



A Screening for Virus Infections in Eight Herds of Semi-domesticated Eurasian Tundra Reindeer (*Rangifer tarandus tarandus*) in Norway, 2013–2018

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Background: Previous serological screenings have indicated that Eurasian semi-domesticated tundra reindeer (*Rangifer tarandus tarandus*) in Finnmark, Northern Norway, are exposed to alphaherpesvirus, gammaherpesvirus and pestivirus. Alphaherpesvirus (i.e., Cervid herpesvirus 2; CvHV2) has been identified as the transmissible component of infectious keratoconjunctivitis (IKC). Limited knowledge exists on the presence and prevalence of virus infections in other herding regions in Norway, which are hosting ~67,000 semi-domesticated reindeer and have contact with other species and populations of wildlife and livestock than those present in Finnmark.

Methods: Blood samples ($n = 618$) were obtained over five winter seasons (2013–2018), from eight different herds representing summer pasture districts in Tana, Lakselv, Tromsø, Lødingen, Hattfjelldal, Fosen, Røros, and Filefjell, distributed from North to South of the reindeer herding regions in Norway. Blood samples were investigated for specific antibodies against five viral pathogen groups, alphaherpesvirus, gammaherpesvirus (viruses in the malignant catarrhal fever group; MCFV), pestivirus, bluetongue virus (BTV), and Schmallenberg virus (SBV), by using commercial multispecies serological tests (ELISA). In addition, swab samples obtained from the nasal mucosal membrane from 486 reindeer were investigated by PCR for parapoxvirus-specific DNA.

Results: Antibodies against alphaherpesvirus and MCFV were found in all eight herds, with a total prevalence of 42% (range 21–62%) and 11% (range 2–15%), respectively. Anti-Pestivirus antibodies were detected in five of eight herds, with a total prevalence of 19% (range 0–52%), with two of the herds having a particularly high seroprevalence. Antibodies against BTV or SBV were not detected in any of the animals. Parapoxvirus-specific DNA was detected in two animals representing two different herds in Finnmark.

Conclusions: This study confirmed that alphaherpesvirus and MCFV are enzootic throughout the geographical reindeer herding regions in Norway, and that pestivirus is present in most of the herds, with varying seroprevalence. No exposure to BTV and SBV was evident. This study also indicated that semi-domesticated reindeer in Finnmark are exposed to parapoxvirus without disease outbreaks being reported from this region.

Keywords: alphaherpesvirus, bluetongue virus, gammaherpesvirus, parapoxvirus, pestivirus, schmallenberg virus, serology

INTRODUCTION

Reindeer husbandry is of major importance for livelihoods and for cultural values in Norway. For the five winter seasons of 2013–2018 (this study), the total number (all numbers being mean over five winters, counted March 31st, after slaughter and before calving) of Eurasian tundra reindeer (*Rangifer tarandus tarandus*) was reported to be 217,824 animals, with 74,441 animals slaughtered per year, representing 1,689 tons of meat. In Norway, reindeer herding is for the most part conducted by Sami herders, except for a few herds in the southernmost part of the reindeer herding region. In most areas in the country, reindeer herding is characterized as a mountain tundra type, with intermediate to long seasonal animal migration, using inland tundra pastures during winter. While in a few regions a coastal-oriented type is conducted, with local seasonal migrations, using coastal and snow free areas during winter (1). In many regions, reindeer share pasture with sheep, and to some extent goats. In addition, they may have contact with wild ruminants, such as moose (*Alces alces*), roe deer (*Capriolus capriolus*), and in some regions red deer (*Cervus elaphus*). A shortening daylength during fall increase melatonin levels in reindeer, which is associated with onset of puberty in young animals, oestrus in females, and rutting in males (2). Reindeer normally have a single offspring, born in spring. Reindeer are gregarious animals, forming large herds. The production is for the most part based on natural pastures, but the practice of supplementary feeding is increasing and may contribute to increased animal-to-animal contact and poor hygiene on feeding spots which may facilitate transmission of potential pathogens (3).

During 2013–2018 (this study), the average annual animal losses were reported to be 60,925 calves and 21,877 adult reindeer. The majority of these losses was reported to be due to predators, being on average for the period 89% of the lost calves and 77% of the lost adult reindeer. Loss of animals with unknown cause was reported to be 8% for calves and 13% for adult reindeer (4).

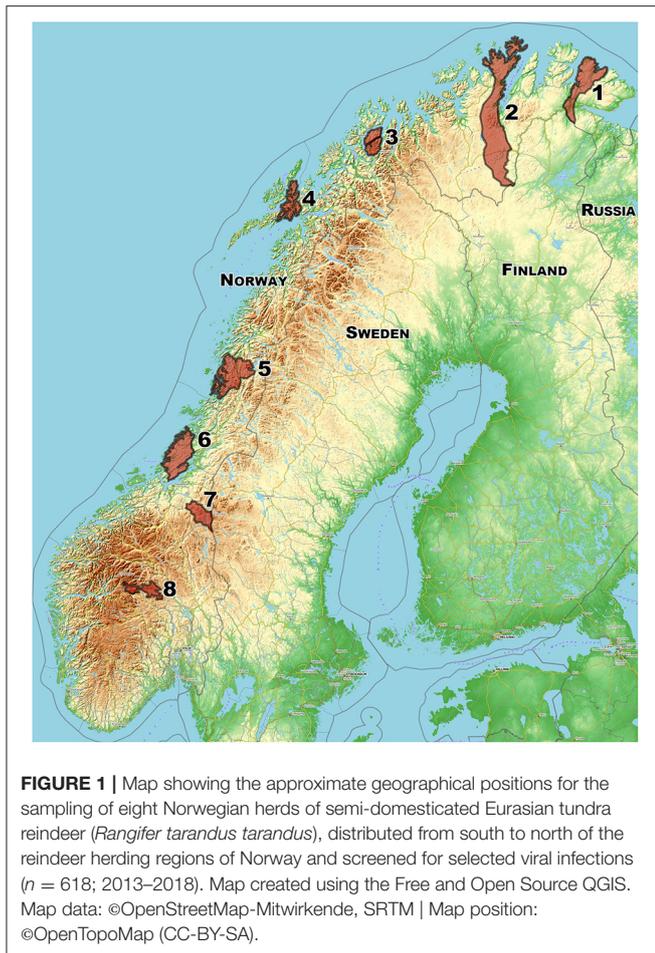
There have been no reports of major mortalities due to specific infectious diseases during the five winter seasons covered by this study. Previous serological screenings of semi-domesticated reindeer indicated that reindeer alphaherpesvirus, gammaherpesvirus (malignant catarrhal fever virus group; MCFV), and pestivirus are enzootic in all the investigated herds of Finnmark, Northern Norway (5–10), hosting ~73% of all semi-domesticated reindeer in Norway.

