture, 79.6% and 83.9% of lambs and

Table 3. Reciprocal antibody titres against *E. equi* in 361 lambs and 388 adult sheep (>1.5 years) in Norway.

Titre values	Number of lambs	Number of adults	Total	(%)
<40	232	247	479	(64)
40	5	5	10	(1)
80	11	10	21	(3)
160	18	21	39	(5)
320	36	36	72	(10)
640	37	33	70	(9)
1280	12	23	35	(5)
2560	8	7	15	(2)
5120	2	5	7	(1)
10240	0	0	0	(0)
20480	0	1#	1	(0)
Total	361	388	749	(100)

The highest titre recorded was in a 3.5-year-old sheep.

adults were found seropositive, respectively. Significant difference in seroprevalence between animals of different ages was not found (Table 4).

Mean antibody titre ($\log_{10} \pm$ SD) in seropositive lambs and adults were 2.59 ± 0.449 and 2.70 ± 0.481, respectively. However, no significant differences in mean antibody titres between different age groups of seropositive animals were observed (Table 4).

In addition, no significant differences in either seroprevalence or mean antibody titre values were found between flocks treated or not treated with insecticides / repellents (data not shown).

The present investigation indicates that 94% of sheep flocks on *Ixodes* pastures were infected with a granulocytic *Ehrlichia* infection. The association between seropositive flocks and *Ixodes* infested pasture shows a very high degree of agreement ($p<0.00001$) (Table 5).

Discussion

Strong serological cross-reactions between *E. equi, E. phagocytophila* and the agent causing human granulocytic ehrlichiosis (HGE) have been reported (*Dumler et al.* 1995, *Nicholson et al.* 1997, *Pusterla et al.* 1997). It is therefore possible to use any of the 3 closely related *Ehrlichia* antigens to get acceptable results in serosurveys. The titre to a heterologous strain of *Ehrlichia* is normally less than against the homologous strain, but the IgG titres may also differ noticeably depending on the source of the antigen (*Bjoersdorff et al.* 1999, *Walls et al.* 1999). The sensitivity of the present test could perhaps have been increased by use of a more proper antigen, but unfortunately *E. phagocytophila* was not available for use as antigen in this study.

All blood samples were collected in October/November. Earlier investigations indicate that

Table 4. Seroprevalence and mean antibody titres ($\log_{10} \pm$ SD) to granulocytic *Ehrlichia* in sheep of different ages that had been grazing on *Ixodes* pastures.

Age	Seroprevalence	Mean titre values*	Number
<1 year	0.80	2.59 ± 0.449	129
1.5 years	0.82	2.59 ± 0.418	37
2.5 years	0.85	2.68 ± 0.387	22
>3 years	0.84	2.79 ± 0.527	82

* Only positive sera included

Table 5. Comparison of *E. equi* serology and tick pasture in relation to the number of seropositive sheep flocks in Norway.

Pasture	Seropositive flocks	Seronegative flocks	Total
Ixodes-infested	32	2 *	34
Ixodes-free	0	41	41
Total	32	43	75

Yates corrected $\chi^2 = 63.51$ ($p<0.00001$)

* Both flocks were grazing on pasture with an unknown distribution of *I. ricinus;* one flock had been prophylactically treated with synthetic pyrethroids

the antibody titres can be detected for at least 6 months in sheep after the primary infection (*Paxton & Scott* 1989), also when *E. equi* was used as antigen in the serological test (*Stuen et al.* 1998). In humans, serological titres may last for at least 30 months after an acute HGE infection (*Bakken et al.* 1997). In horses, serological investigations indicate that a positive antibody titre to *E. equi* could persist for more than 12 months in naturally infected horses (*Artursson et al.* 1999). The persistence of *Ehrlichia* antibodies therefore indicates that animals infected during the grazing season would be found seropositive the following autumn and winter.

