A serological survey for brucellosis in reindeer in Finnmark county, northern Norway

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Abstract: During September-December, 1990 to 1994, serum samples from a total of 5792 semi-domesticated reindeer (*Rangifer tarandus tarandus*) from Finnmark county, northern Norway, were screened for brucellosis on an indirect ELISA. There were no serologically positive animals. Twenty six of the animals had levels of antibodies detectable on the ELISA and were classed as suspicious, but the ELISA optical density readings were low compared to the readings for reindeer that were both culture positive and seropositive for *Brucella suis* biovar 4. When assayed on the standard tube agglutination test (STAT), all the 26 animals were seronegative. When absorbed with cells of *Yersinia enterocolitica* O-9, the antibody detectable on the ELISA could be removed to a great extent from most of the sera, indicating previous or ongoing exposure to bacteria serologically cross-reacting with *Brucella* in these animals. We concluded that brucellosis was not present among reindeer in Finnmark during this study. This is supported by the absence of any reports of brucellosis among reindeer in Norway.

Key words: *Rangifer tarandus tarandus, Brucella suis* biovar 4, ELISA, screening.

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Introduction

Brucellosis in reindeer is typically caused by *Brucella suis* biovar 4 (Corbel, 1990). The agent causing the disease in Soviet reindeer has been referred to as *Brucella rangiferi* (e.g. Zabrodin, 1973). Meyer (1966) concluded that the bacteria causing brucellosis in Soviet reindeer and in reindeer and humans in Alaska and Canada were the same species and biovar.

Most *Brucella* infected reindeer show no clinical signs of the disease (O'Reilly & Forbes, 1994; Ferguson, 1997). When clinical signs are present, the disease is characterized by abortion, retained placenta and metritis in the female, and by orchitis and epididymitis in the male (Rausch & Huntley, 1978). In both sexes, abscess-formation in joints and internal organs is sometimes observed. The disease is often manifest as bursitis and lameness (Neiland *et al.*, 1968; Syroechkovskii, 1995).

Brucellosis is enzootic in reindeer in arctic areas of Russia, Canada and Alaska (Dieterich, 1981; Zabrodin, 1984; Tessaro & Forbes, 1986; Brinley Morgan & Corbel, 1990; Forbes, 1991; O'Reilly & Forbes, 1994; Ferguson, 1997). In Russia, brucellosis in reindeer is known especially from the Taimyr (Syroechkovskii, 1995) and the Chucki

peninsulas and from Yakutia (Kalinovskii *et al.*, 1995). Brucellosis in Taimyr and on Baffin Island is a typical zoonosis, with sick domestic and wild reindeer as the main sources of infection (Kalinovskii *et al.*, 1995; Ferguson, 1997). Brucellosis is found in wolves (*Canis lupus*), polar foxes (*Alopex lagopus*), wolverines (*Gulo gulo*), brown bears (*Ursus arctos*) and ermines (*Mustela erminea*) as a consequence of their association with wild and domestic caribou and reindeer (Zarnke, 1983; Syroechkovskii, 1995).

The majority of cases of brucellosis in Russia are found in other species than reindeer and are caused by infection with species other than *B. suis* biovar 4. In 1993, nearly half of the registered cases among humans and cattle were in the northern part of Caucasus (Kalinovskii *et al.*, 1995). Finnmark is the northernmost county of Norway and borders on Russia, with the Kola Peninsula stretching eastwards at the Russian side. Little is known about the dissemination of brucellosis in the western parts of Russia (O'Reilly & Forbes, 1994). According to Syroechkovskii (1995), there has been no evidence of brucellosis among wild reindeer in the Kola Peninsula since 1930.

There is a possibility that brucellosis can be introduced to norwegian reindeer through exchange of reindeer and reindeer products across the border between Russia and Norway. For this reason, a serologic screening project was initiated to provide data on the occurrence of antibodies to *Brucella* among reindeer in Finnmark.