The alphaherpesvirus circulating in reindeer in Fennoscandia has been identified as Cervid herpesvirus 2; CvHV2, which

is grouped with other virus species in the subfamily *Alphaherpesvirinae*, genus *Varicellovirus*, that infect cattle, goats, and cervids. Serological screenings have indicated that CvHV2, or similar alphaherpesviruses, are enzootic in most of the investigated reindeer and caribou populations, including Sweden, Finland, Alaska (USA) and Canada (11–13). A serosurvey conducted among semi-domesticated reindeer in Finnmark, Norway (2004–2006, $n = 3,062$), showed that seroprevalence increased with age, being 8% in calves (≤ 1 year) and 49% in adults (> 1 year) (7). It has been shown that IKC may cause clinical symptoms characteristic for infectious keratoconjunctivitis (IKC) in semi-domesticated reindeer (14), often associated with secondary bacterial infections (15). IKC may affect single animals in a herd, but may also appear as regular outbreaks, affecting animal welfare and causing mortality, especially in young and immunologically naive animals (16).

The disease malignant catarrhal fever (MCF) is caused by MCFV, a group of gammaherpesviruses that primarily affect domestic and wild ruminants (17, 18). Antibodies against MCFV were previously found in semi-domesticated reindeer ($n = 3,339$) in Finnmark, with a prevalence of 3.5% (8). The risk of exposure was significantly higher for adults (> 1 year) than for calves (≤ 1 year), higher in the eastern compared to the western part of the studied area and increased with increasing population density (8). Further, a clinical case of sheep-associated MCF has been 208 diagnosed in a semi-domesticated reindeer (16). One clinical 209 reindeer case of malignant catarrhal fever has been reported (16), 210 but the overall impact of such infections in the reindeer herds is 211 not known.

Pestivirus is a genus in the *Flaviviridae* family, with four well-defined species causing disease in livestock; Pestivirus A and B (former BVDV-1 and BVDV-2, respectively) associated with cattle, Pestivirus C in swine (former classical swine fever virus; CSFV) and Pestivirus D in sheep (former border disease virus; BDV). In a study of 48 carcasses of predominantly emaciated semi-domesticated reindeer from Finnmark, pestivirus antibodies were detected in 33% of the animals by using a virus neutralization test (VNT) (6). A larger screening in Finnmark of apparently healthy reindeer sent to slaughter (2004–2008, $n = 3,339$) revealed a prevalence of 13% (9). In Sweden, several studies have shown a seroprevalence ranging from 0 to 35% among different reindeer herds (19, 20). A recent screening (2016–2017) revealed a mean seroprevalence of 49% in three Swedish herds and 41% in three Norwegian herds (21). In wild Eurasian tundra reindeer (*R. t. tarandus*)



in southern Norway, a seroprevalence of 4.2% was found (22), whereas a seroprevalence ranging from 34 to 78% has been described in different subspecies (*R. t. granti*, *R. t. caribou*, and *R. t. groenlandicus*) and populations of migratory caribou herds across Canada and in western Greenland (13).

Parapoxvirus (family *Poxviridae*) may cause proliferative processes in the skin and oral mucosa of reindeer, a disease called contagious ecthyma (CE) (16). Contagious ecthyma has been reported in semi-domesticated reindeer in Sweden (23), Norway [experimental animals; (24)] and Finland, the latter country having experienced severe outbreaks affecting tens and hundreds of animals, sometimes with high mortality (25). The single reported serious outbreak of CE under regular reindeer herding conditions in Norway appeared in Nordland in April 2000. It was caused by Orf virus (ORFV) and affected about 30 animals (26). Since reindeer's humoral immune response against parapoxvirus is assumed to be short lived, as in sheep (27), serological tests may be of restricted value. Active infections may be detected by the amplification (polymerase chain reaction; PCR) of parapoxvirus-specific DNA in tissue or swab samples, and phylogeny studies based on amplicon DNA sequences may help characterizing the circulating virus (28).

Bluetongue virus (BTV; genus *Orbivirus*, family *Reoviridae*) is transmitted by biting midges (*Culicoides* spp.) and may cause non-contagious acute disease in naïve sheep, inducing fever, excessive salivation, and oedema and cyanosis of the tongue and lips. BTV also infects cattle and a wide range of other domestic animals. Wild cervids are regarded as important in the epidemiology of BTV (29). Due to a rapid northward distribution of BTV in Europe, BTV appeared in Denmark (2007), Sweden (2008), and Norway (2009), but exposure of reindeer has not been documented (16). However, antibodies against BTV were detected in captive reindeer in Germany, with a prevalence of 3.4% ($n = 119$) (30).

Schmallenberg virus (SBV; genus *Orthobunyavirus*, family *Bunyaviridae*) appeared in dairy cattle in Germany in 2011, quickly spreading to 27 European countries during in the following couple of years (31, 32), including Norway (33). As for BTV, no exposure of semi-domesticated or wild reindeer has been reported for SBV, but antibodies were detected in captive reindeer in Germany, with a prevalence of 59% ($n = 115$) (30), indicating that reindeer are susceptible to infection.

