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ANTIBODIES TO RUMINANT ALPHA-HERPESVIRUSES AND PESTIVIRUSES IN NORWEGIAN CERVIDS

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ABSTRACT: A serologic survey revealed that Norwegian populations of free-ranging reindeer (*Rangifer tarandus tarandus*), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), and moose (*Alces alces*) have been exposed to alpha-herpesviruses and pestiviruses. A total of 3,796 serum samples collected during the period 1993–2000 were tested in a neutralization test for antibodies against bovine herpesvirus 1 (BHV-1) or cervid herpesvirus 2 (CerHV-2), and 3,897 samples were tested by a neutralization test and/or enzyme-linked immunosorbent assay for antibodies against bovine viral diarrhea virus (BVDV). Antibodies against alpha-herpesvirus were found in 28.5% of reindeer, 3.0% of roe deer, and 0.5% of red deer, while all moose samples were negative. In reindeer, the prevalence of seropositive animals increased with age and was higher in males than females. Antibodies against BVDV were detected in 12.3% of roe deer, 4.2% of reindeer, 2.0% of moose and 1.1% of red deer. The results indicate that both alpha-herpesvirus and pestivirus are endemic in reindeer and pestivirus is endemic in roe deer in Norway. The viruses may be specific cervid strains. Seropositive red deer and moose may have become exposed as a result of contact with other ruminant species.

Key words: *Alces alces*, alpha-herpesvirus, *Capreolus capreolus*, cervids, *Cervus elaphus*, pestivirus, *Rangifer tarandus tarandus*, serosurvey.

INTRODUCTION

Of the ruminant alpha-herpesviruses, bovine herpesvirus 1 (BHV-1) is the best characterized. However, other alpha-herpesviruses have been isolated from red deer (*Cervus elaphus*) and reindeer (*Rangifer tarandus tarandus*): cervid herpesvirus 1 (CerHV-1; Inglis et al., 1983) and cervid herpesvirus 2 (CerHV-2; Ek-Kommonen et al., 1986), respectively. Antibodies to alpha-herpesvirus were found in several species of cervids, including caribou (*Rangifer tarandus*; Zarnke, 1983), red deer (Pospisil et al., 1996), roe deer (*Capreolus capreolus*; Baradel et al., 1988), and moose (*Alces alces*; Zarnke and Yuill, 1981). Serologic cross reactions occur between BHV-1 and those isolated from cervids (Lyaku et al., 1992).

The pestiviruses have been divided into bovine viral diarrhea virus (BVDV) types 1 and 2, classical swine fever virus, and border disease virus, which are antigenically closely related. Pestiviruses have been isolated from several ruminant species, including red deer (Nettleton and Herring,

1980; Baradel et al., 1988), roe deer (Frölich and Hofmann, 1995), and reindeer (Becher et al., 1999). Seropositive individuals for pestivirus have been described in red deer and roe deer (Frölich, 1995), caribou (Elazahary et al., 1981), and moose (Thorsen and Henderson, 1971).

Monoclonal antibody studies and genomic analyses have indicated that there are four groups of pestiviruses. Most isolates from wild ruminants have been classified with the majority of bovine isolates (Edwards and Paton, 1995). However, later studies based on immunologic assays and gene sequence analyses have allocated certain free-ranging ruminant isolates to unique groups, indicating they are distinct species (Dekker et al., 1995; van Rijn et al., 1997; Fischer et al., 1998; Becher et al., 1999; Harasawa et al., 2000; Avalos-Ramirez et al., 2001).

The pathogenicity of alpha-herpesviruses and pestiviruses for cervids is uncertain, though there are reports of ocular disease in red deer calves due to CerHV-1 (Inglis et al., 1983; Nettleton et al., 1986) and

mild signs in red deer after experimental infection with this virus (Reid et al., 1986). There is only one report of successful infection of cervids with BHV-1 from cattle, an experimental infection of mule deer (*Odocoileus hemionus*; Chow and Davies, 1964). This suggests alpha-herpesviruses do not easily transmit between ruminant species. Experimental infection of reindeer with BVDV mainly caused enteric signs (Morton et al., 1990), while BVDV and border disease virus infection only caused seroconversion in red deer (Nettleton, 1990).