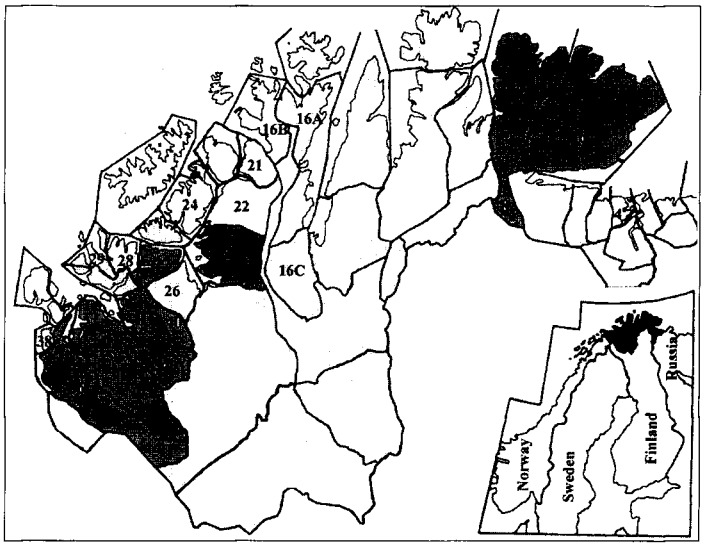

Fig. 1. Reindeer pasture districts in Finnmark (after Reindriftsforvaltningen, 1994). The borders between the districts are shown. The reindeer tested for *Brucella* antibodies came from the districts with the administrative numbers shown. About 95% of the animals came from the districts shown with darker shadowing.

Materials and methods

Serum samples

Blood samples were collected from semi-domesticated reindeer (*Rangifer tarandus tarandus*) at the reindeer slaughterhouse (Reinprodukter A/S) in Guovdageaidnu (Kautokeino), Finnmark, during September-December, 1990 to 1994. Samples were taken from 5792 animals as they came in for slaughter, from 19 pasture districts (Reindriftsforvaltningen, 1994) in Finnmark county (Fig. 1). About 95% of the animals came from ten of the districts (Table 1). Over time, sampling intensified especially in 1993 and 1994 (Table 2).

The animals were shot in the head with a cartridge hammer and bled immediately after. The samples were taken at that time in 10 ml silicone

Table 1. Blood samples collected for *Brucella* antibody testing from pasture districts (Fig. 1) in Finnmark, during Sep–Dec, 1990–1994.

Pasture district	Number of animals sampled	Proportion of all samples
6	1197	20.7
23	960	16.6
35	830	14.3
34	821	14.2
33	554	9.6
7/8	333	5.7
36	333	5.7
27	231	4.0
40	230	4.0
4, 16, 19, 21, 22, 24, 26, 28, 29, 38	303[1]	5.2
Total	5792	100

[1] The number of animals from each district ranged between 1 and 100.

Table 2. Age and year of sampling of reindeer tested for *Brucella* antibodies.

Year	Calves	Adults[1]	Total
1990	-[2]	-	28
1991	526	181	707
1992	376	99	475
1993	362	790	1152
1994	639	2791	3430
Total	1903	3861	5792

[1] ≥ 1 year old.
[2] -: not determined.

coated blood collecting tubes (Venoject, Terumo, Leuven, Belgium). Serum was collected (centrifugation 1000 g, 10 min) and stored at - 20 °C for subsequent analysis.

Control sera
Serum samples from 34 reindeer (*Rangifer t. groenlandicus*) that were culture positive for *B. suis* biovar 4 were used as positive controls. All were positive in the screening ELISA used and in the standard tube agglutination test (STAT) (Åsbakk *et al.*, in press). Two of these 34 sera, a strong positive and a weaker positive in the screening ELISA, were run on every plate to control for interplate variation in the screening ELISA.

All the 34 reindeer were from the herd at Tuktoyaktuk, North West Territories, Canada. They were part of a group of 288 reindeer transported from the arctic to brucellosis-free southern zones of traditional agriculture for game ranching purposes. The animals were quarantined after arrival and tested for brucellosis, and 33 of the 34 animals were positive on the buffered plate agglutination test (BPAT) when tested within one month prior to slaughter. One of the animals was not tested on the BPAT, but was positive on the Brucellosis Card Test® (BCT) (kit purchased from Becton Dickinson, BBL Microbiology Systems, Cockeysville, Md, USA was used) on the day of slaughter (Forbes & Tessaro, 1993).