The virus infections mentioned above were selected for this study since there are evidence that reindeer are exposed to these viruses, showing that reindeer are susceptible to infection. Alpha- and gammaherpesviruses and parapoxviruses may cause specific diseases in reindeer, whereas such knowledge is scarce or absent for pestivirus, BTV and SBV. However, comparable studies and reports on domestic animals and wild ruminants suggest that also reindeer may be clinically affected upon exposure. Except for the region of Finnmark, little is known about the exposure of semi-domesticated reindeer to viral pathogens in the other reindeer herding regions in Norway, hosting about 67,000 animals. The aim of this study was to investigate the exposure of semi-domesticated reindeer to six viral pathogens in eight different herding districts, representing a geographical north to south gradient through the reindeer herding regions of Norway and, at the same time, addressing possible temporal trends during the sampling period of five winter seasons.

MATERIALS AND METHODS

Animals and Sampling

Eight different semi-domesticated reindeer herds were sampled during late fall and winter (October - April) in five consecutive winter seasons, during the period 2013–2018. Whenever possible, samples were obtained from 10 calves (4–10 months old; both sexes) and 10 adult females (>1 year) from each of the eight herds (Figure 1). A total of 618 animals were sampled, 294 adults and 324 calves of the year (Table 1). The animals were either sampled live and released back into the herd during routinely herding practices or sampled before or during the slaughter process. Blood samples were collected from the jugular vein in blood tubes (BD Vacutainer®; BD, Plymouth, UK), using a venoject needle (Terumo, Leuven, Belgium) for live animals, or by collecting blood directly into open tubes during bleeding of slaughtered animals. Serum was prepared by centrifugation (10 min, 3,000 g) and stored at -20°C until further analyses. Swab samples (Applimed SA, Châtel-St-Denis, Switzerland) were

obtained from 486 reindeer by gently rubbing the mucosal membrane of the nose ~5 cm inside one of the nostrils. Swabs were placed in 1.8 ml cryotubes with 800 μ l of Eagle's Minimum Essential Medium (EMEM) containing antibiotics (10,000 U/ml penicillin and 10 mg/ml streptomycin; 1 ml/l of gentamicin 50 mg/ml and 10 ml/l of amphotericin B 250 μ g/ml; EMEMab 10 ml/l), frozen and stored at -80°C until analysis.

Analyses

A total of 618 reindeer were sampled for blood. The number of animals tested for antibodies against each pathogen, however, varied somewhat due to the limited volume of samples for some of the animals.

Alphaherpesvirus

Serum samples ($n = 570$) were investigated for anti-alphaherpesvirus antibodies using a commercial bovine enzyme-linked immunosorbent assay (ELISA) based on BoHV1 glycoprotein B (gB) as antigen (Table 2). The kit has previously been validated against a virus neutralization test (VNT) for analyzing reindeer serum samples for anti-alphaherpesvirus antibodies (34). All serum samples were tested in duplicate and evaluated against bovine (provided with the kit) and reindeer (35) positive-control sera.

MCFV

Serum samples ($n = 550$) were investigated for the presence of specific antibodies against viruses from the MCFV-group by a direct competitive-inhibition ELISA (cELISA) (36, 40), applied for reindeer serum samples as described previously (8) (Table 2). The cELISA is based on a monoclonal antibody (15-A) against an epitope highly conserved among all identified MCFV, including OvHV-2, CpHV-2, and CpHV-3 that are known also to infect and cause disease in cervids. Sera were tested as described for a previous screening of reindeer serum samples in Finnmark (8).

Pestivirus

Serum samples ($n = 570$) were tested for anti-pestivirus antibodies using a commercial blocking ELISA (bELISA; Table 2) designed for domestic ruminants which is based on the p80/125 non-structural protein as antigen, presumably shared between all strains of Pestivirus A and B (BVDV) and D (BDV) (37). This kit has previously been used for testing red deer (*Cervus elaphus*) and other wild ruminants for pestivirus antibodies, including reindeer (9, 20, 22). Further details, including evaluation of the kit for testing reindeer serum samples by a virus neutralization test (VNT), is published elsewhere (9).

Bluetongue Virus

Serum samples ($n = 479$) were investigated for antibodies against BTV using a commercial competitive ELISA based on recombinant VP7 as antigen (Table 2), which is conserved among BTV serotypes. According to the producer, the kit is suitable for testing multiple species, including sheep, goat, buffalo, deer, and others (38, 41), and has also been used to test reindeer serum samples (30). Sera were tested as described for a previous screening of reindeer serum samples (30).

Schmallenberg Virus

Serum samples ($n = 470$) were investigated for antibodies against SBV using a commercial competitive ELISA based on recombinant SBV nucleoprotein antigens for multispecies testing (Table 2). This kit has been used to detect anti-SBV antibodies in red deer (*Cervus elaphus*), fallow deer (*Dama dama*), moose (*Alces alces*), and roe deer (*Capreolus capreolus*) (42), as well as reindeer (30). Sera were tested as described for a previous screening of reindeer serum samples (30).

Parapoxvirus

DNA was isolated from nasal swab samples with a Maxwell 16 Buccal Swab LEV DNA purification kit (Promega, Madison, WI, USA). PCRs targeting the *B2L* gene (43) and the parapoxvirus gene region encoding a protein inhibiting granulocyte-macrophage colony-stimulating factor and interleukin-2 (*GIF*) (44) were conducted (28). Isolated DNA from a skin lesion of a goat with CE (Norwegian Veterinary Institute) was used as a positive control. PCR amplicons were prepared by enzymatic removal of unused dNTP and primers (ExoSAP-ITTM; Amersham Pharmacia Biotech, Sweden) and subsequently sequenced (BigDye[®] Terminator v3.1 cycle sequencing kit; Applied Biosystems, Norway) in an Applied Biosystems 3130 XL Genetic Analyzer (Applied Biosystems).