In cattle, BHV-1 and BVDV may result in disease problems; they cause infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea (BVD), respectively. Bovine herpesvirus 1 may also cause conditions like infectious pustular vulvovaginitis/balanoposthitis and abortion (Engels and Ackermann, 1996; Smith, 1997). Bovine viral diarrhea virus can be the cause of mucosal disease (MD) and hemorrhagic syndrome, but the economically most important manifestations are related to infection in pregnant animals, resulting in embryonic death, abortion, and congenital defects. Persistently infected (PI) calves may be born and serve as the main reservoir of infection to other animals (Baker, 1995).

The prevalence of BHV-1 infection is very low in the Nordic countries. A Norwegian surveillance and control program has been in place since 1993 and only one seropositive herd has been identified (Nyberg et al., 2001). There are also control programs for BVDV in the Nordic countries (Bitsch and Rønsholt, 1995). In Norway, the prevalence of BVDV-seropositive herds has decreased from 37% in 1993, the first year of the surveillance and control program, to 12% in 2000 (Nyberg, 2001). An infectious reservoir of alpha-herpesvirus or pestivirus in a free-ranging cervid population may constitute a risk of transmission to cattle, particularly when wild and domestic animals share pastures. Alpha-herpesvirus isolated from red deer

(CerHV-1) caused a weak immune response in cattle (Rønsholt et al., 1987) and a strain from reindeer (CerHV-2) caused mild rhinitis (Nettleton et al., 1988). Seroconversion in cattle can interfere with surveillance and control programs.

The aim of the present study was to find out whether Norwegian reindeer, roe deer, red deer, and moose are exposed to alpha-herpesviruses and pestiviruses by testing blood samples for antibodies. The study was performed as part of the National Health Surveillance Program for Cervids (HOP) at the National Veterinary Institute (NVI; Oslo, Norway).

MATERIALS AND METHODS

Collection and preparation of serum samples

Blood samples were collected during 1993–2000 from free-ranging reindeer in southern Norway, from red deer in western and central Norway, and from roe deer and moose in a majority of counties representing all regions of Norway. The broad latitudes and longitudes for Norway are 57°58'–71°08'N, 4°56'–31°10'E. More than 90% of the blood samples were obtained from animals shot during regular hunting. Of the remainder, a total of 70 roe deer samples were collected from animals found dead or shot for animal welfare reasons after being injured either in traffic accidents (49 animals) or in other ways. Blood samples from 105 red deer and 133 moose were obtained in connection with immobilization of animals as part of population studies. Together with each blood sample, a form giving information about location, date, species, sex, and age (calf, yearling, or adult) was sent by mail to NVI. At the laboratory, samples were centrifuged for 10 min at $1,400 \times G$, and serum was collected and stored at -20°C or -40°C until testing. Some serum samples collected were excluded from the study due to severe hemolysis or other poor quality factors. Blood samples from immobilized cervids were centrifuged and the serum collected and frozen at -20°C until submission to NVI.

"County" was used to designate the geographic origin of roe deer, red deer, and moose. The free-ranging reindeer in southern Norway comprise several distinct populations located in separate mountain areas, and the results are related to the actual area or population.

Virus neutralization tests

All serum-samples were analyzed by virus neutralization (VN). A rabbit kidney cell line

(RK 13) was used for testing of samples collected from reindeer. Eagle medium with Earle's buffered saline solution (EMEM; Eagle, Bio Whittaker, Wokingham, UK) served as medium, with gamma-irradiated and heat-inactivated (56 C for 30 min) bovine fetal serum at a concentration of 10% for cell growth and 2% for maintenance. Fifty mg gentamicin sulfate (Bio Whittaker) were added to 1,000 ml medium.