Serum samples from three animals from the Kaamanen experimental reindeer herd in Finnish Lapland were used as negative controls in the screening ELISA. The animals of this herd are individually tagged and there has been no clinical or epidemiological evidence of brucellosis in the herd. All three were negative in the screening ELISA and in the STAT (Åsbakk *et al.*, in press).

Screening ELISA
A screening ELISA utilizing s-LPS from *B. abortus* (strain 413) as antigen and biotin-labeled rabbit antibody to reindeer immunoglobulin as detecting antibody (Åsbakk *et al.*, in press) was used. Each test serum, along with the positive and negative controls, were run in three parallel wells. Other wells contained 0.01 M phosphate-buffered saline, pH 7.2, supplemented with 0.05% Tween-20 (PBS-T), instead of serum. ELISA optical density (OD) readings greater than 0.104 were interpreted as suspicious, and OD readings greater than 0.161 were interpreted as serologically positive (Åsbakk *et al.*, in press).

Standard tube agglutination test (STAT)
STAT was performed according to Malkin *et al.* (1968) in wells of microtitre plates with V-shaped bottoms. Inactivated *B. abortus* was used as antigen (*B. abortus* SAT antigen) (Åsbakk *et al.*, in press). The sera were tested in dilutions 1:25, 1:50, 1:100 and 1:200.

Bacteria for absorption of antibody from serum
Yersinia enterocolitica O-9 was sub-cultured on conventional blood agar medium at 37 °C, harvested in physiological saline, and heated at 70 °C for 2 hr. The bacteria were washed in PBS, packed by centrifugation (8000 g, 10 min), and re-suspended in PBS to give 60% transmission of light at 540 nm. *Francisella tularensis* was sub-cultured on glucose-cystein-blood agar (Bevanger *et al.*, 1988), harvested, heat-treated and re-suspended as described for *Y. enterocolitica.*

Absorption of sera
Three volumes of reindeer (test) serum diluted 1:10 with PBS were mixed with one volume of the preparation of *Y. enterocolitica* or *F. tularensis.* Control tubes contained diluted test serum and PBS (3:1, v/v). The tubes were incubated (first 2 hr at 20 °C, then overnight at 4 °C, under mixing end-over-end) and centrifuged (8000 g, 10 min). The supernatant was diluted with PBS at a final serum dilution of 1:100 and tested on the screening ELISA.

Results

Of the 5792 animals tested on the ELISA, no animals were serologically positive. Twenty six were found suspicious. Twenty-five of these 26 were adults. This result in Pearson's Chi-square test

Table 3. Effects of absorption with *Yersinia enterocolitica* O-9 on the ELISA results for 26 sera suspicious for *Brucella* antibodies.

	Percent reduction in ELISA optical density				
	0	5 - 20	21 - 49	50 - 75	76 - 87
Number of sera	5	3	6	6	6
Number of sera remaining suspicious	5	3	1	0	0

returned the probability $P = 0.0015$ that the frequency of suspicious animals among the calves of the sample population (5764 animals with known age) equaled the frequency of suspicious animals among the adults of the sample population. When assayed on the STAT, the sera from all the 26 animals were negative in all dilutions.

When the 26 suspicious sera were tested on the ELISA after absorption with *Y. enterocolitica* O-9, a reduction of the OD readings was observed for 21 sera (Table 3). Nine sera remained suspicious after the absorption. Absorption with *F. tularensis* gave no reduction in OD for any of the 26 sera.

When the 34 positive control sera were tested on the ELISA after absorption with *Y. enterocolitica* O-9, the OD for 17 was reduced, by a mean of 9.4% (range 0.4-28%), and all remained positive (OD≥ 0.161). The remaining sera showed no reduction. The absorption with *F. tularensis* gave no reduction for any of the 34 sera.

Discussion

None of 5792 reindeer from Finnmark county were positive for brucellosis during ELISA testing. The OD readings for 26 animals with suspicious results were far below the readings for the positive controls and close to the readings for the negative controls.

The reindeer of Finnmark are semi-domesticated animals owned and herded by Sami people. The reindeer density is high, often exceeding 10 reindeer/km^2 and causing problems with overgrazing of the lichen pastures in parts of the county (Johansen *et al.*, 1995; Nordic Council of Ministers, 1996). The animals annually migrate from the inland winter areas to the coastal areas (summer pastures) in spring, and back in autumn (Vorren, 1962; Paine, 1994). During these movements the possibility of transmission of infection among the herds can be expected to be high due to mingling and contact among flocks. However, close observation of the herds is possible at this time and clinical signs of brucellosis would probably be noticed if present. Clinical signs of brucellosis have not been reported by herders.