Statistical Analyses

All analyses were performed using the *glm* function in R 4.1.0 (45). The antibody screening for each virus infection was individually assessed with a binomial outcome (negative/positive result in the test, coded as 0 or 1, respectively) and a logit link, after removing rows with missing data in the explanatory variables. Explanatory variables comprised each individual's age (coded as calf or adult), sex (male or female), latitude, and sampling season (year). Age and sex were categorical variables, and latitude and sampling season were numerical variables. Goodness of fit and overdispersion were checked during the modeling, and the significance level was set at $P < 0.05$ for the explanatory variables.

RESULTS

Results from the serological screening for antibodies against alphaherpesvirus, MCFV and pestivirus are displayed in Table 1 and Figure 2. In Table 1, prevalence values are presented as a percentage with 95% confidence interval (CI) in square brackets.

Seroprevalence for alphaherpesvirus [overall 240/570; 42% (38;46)] was significantly higher in adult animals [197/277; 71% (66;76)] as compared to calves [43/293; 15% (11;19), $p < 0.01$], whereas no significant difference was found between males and females when correcting for age (Figure 3). There were no latitudinal or temporal trends in the alphaherpesvirus seroprevalence, although the three northernmost herds (i.e., Tana, Lakselv, and Tromsø) showed a high variability in seroprevalence, ranging from 21 to 63%.

Seroprevalence for MCFV [58/550; 11% (8;13)] was significantly higher in adults [44/270; 16% (12;21)] as compared to calves [14/280; 5% (3,8), $p < 0.01$] whereas no significant

TABLE 1 | Serological investigation of semi-domesticated Eurasian tundra reindeer (*Rangifer t. tarandus*) from eight different reindeer summer pasture districts in Norway, 2013–2017, presented as seropositive/animals tested, and seroprevalence (%) with confidence interval in square brackets for females (F), males (M), and overall (O).

Geographical region ^a	Sex	Alphaherpesvirus	Gammaparvovirus	Pestivirus
1. Tana	F	61/88; 69% (59;78)	4/88; 5% (2;11)	11/88; 13% (7;21)
	M	1/11; 9% (2;38)	0/11; 0% (0;11)	3/11; 27% (10;57)
	O	62/99; 62% (53;72)	4/99; 4% (2;10)	14/99; 14% (9;22)
2. Lakselv	F	28/46; 61% (46;74)	7/46; 15% (8;28)	0/46; 0% (0;7)
	M	4/13; 31% (13;58)	1/13; 7% (1;33)	0/13; 0% (0;23)
	O	32/59; 54% (42;66)	8/59; 14% (7;24)	0/59; 0% (0;6)
3. Tromsø	F	12/34; 35% (21;52)	1/35; 3% (1;15)	6/34; 18% (8;34)
	M	2/33; 6% (2;20)	3/35; 9% (3;22)	0/33; 0% (0;10)
	O	14/67; 21% (13;32)	4/70; 6% (2;14)	6/67; 9% (4;18)
4. Lødingen	F	10/17; 59% (36;78)	1/17; 6% (1;27)	3/17; 17% (6;41)
	M	1/13; 8% (1;33)	0/13; 0% (0;22)	0/13; 0% (0;22)
	O	12 ^b /42 ^b ; 29% (17;44)	1 ^b /42 ^b ; 2% (0;12)	3 ^b /42 ^b ; 7% (2;19)
5. Hattfjelldal	F	25/64; 39% (28;51)	9/51; 18% (10;30)	35/64; 55% (43;66)
	M	10/46; 22% (12;36)	4/38; 11% (4;24)	22/46; 48% (34;62)
	O	35/104; 34% (25;43)	13/89; 15% (9;23)	57/110; 52% (43;61)
6. Fosen	F	19/36; 53% (37;68)	7/34; 21% (10;37)	0/36; 0% (0;10)
	M	7/24; 29% (15;49)	1/24; 4% (1;20)	0/24; 0% (0;14)
	O	26/60; 43% (32;56)	8/58; 14% (7;25)	0/60; 0% (0;6)
7. Røros	F	24/57; 42% (30;55)	9/57; 16% (9;27)	24/57; 42% (30;55)
	M	7/25; 28% (14;48)	3/25; 12% (4;30)	6/25; 24% (11;43)
	O	31/82; 38% (28;49)	12/82; 15% (8;24)	30/82; 37% (27;47)
8. Filefjell	F	11/31; 36% (21;53)	5/31; 16% (7;33)	0/31; 0% (0;11)
	M	18/32; 56% (39;72)	3/32; 9% (3;24)	0/32; 0% (0;11)
	O	29/65 ^b ; 45% (33;57)	8/65 ^b ; 12% (6;22)	0/65 ^b ; 0% (0;5)
Overall	F	190/373; 51% (46;56)	43/359; 12% (9;16)	79/373; 21% (17;26)
	M	50/197; 25% (20;32)	15/191; 8% (5;13)	31/197; 16% (11;21)
	O	240/570 ² ; 42% (38;46)	58/550 ² ; 11% (8;13)	110/570 ² ; 19% (16;23)