The present study shows that granulocytic *Ehrlichia* infected sheep are found on the coast of southern Norway from Vestfold to Sør-Trøndelag (as far north as 63°38´N). No antibodies to granulocytic *Ehrlichia* were found on the southeast, east or northern parts of Norway. The distribution of seropositive animals in this study is in accordance with the distribution of *I. ricinus* in Norway, although scattered populations of *I. ricinus* have been found as far north as Brønnøysund (65°30´N) (*Mehl* 1983).

The present results are also in accordance with earlier reports on the distribution of clinical cases of TBF in domestic animals (*Stuen* 1997). In addition, in June 1997, cattle was found infected with *E. phagocytophila* for the first time in Stadsbygd (north of Trondheimsfjorden-63°32´N), in an area where *Babesia divergens* in cattle is common (*Schei,* personal communication). The present study indicates that the area around Trondheimsfjorden is so far the northernmost limit of *Ehrlichia* infections in domestic animals in Norway.

In comparison, babesiosis in cattle in Norway has been observed as far north as in Nordland county (65°47´N) (*Stuen* 1997). This difference in northern distribution between babesiosis in cattle and ehrlichiosis in sheep, may be due to differences in the maintenance of the respective infections in hosts or vectors. Sheep, wild deer and small rodents have been proposed as reservoir hosts for granulocytic *Ehrlichia* infection in Europe (*Ogden et al.* 1998a, *Brouqui* 1999), while *B. divergens* is regarded to be rather host specific (*Gray & Murphy* 1985). Both *B. divergens* and *E. phagocytophila* may cause persistent infection in cattle and sheep, respectively (*Joyner & Davies* 1967, *Foggie* 1951, *Stuen et al.* 1998), so both infections could be brought from endemic areas by both ticks or hosts. Both microorganisms are transmitted by *I. ricinus,* the only tick in Norway known to transmit infections to animals (*Mehl et al.* 1987). *E. phagocytophila* is transmitted transstadially in *I. ricinus,* and ovarial transmission has not yet been observed (*MacLeod & Gordon* 1933, *Ogden et al.* 1998b). In contrast, *B. divergens* infection could persist in *I. ricinus* for at least 2 generations even in the abscence of cattle (*Donnelly & Pierce* 1975, *Gray & Murphy* 1985). These observations might indicate a greater chance for maintenance of a *B. divergens* infection than a granulocytic *Ehrlichia* infection in *I. ricinus* populations in areas where competent hosts are sparsely scattered, as along the coast of northern Norway.

In the present study, 32 out of 34 flocks that grazed on tick infested pastures were infected with granulocytic *Ehrlichia.* The association between seropositive flocks and *Ixodes* infested pastures indicates a high degree of agreement. In 78% of the seropositive flocks, more than 80% of the sheep were seropositive. These results indicate a widespread *Ehrlichia* infection in areas where *I. ricinus* populations are present. Observations done in UK indicate a nearly 100% probability that a susceptible sheep will acquire granulocytic *Ehrlichia* infection on tick infested pasture (*Ogden et al*. 1998a). Earlier investigations indicate that the prevalence of granulocytic *Ehrlichia* infection in populations

of *I. ricinus* varies between different countries (*Brouqui* 1999). However, no information on the prevalence of *Ehrlichia* infection in *I. ricinus* populations in Norway is available.

No significant differences in antibody titres between different age groups of sheep were observed in this study. The titre values are in accordance with *E. equi* titres, found in experimentally *E. phagocytophila* infected lambs, 2 months after the initial infection (*Stuen et al.* 1998).

No effect of acaricide treatment was observed on the prevalence of infection or the titre values in *Ehrlichia* infected sheep. Most lambs / sheep were treated only once with acaricides on tick pastures. Earlier observations indicate that synthetical pyrethroids only give 2 to 3 weeks of full protection against ticks (*Mitchell et al.* 1986, *Henderson et al.* 1987). In addition, lambs grazing on tick pastures may seroconvert to *E. phagocytophila* after 3 weeks of tick exposure, although synthetical pyrethroids have been applied (*Hardeng et al.* 1992).