Reported seroprevalences for brucellosis in reindeer (Zabrodin, 1973; Dieterich, 1981; Syroechkovskii, 1995; Ferguson, 1997) vary over a wide range. In populations with seroprevalence rates of 15% or more, animals with signs of brucellosis were commonly seen (Dieterich, 1981; Ferguson, 1997). A high incidence of brucellosis in caribou has been associated with increased cases in humans (Ferguson, 1997). No evidence of brucellosis has been found among Sami herders or workers in reindeer slaughter houses in Finnmark county or other parts of Norway.

The selection of reindeer for slaughter may be biased for animals showing signs of disease. Thus there is a possibility that females with reproductive problems and animals with other signs of reduced health may preferentially be selected for slaughter the following autumn. Therefore females with brucellosis, if present in the herd, are likely to be represented in the slaughtered population.

If the ELISA reactions for the 26 suspicious reindeer had been due to contact with *Brucella,* the STAT results indicated that contact was not recent. The STAT detects antibodies of the IgM class most efficiently, and is usually positive early in the course of primary infection, with titres decreasing as IgM levels fall and early IgG levels are still low. The ELISA results could indicate levels of anti-*Brucella* antibodies below the detection limits of the STAT. More likely, however, previous or ongoing exposure to serologically cross-reacting bacteria may have caused the 26 reindeer to give the suspicious test results. *Y. enterocolitica* O-9 and several other bacterial species, including *F. tularensis,* are known to cross-react serologically with *Brucella* strains (Perry & Bundle, 1990). The OD for 21 of the 26 suspicious sera were reduced after absorption with *Y. enterocolitica* O-9, and 17 were no longer suspicious. Absorption with other potentially cross-reacting bacteria may have further reduced the number of suspicious samples. Our conclusion that the suspicious results were caused by serologically cross-reacting agents was supported by the finding that 25 of the 26 suspicious reindeer were adults. Older animals have an increased opportunity to be exposed to cross-reacting bacteria. The conclusion is also supported by the fact that absorption with *Y.*

enterocolitica O-9 did not change the results for the positive controls.

Infectious diseases tend to spread and would be expected to cluster somewhat within a herd or population. If brucellosis was present, the population would likely contain several diseased individuals particularly since the reindeer density in Finnmark is high. The total population of reindeer in Finnmark in 1993 was counted to approximately 151 000 animals (Reindriftsforvaltningen, 1994). Given the population size, sample sizes of 298 and 457 teindeer would be required to be 95% and 99% certain, respectively, that at least one animal in the sample would be diseased if the disease was present at or above a prevalence of 1% (Martin *et al.*, 1987). Higher prevalences would require smaller sample sizes. We found that the 5792 reindeer were negative for brucellosis, and from this we calculated that the maximum number of diseased animals expected in the population of 151 000 reindeer, with probability (confidence level) of 95% and 99%, would be 77 and 118 animals, respectively (Martin *et al.*, 1987). This corresponds to prevalences of 0.05% and 0.08%, respectively. The lowest seroprevalence reported among known infected reindeer herds is, to our knowledge, 1.1% (in Taimyr, Syroechkovskii, 1995). In other areas the lowest reported prevalences are higher (Zabrodin, 1973; Dieterich, 1981; Syroechkovskii, 1995; Ferguson, 1997). In addition, the sample population was obtained at slaughter, and since disease may influence the withdrawal of the animal in the first instance, it can be assumed that the level of disease in this population of culled animals is higher than in the source population. The sample population (5792 animals) was large compared to what was required for the confidence levels 95% and 99%, and all the animals were found negative. The ELISA results, together with the negative results on the STAT and the lack of historical evidence of brucellosis among reindeer in Norway (the last case of brucellosis in Norway was in cattle in 1953; Sandvik & Næss, 1993), strongly indicate that none of the animals had been in contact with *Brucella* spp. We conclude that brucellosis was not present among reindeer in Finnmark at the time of study.

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