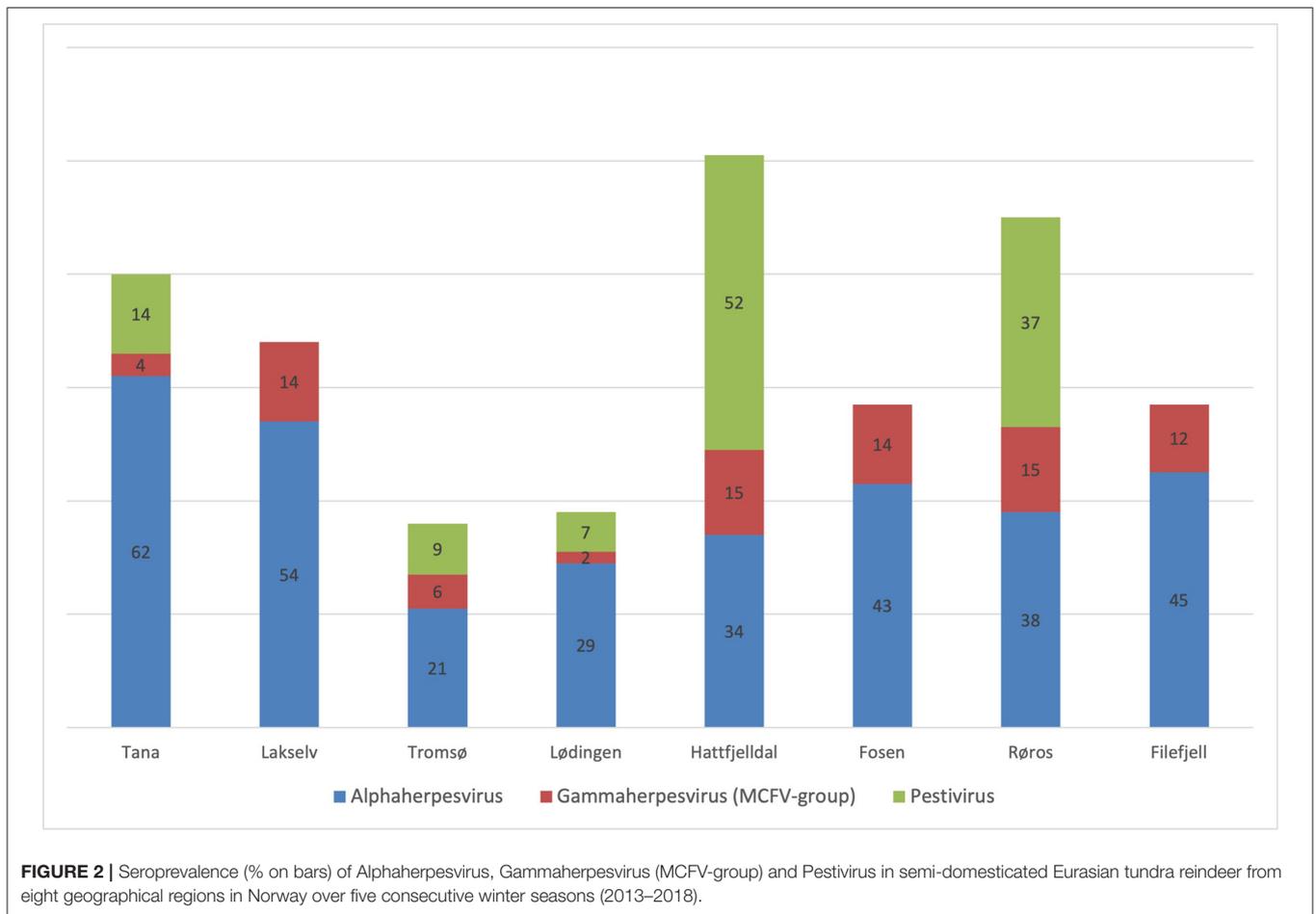
^aGeographical Regions (Counties): 1. Tana (Troms and Finnmark), 2. Lakselv (Troms and Finnmark), 3. Tromsø (Troms and Finnmark), 4. Lødingen (Nordland), 5. Hattfjelldal (Nordland), 6. Fosen (Trøndelag), 7. Røros (Trøndelag), 8. Filefjell (Innlandet).

^bOf the 42 animals from Lødingen, 12 calves were sampled with no record of sex, of which one was seropositive for alphaherpesvirus. None of the 12 calves had antibodies against any of the other pathogens tested. Similarly, of the 65 animals sampled in Filefjell, two were sampled with no record of sex, and being seronegative for all tests.

TABLE 2 | Serological tests used to investigate semi-domesticated Eurasian tundra reindeer (*Rangifer t. tarandus*) from eight reindeer herding districts in Norway (2013–2015) for antibodies against viral pathogens.

Viral pathogen	Test and producer	Test principle	Antigen	References
Alphaherpes	SERELISA BHV-1 gB Synbiotics, France	Blocking ELISA	Glycoprotein B	(34)
MCFV	Non-commercial	Competitive-inhibition ELISA	AIHV-1*	(36)
Pesti	SERELISA® BVD p80 Synbiotics, France	Blocking ELISA	p80	(37)
Bluetongue	ID Screen® Bluetongue Competition. ID Vet, Grabels, France	Competitive ELISA	Recombinant, VP7	(38)
Schmallenberg	ID Screen® Schmallenberg virus competition multispecies. ID Vet, Grabels, France	Indirect ELISA	Recombinant SBV antigen	(39)

*The MCFV cELISA is based on a monoclonal antibody (15-A) against an epitope highly conserved among all viruses in the MCF group, including AIHV1, AIHV2, OvHV2, CpHV2, CpHV-3, and Hippotragine herpesvirus 1 (HIHV1).



difference was found between males and females (**Figure 4**). There was a significant increase in overall seroprevalence from ~10% during 2013–2016, to 22% in 2017 ($p < 0.01$). In addition, there was a trend with latitude, where three of the four northernmost herds (i.e., Tana, Lødingen, and Tromsø) showed a lower seroprevalence than their southern counterparts ($p < 0.01$).

Seroprevalence for pestivirus [110/570; 19% (16;23)] was significantly higher in adults [68/277; 25% (20;30)] as compared to calves [42/293; 14% (11;19), $p < 0.01$] (**Figure 5**), whereas no significant difference was found between males and females. Furthermore, there was an increasing trend in overall seroprevalence with time, from 4% in 2013 to 43% in 2017, but with high variability along the latitudinal gradient.