Only half of the seropositive flocks had a known history of TBF, indicating that granulocytic *Ehrlichia* infection is underdiagnosed in sheep flocks on tick infested pastures in Norway. This statement is supported by the fact that only 20 of 32 seropositive flocks (62.5%) had been treated prophylatically against TBF. Disease problems associated with tick infested pasture were only recorded in 6 flocks during the year of sampling; 4 of these had been treated with synthetical pyrethroids. These results indicate that some strains of granulocytic *Ehrlichia* may have low virulence in sheep, as observed earlier by *Foggie* (1951), *Tuomi* (1967), *Stannard et al.* (1969) and *Stuen et al.* (1998). *Foggie* (1951) and *Tuomi* (1967) also observed that isolates of *E. phagocytophila* from cattle and sheep in different geographic areas of infection may vary considerably with regard to their ability to cross-protect. Antigenic diversity has also been observed in isolates of the HGE agent (*Asanovich et al.* 1997).

Mild or subclinical *E. phagocytophila* infection may also be due to breed variations in susceptibility to a TBF infection, as has earlier been reported in sheep (*Scott* 1983). However, to the authors knowledge, no such breed differences have been observed in Norwegian sheep breeds. Few recorded disease problems may also indicate a recent introduction of TBF in the flock, since most primary infections of TBF in the field are not observed due to unobtrusive clinical signs (*Scott* 1983). The main disease problems associated with TBF are seen in lambs, and in sheep purchased from tick-free areas and put onto tick infested pastures.

In conclusion, the present results indicate that granulocytic *Ehrlichia* infection is abundant on tick infested pastures in Norway. The total sheep population in Norway during summer time is around 2.4 million, and the average flock size is approximately 100 sheep (*Trodahl* 1998). In 1996, more than 5100 flocks were treated prophylatically against TBF with tick repellents / insecticides (Norwegian Animal Disease Report 1996). However, in the present investigation only around 60% of the seropositive flocks had been prophylatically treated against tick infestation; all animals were treated in 75% of these flocks. These results indicate that more than 850 000 sheep in Norway are grazing on *I. ricinus* infested pastures and may be exposed to infection with *E. phagocytophila.* It is therefore probable that TBF infection in sheep may have a wider distribution in Norway than earlier believed.

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Sammendrag

Serologisk undersøkelse med hensyn på granulocyttær Ehrlichia infeksjon hos sau i Norge.

Serologisk undersøkelse med hensyn på antistoffer mot *Ehrlichia equi* ble foretatt på 749 sauer, fordelt på 75 flokker fra hele landet. Totalt ble 361 lam (6-7 måneder gamle) og 388 voksne (>1,5 år) undersøkt. Seropositive dyr ble funnet fra kysten av Sør-Norge fra Vestfold til Sør-Trøndelag (så langt nord som 63°38´N). Trettito flokker var seropositive, men granulocyttær ehrlichiose (sjodogg) hadde bare vært diagnostisert i halvparten av disse. I 78% av de seropositive flokkene var mer enn 80% av sauene seropositive. Totalt var 35,7% og 36,3% av henholdsvis lam og voksne seropositive. Av de sauene som hadde gått på Ixodes-infisert beite var imidlertid 79,6% av lammene og 83,9% av de voksne dyra seropositive. I middel var titret ($\log_{10} \pm$ SA) hos seropositive lam og voksne henholdsvis 2,59 (± 0,449) og 2,70 (± 0,481). Det var ingen signifikant forskjell i seroprevalens og titer mellom sau av ulik alder. Nittifire prosent av flokkene på *Ixodes*-beite var infisert med granulocyttær *Ehrlichia.* Det var sterk assosiasjon mellom seropositive flokker og forekomst av *Ixodes* ($p<0,00001$). Undersøkelsen tyder på at granulocyttær ehrlichiose hos sau er underdiagnostisert i Norge.

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