Aliquots of 100 µl EMEM, each containing a calculated number of 400 50% tissue culture infectious doses (TCID₅₀) of CerHV-2 (Ek-Kommonen et al., 1986), were mixed with 100 µl volumes of undiluted test serum samples and left overnight in the dark at 4 C. From each of the virus/serum mixtures, 50 µl volumes were inoculated into each of two wells of microtiter plate cell culture wells containing confluent monolayers of RK 13 cells with 150 µl of maintenance medium. A control titration was made of the previously calculated test virus dose. The plates were incubated in 5% CO₂ at 37 C and read for cytopathic (CP) effect after 3–4 days.

Sera that reacted with CP-inhibition at the screening dilution of 1:4 were retested with titration in double dilutions to the end point. Results were based on readings of the retest. Inhibition in one or both wells at a given dilution was regarded as antibody positive for that dilution.

For sera from roe deer, red deer, and moose, BHV-1 was used as the test herpesvirus and was cultured in bovine fetal turbinate cells as for BVD virus (see below).

In a validation test, 30 reindeer samples were tested by VN with BHV-1. Of these, 20 samples showed identical results with the two different antigens (10 negative, 10 positive; titers from 1:8 to 1:256), seven differed one dilution step between the tests (five were higher with BHV-1, two with CerHV-2), and three differed two dilution steps (two were higher with BHV-1, one with CerHV-2).

The same cell culture ingredients and general procedures were used for VN tests for antibodies against BVDV, except that a locally prepared culture of bovine fetal turbinate cells (FBT), kept as replicate batches in liquid nitrogen, were used up to the 7th passage. The virus used until the end of 1998 was the NADL-strain of BVDV, after which a Norwegian CP strain (MD2157/66) served as standard. The virus-serum mixtures were left to react for 60 min in the dark at room temperature, and the readings were made after 7–8 days.

ELISA for the detection of antibodies to pestivirus

A blocking enzyme-linked immunosorbent assay (ELISA; Serelisa BVD/MD Ab, Synbiot-

ics, Lyon, France) based on the non-structural protein P80/125 was used as the only serologic method in 57 red deer samples, of which 19 samples came from the county of Møre og Romsdal and 38 from Hordaland. In addition, 66 red deer samples were analyzed by ELISA following the VN, due to toxic effects of these sera on FBT cells. These samples were from the counties Sør-Trøndelag (17), Møre og Romsdal (20), and Sogn og Fjordane (29). Positive readings for the ELISA were set as recommended by the manufacturer, at 50% inhibition.

The ELISA was compared to the VN-test in a validation test. Of 42 samples tested, 34 were positive both in the ELISA and VN (titers from 1:2 to 1:128), while seven were negative in both tests. One sample had a VN-titer of 1:4, but was negative in the ELISA (32.5% blocking).

Statistical analysis

Chi-square tests were used to evaluate intra-species differences in prevalences of seropositive animals between sexes and age categories.

RESULTS

Numbers of animals seropositive to alpha-herpesvirus and BVDV are described in detail for each animal species in Tables 1–4. The highest seroprevalence (28.5%) to alpha-herpesvirus was found in free-ranging reindeer (Table 1). There were considerable variations in seroprevalences between the different reindeer populations. Significant differences by sex and age categories were found for reindeer seropositive to CerHV-2 ($P < 0.001$ for both analyses; Table 5). Almost 50% of the adult animals were positive. Even for BVDV, with an overall seroprevalence of 4.2%, there was a trend to higher seroprevalence in males than females (not significant).

In roe deer, 12.3% were positive for antibodies against BVDV and 3.0% were seropositive for BHV-1 (Table 2). Up to 25% of animals tested were positive in single counties for both infections; however the numbers tested were small in some counties. There was no significant difference relating to sex or age category, but the tendencies were similar to the results for reindeer (Table 5).

Three samples (0.5%) from red deer

TABLE 1. Results of serum neutralization tests of samples from reindeer collected in different Norwegian populations or areas, 1999 and 2000, for antibodies against reindeer herpesvirus and bovine viral diarrhea virus (BVDV).