None of the animals investigated had detectable antibodies against SBV or BTV.

PCR screening of nasal swabs for parapoxvirus revealed parapoxvirus-specific DNA of the *GIF* gene region in two nasal swab samples tested [2/486; 0.4% (0;1)], one from each of the two herds in Finnmark, both animals being female calves. PCR amplicons from one of these animals (Tana) were sequenced (consensus sequence, 296 nt, GenBank accession number MZ064575). A blast search (NCBI) indicated >99%

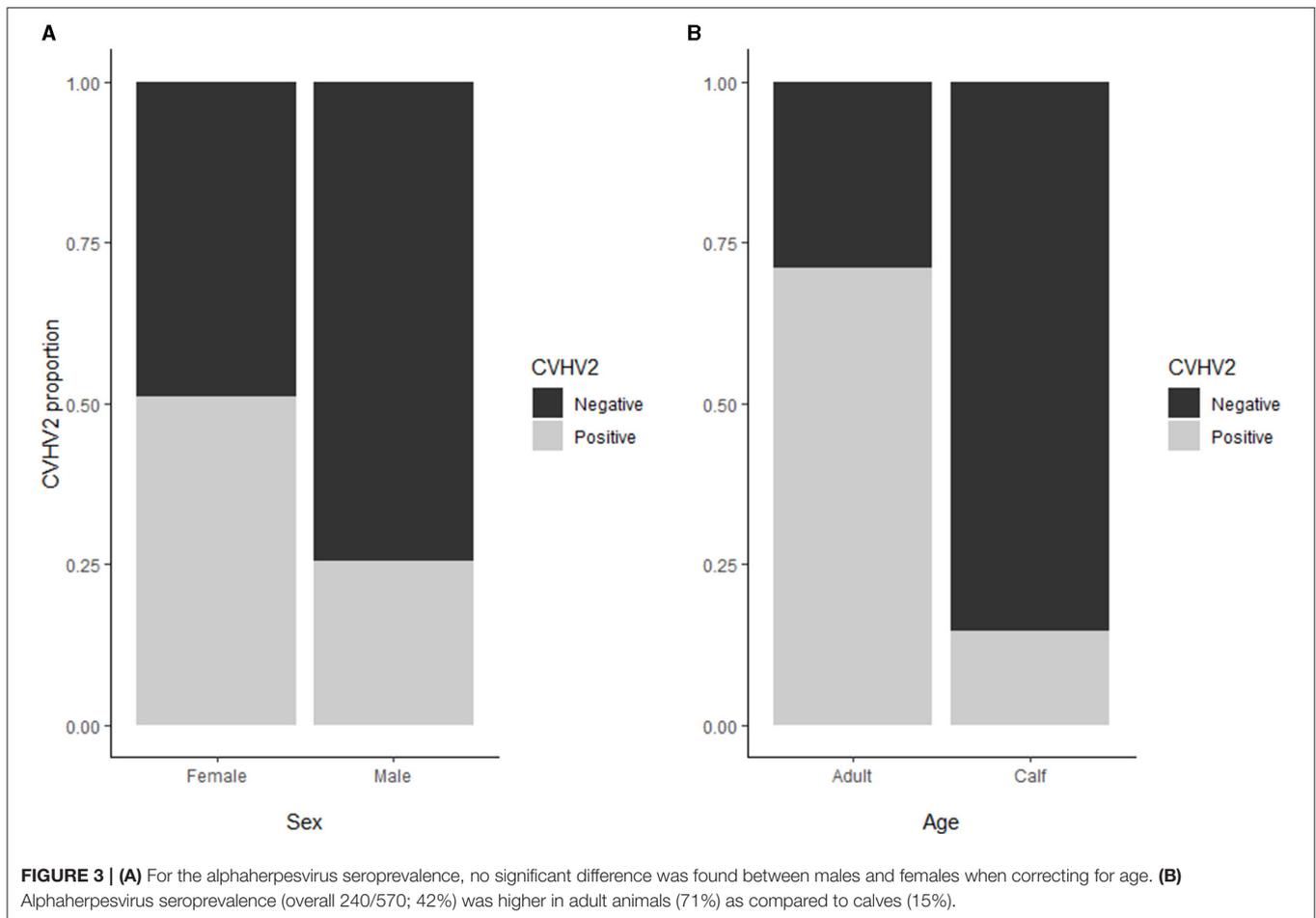
sequence homology with a large number of ORFV isolates, including isolates from reindeer and caribou.

Thirteen reindeer, six from herd 5 (Hattfjelldal) and seven from herd 7 (Røros) had antibodies against alphaherpesvirus, MCFV and pestivirus at the same time, whereas parapoxvirus-specific DNA was not detected in any of these animals. Of the 13 animals, all except one were adults, 10 females, one male, and two with unrecorded sex.

DISCUSSION

This is the first thorough screening of viral infections in semi-domesticated reindeer in Norway, conducted over a period of five consecutive winter-seasons and representing the different geographical regions and varying ecosystems that are supporting reindeer herding in Norway.

Serum samples were of good quality, since they were collected either from live animals or from animals during the slaughter process and centrifuged for serum collection the same day. Further, the serological tests used have been validated and/or used for testing reindeer samples in previous studies (**Table 2**). Samples were obtained mostly during the main slaughter season of reindeer, after rut. Since the main slaughter season of bulls is



prior to rut, the number of adult bulls in this study was restricted to 37, as compared to 198 females, which represents a gender bias in the sampling regime for this study.