Population or area	Number of reindeer herpesvirus positive/ number tested (%)	Number of BVDV positive/ number tested (%)
Forelhogna	165/641 (25.7%)	9/638 (1.4%)
Hardangervidda	33/43 (77%)	18/35 (51%)
Setesdal Austhei	3/10 (30%)	1/10 (10%)
Setesdal Ryfylke	12/29 (41%)	5/26 (19%)
Førdefjella	0/17	0/16
Snøhetta	8/43 (19%)	0/38
Sølnkletten	16/48 (33%)	1/47 (2%)
Total	237 ^a /831 (28.5%)	34 ^b /810 (4.2%)

^a Titers of reindeer herpesvirus positives, number: 1:4, 7; 1:8, 15; 1:16, 27; 1:32, 40; 1:64, 39; $\geq$ 1:128, 109.

^b Titers of BVDV positives, number: 1:8, 7; 1:16, 9; 1:32, 4; 1:64, 6; $\geq$ 1:128, 8.

(Table 3) tested positive for antibodies to BHV-1, and seven (1.1%) tested positive for BVDV-antibodies. Two of the latter were positive by ELISA. In moose (Table 4), 2.0% seropositive samples for BVDV were detected, while no moose tested positive for anti BHV-1 antibodies.

DISCUSSION

Antibodies to ruminant alpha-herpesviruses were found in a significant proportion of animals only in reindeer (28.5%),

while 3.0% of the roe deer, only 0.5% of the red deer, and none of the moose samples were positive. Antibodies to BVDV were detected in all four free-ranging cervid species studied; roe deer (12.3%), reindeer (4.2%), moose (2.0%), and red deer (1.1%). These findings confirm that cervids are susceptible to infection with pestivirus and alpha-herpesvirus and that they are exposed to such viruses in the wild in Norway.

Serologic surveys do not, however, give

TABLE 2. Results of serum neutralization tests of samples from roe deer collected in Norwegian counties, 1999 and 2000, for antibodies against bovine herpesvirus 1 (BHV-1) and bovine viral diarrhea virus (BVDV).

County	Number of BHV-1 positive/ number tested (%)	Number of BVDV positive/ number tested (%)
Nordland	0/6	2/8 (25%)
Nord-Trøndelag	1/35 (3%)	2/28 (7%)
Sør-Trøndelag	1/79 (1%)	14/89 (16%)
Møre og Romsdal	2/52 (4%)	5/61 (8%)
Hordaland	2/8 (25%)	1/8 (13%)
Rogaland	0/3	0/4
Vest-Agder	0/66	8/61 (13%)
Aust-Agder	2/97 (2%)	8/102 (8%)
Telemark	0/9	0/9
Vestfold	2/74 (3%)	9/85 (11%)
Buskerud	1/21 (5%)	5/23 (22%)
Hedmark	2/17 (12%)	4/20 (20%)
Oslo og Akershus	2/83 (2%)	10/82 (12%)
Østfold	3/52 (6%)	10/55 (18%)
Total	18 ^a /602 (3.0%)	78 ^b /635 (12.3%)

^a Titers of BHV-1 positives, number: 1:4, 9; 1:8, 2; 1:16, 1; 1:32, 3; 1:64, 1; $\geq$ 1:128, 2.

^b Titers of BVDV positives, number: 1:8, 10; 1:16, 10; 1:32, 14; 1:64, 8; $\geq$ 1:128, 36.

TABLE 3. Results of examination of serum samples from red deer collected in Norwegian counties, 1993–2000, for antibodies against bovine herpesvirus 1 (BHV-1) by virus neutralization test and bovine viral diarrhoea virus (BVDV) by virus neutralization and/or enzyme-linked immunosorbent assay (ELISA).

County	Year collected	Number of BHV-1 positive/ number tested (%)	Number of BVDV positive/ number tested (%)
Sør-Trøndelag	1995, 1998	1/114 (0.9%)	4 ^a /135 (3.0%)
Møre og Romsdal	1993–2000	1/212 (0.5%)	2 ^a /232 (0.9%)
Sogn og Fjordane	1998–2000	1/194 (0.5%)	1/221 (0.5%)
Hordaland	1995–99	0/43	0/44
Rogaland	1997–99	0/26	0/26
Total		3 ^b /589 (0.5%)	7 ^c /658 (1.1%)

^a One of the test positives in each county was by blocking ELISA, 92% positive and 69% positive.

^b Titers of BHV-1 positives, number: 1:16, 1; 1:64, 2.

^c Titers of BVDV positives, number: 1:4 – 1; 1:8, 1; 1:32, 2; 1:64, 1.

any information regarding which of antigenically closely related viruses caused seroconversion, or whether the origin is a domestic or wild ruminant reservoir. There is a great degree of serologic cross-reaction between the ruminant alpha-herpesviruses (Lyaku et al., 1992) and pestiviruses (Moennig and Plagemann, 1992).

In the present study, the NADL-strain of BVDV used in the neutralization test for pestivirus-antibodies was replaced at the end of 1998 by a Norwegian CP strain, isolated from a calf with mucosal disease. The latter strain gave somewhat higher neutralizing titers in seropositive goats than the NADL-strain, as reported by Løken

(1990). Different alpha-herpesvirus strains were used in the VN-test for different animal species. It was assumed that the reindeer strain was more relevant for screening of free-ranging reindeer. Validation tests demonstrated high degrees of agreement in results, indicating close serologic relationships both between the two strains of alpha-herpesviruses used and the BVDV strains, respectively, indicating that use of different strains is not likely to have influenced the outcome of the study.

Results of the present study indicate that an alpha-herpesvirus is endemic in free-ranging reindeer populations in Norway. Prevalence of seropositive individuals

TABLE 4. Results of serum neutralization tests of samples from moose collected in Norwegian counties, 1994–99, for antibodies against bovine herpesvirus 1 (BHV-1) and bovine viral diarrhoea virus (BVDV).

County	Year collected	Number of BHV-1 positive/ number tested	Number of BVDV positive/ number tested (%)
Nordland	1994, 1996	0/42	0/42
Nord-Trøndelag	1997	0/57	1/58 (2%)
Sør-Trøndelag	1995, 1999	0/104	0/104
Møre og Romsdal	1994	0/27	2/27 (7%)
Aust-Agder	1995	0/470	0/47
Vest-Agder	1995	0/31	0/31
Telemark	1999	0/37	0/37
Buskerud	1995, 1997–98	0/276	3/275 (1.1%)
Oppland	1995	0/428	11/426 (2.6%)
Hedmark	1995, 1999	0/492	10/497 (2.0%)
Akershus	1995	0/233	8/250 (3.2%)
Total		0/1774	35 ^a /1794 (2.0%)

^a Titers of BVDV positives, number: 1:4, 4; 1:8, 10; 1:16, 5; 1:32, 7; 1:64, 6; $\geq$ 1:128, 3.

TABLE 5. Age and sex distribution of free-ranging reindeer and roe deer blood sampled in Norway, during 1999 and 2000, in relation to positive antibody response to ruminant alpha-herpesvirus or bovine viral diarrhea virus (BVDV).

Sex, age category	Reindeer				Roe deer			
	Serology BVDV		Serology alpha-herpesvirus		Serology BVDV		Serology alpha-herpesvirus	
	Total	Positive (%)	Total	Positive (%)	Total	Positive (%)	Total	Positive (%)
Males	400	20 (5.0%)	411	142 (34.6%)	363	50 (13.8%)	342	11 (3.2%)
Females	401	14 (3.5%)	410	93 (22.7%)	261	26 (10.0%)	252	7 (2.8%)
Unclassified sex	9	0	10	2 (20%)	11	2 (18%)	8	0
Adults	369	13 (3.5%)	369	181 (49.1%)	259	32 (12.4%)	285	11 (3.9%)
Yearlings	86	3 (3%)	87	10 (11%)	134	14 (10.5%)	130	3 (2.3%)
Calves	293	5 (1.7%)	300	13 (4.3%)	182	26 (14.3%)	177	3 (1.7%)
Unclassified age	62	13 (21%)	75	33 (44%)	60	6 (10%)	10	1 (10%)
Total	810	34 (4.2%)	831	237 (28.5%)	635	78 (12.3%)	602	18 (3.0%)