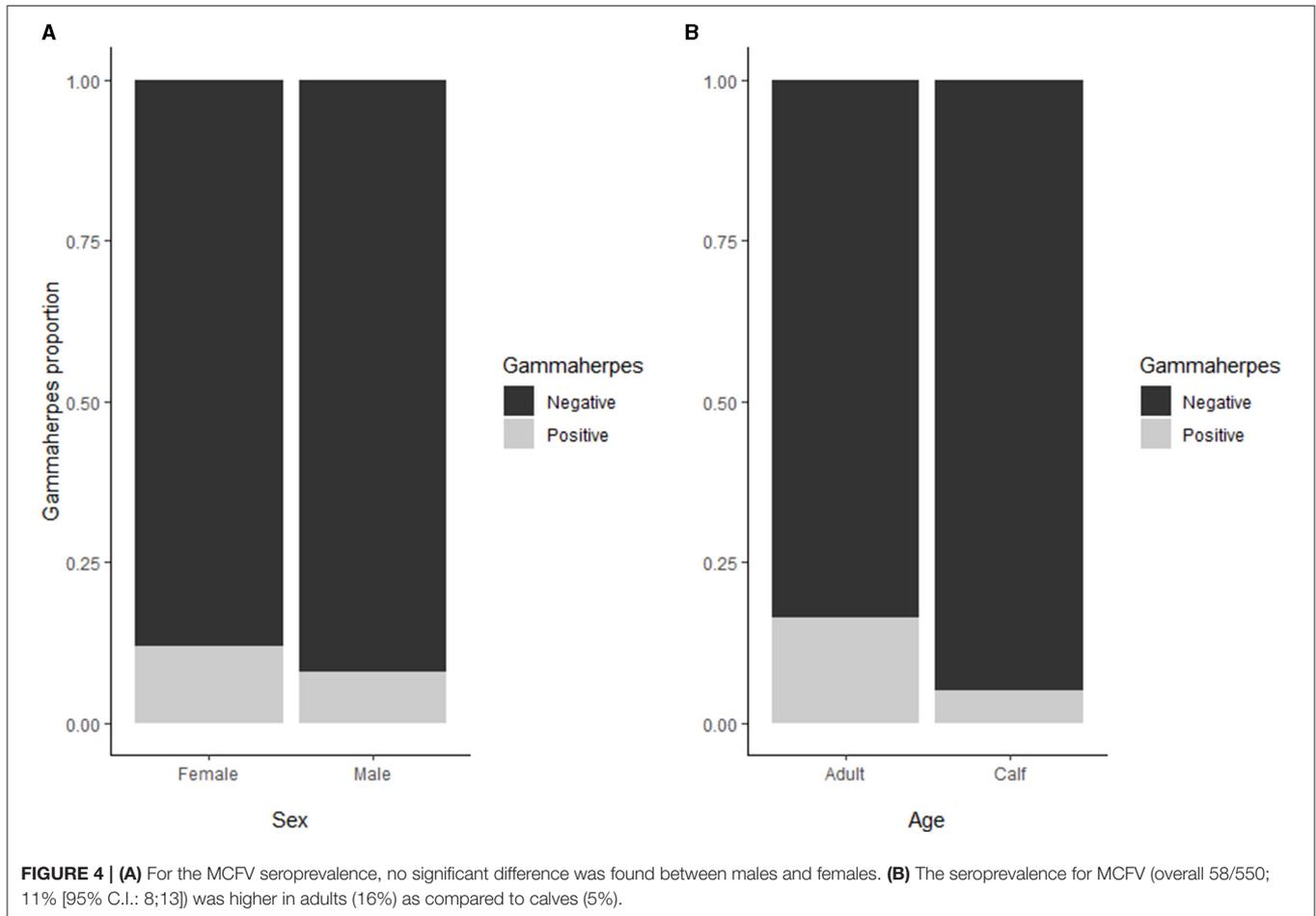
The antibody prevalence against alphaherpesvirus, presumably CvHV2, was 42% (mean, all herds), varying from 21% in Tromsø to 63% in Tana. Similar figures have been published for semi-domesticated reindeer in Finnmark (49%; $n = 3,062$ animals) (7) as well as for seven populations of wild Eurasian tundra reindeer in southern Norway (29%; $n = 831$) (22). Together, these investigations demonstrate that an alphaherpesvirus is enzootic in all the reindeer populations in Norway, both wild and semi-domesticated.

Cervid herpesvirus 2 does presumably not cause disease outbreaks with high mortality, but its general distribution and high prevalence in most investigated reindeer herds makes its pathogenic potential interesting. It has been shown that CvHV2 can be the transmissible pathogen during outbreaks of infectious keratoconjunctivitis (IKC) in reindeer, paving the way for secondary bacterial infections (46), and that the virus alone is able to cause IKC under experimental conditions (14). The virus is also associated with respiratory infections and can be vertically transmitted to the reindeer fetus *in utero* (47, 48), and possibly contribute to abortion and reduced fitness of calves

(11). Further, it has been demonstrated that the virus can experimentally be reactivated from latency, shed and isolated, after administration of glucocorticoids to seropositive animals (47, 49, 50). Thus, the impact of CvHV2 on the population level may be considerable and may be associated with stress and the reactivation of latent viruses.

The stress response in mammals includes activation of the hypothalamic-pituitary-adrenal axis, and the release of adrenal glucocorticoids. Experiments conducted in mice exposed to stress and subsequently inoculated with Herpes simplex virus 1 (HSV1) indicated that an exposure to stress, even prior to the infection, may compromise the individual's ability to mount an effective immune response, giving the pathogen time to replicate and spread (51). Studies in cattle with latent Bovine herpesvirus 1 (BHV1) infections and treated with glucocorticoids (i.e., mimicking stress) indicated that the reactivation process was more efficient compared to the mouse-HSV model, and that the glucocorticoid receptors stimulated viral replication upon reactivation (52).