was quite high, and seroreactors were found in almost all reindeer populations, indicating a widespread distribution of the virus. Reindeer gather in large herds at some times of the year, making the transfer of any infection effective. In cattle, antibody titers persist for years after artificial infection (Kaashoek et al., 1996), indicating a lifelong duration of the response. The cattle population in Norway has been surveyed over the years and has been declared free of BHV-1 infection (Nyberg et al., 2001). This supports the hypothesis that a specific reindeer strain of alpha-herpesvirus exists in Norway. A reindeer herpesvirus has been described previously in Finland (Ek-Kommonen et al., 1986).

Very few red deer seropositive for alpha-herpesvirus were detected. This low prevalence indicates that the red deer strain CerHV-1 described by Inglis et al. (1983), if present, is not common in Norway. The few positive individuals found may be false positives or due to contact with reindeer in neighboring habitats. Even in roe deer, the prevalence was relatively low with 3.0% ($n=18$). The few seropositive individuals were quite evenly distributed between counties, and half of these animals had very low titers of 1:4. These characteristics suggest unspecific/false positives responses. However, two of the animals had very high titers of 1:1,024

and 1:512 indicating a history of an active infection.

The situation regarding pestiviruses seems to be similar to the one found for herpesviruses; there are indications of circulating infections in both reindeer and roe deer. In roe deer, the infection was relatively widespread, with seropositive animals quite evenly distributed between counties. In Germany, a specific roe deer strain of pestivirus has been isolated (Fröllich and Hofmann, 1995). Although this virus is classified as a BVDV-1 (Harasawa et al., 2000), the strain has certain characteristics distinguishable from other BVD-strains (Fisher et al., 1998). Such a roe deer strain may also be present in Norway.

There was considerable variation in seroprevalence to BVDV between populations of reindeer, with a maximum at 50% on the high mountain plateau of Hardangervidda, which is the most important free-ranging reindeer area in Norway. Although the overall number of samples from this population was quite low, the high prevalence indicates that a virus has spread within the population. In 1999–2000, the period when samples from reindeer were collected, the proportion of cattle herds with antibodies against BVDV was 12–14% (Nyberg, 2001). It is less likely that persistently infected cattle, sheep, or infectious material (aborted fetuses and placentae) from do-

mestic animals have caused virus transmission to free-ranging reindeer to such an extent that it could explain the high prevalences found.

An earlier study demonstrated high seroprevalences in semi-domesticated reindeer in Finnmark, the northernmost county in Norway (Stuen et al., 1993). In this county, the density of domestic cattle is very low. Although pestivirus has never been isolated from reindeer, the results of these surveys indicate that a reindeer pestivirus may exist. In moose and red deer, seroprevalences for pestivirus were so low that it is more probable that responders were false positives or that an infection had been transmitted from other animal species.

In the present study, seroprevalence for antibodies against alpha-herpesvirus was higher in reindeer males than females, most likely due to differences in behavior between the sexes. The bucks may have increased frequency of contact with other individuals involving risk for transfer of infectious agents, such as guarding the herd, fighting with rivals, and promiscuous mating. The same tendency of higher seroprevalences in males was seen with pestivirus in reindeer and to both pestivirus and alpha-herpesvirus in roe deer (not significant). Additionally, the risk of being seropositive for alpha-herpesvirus increased with age in reindeer, and there was a similar trend in roe deer (not significant). In general, the risk of having been infected with a specific, endemic infection will increase with age. However, this age-dependency may be more evident for alpha-herpesvirus spreading during time limited epizootics, than for pestivirus presumably spreading from persistently infected animals over a prolonged time period in a herd or geographic area.

We conclude that pestivirus is probably endemic among roe deer in Norway, and both pestivirus and alpha-herpesvirus are probably endemic in reindeer. These virus strains may be specific cervid strains. The presence of virus-excreting free-living cervids may constitute a risk for infection in

cattle, which may lead to seroconversion and confuse surveillance and control programs for IBR and BVD. Efforts will be made to isolate the viruses from free-ranging cervids in Norway.

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