These mechanisms have not been studied in detail for reindeer and CvHV2. However, it is likely that stimulation of the glucocorticoid receptors as a response to stress may reactivate latent CvHV2 infections. Further, stress would likely

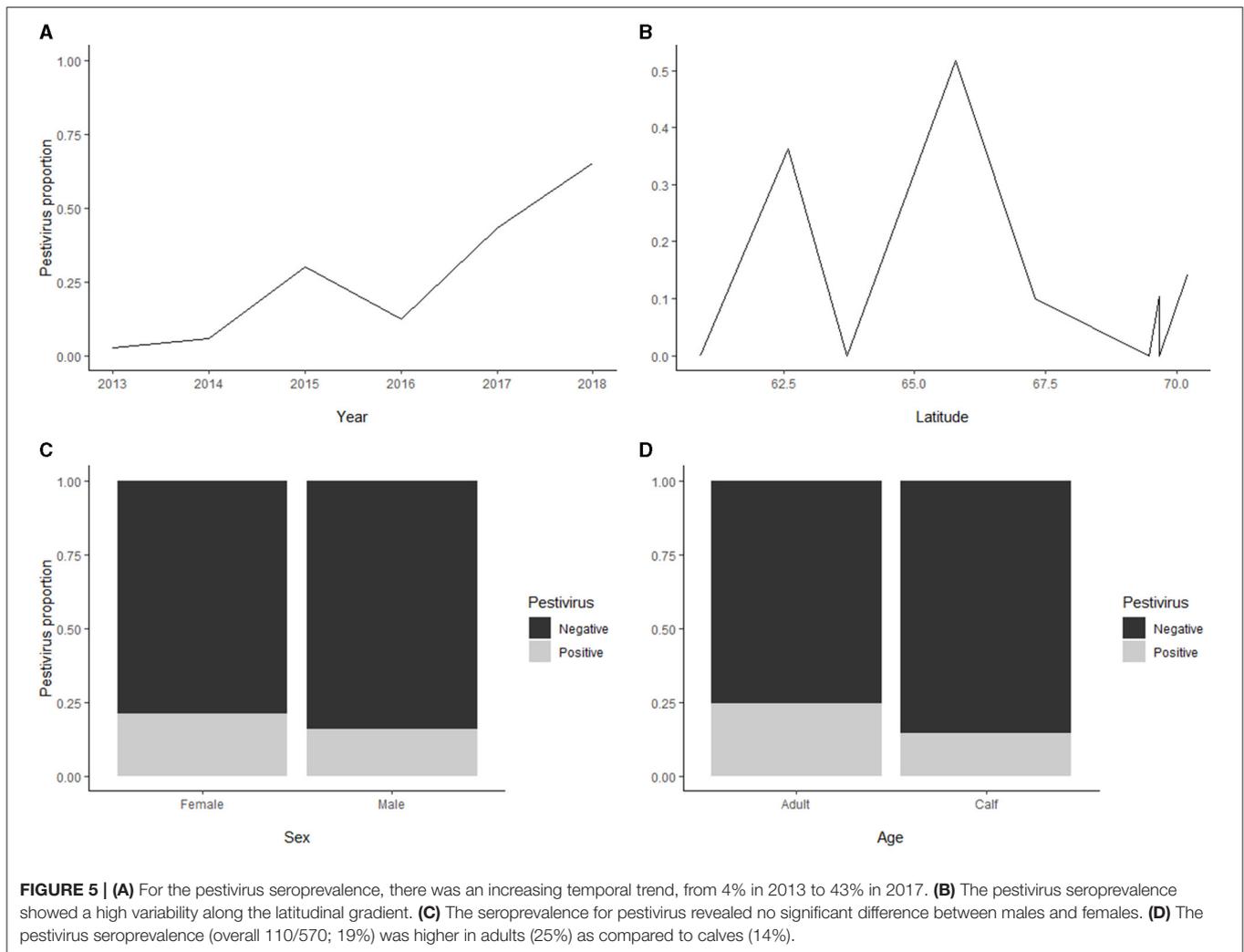


also make previously unexposed individuals (e.g., calves) more prone to develop disease and, with more severe symptoms, as in fact is commonly observed during outbreaks of infectious keratoconjunctivitis (46). The enzootic status of the virus documented here makes these stress-related mechanisms relevant for the whole reindeer herding region and for herding and management methods. This may be especially relevant when addressing potential effects of anthropogenic encroachments and fragmentation of reindeer pasture and climate change mitigation by increased feeding.

The seroprevalence against MCFV was 11%, ranging from 3% in Lødingen to 15% in Røros. For Lakselv (14%) in Finnmark and the three southernmost herds (Fosen 14%, Røros 15%, and Filefjell 13%), the seroprevalence was considerably higher than the 4% reported in Finnmark in a previous study [$n = 3,339$; (8)]. The disease MCF has been diagnosed in one semi-domesticated reindeer in Norway, displaying hair loss and thickening of the skin, with crusts on the muzzle, axillary regions and distal parts of the legs, and with presence of the bacterium *Trueperella pyogenes*. Both eyes displayed swollen eyelids and fibrinopurulent eye flood, as well as corneal opacity. Detection of Ovine herpesvirus 2 (OvHV2) specific DNA in the central nervous system (CNS) and nasal and conjunctival swabs by

PCR verified the diagnosis (16). Pathological lesions in moose (*Alces alces*), roe deer (*Capreolus capreolus*), and red deer (*Cervus elaphus*), along with the detection of OvHV2 and Caprine herpesvirus 2 (CpHV2) specific DNA in the CNS of affected animals by PCR (53) demonstrated that also these cervid species are affected by MCF-viruses in Norway.

The overall seroprevalence against MCFV increased from ~10% for the period 2013–2016, to 23% in 2017. Although few animals constitute the baseline for these numbers, this may indicate the start of a trend that needs to be monitored in future studies. Whether reindeer in general are exposed to OvHV2, CpHV2 or other hitherto unidentified gammaherpesviruses that might be reindeer-associated remains to be proven. However, in a recent study, samples from semi-domesticated ($n = 39$) and wild ($n = 35$) Norwegian reindeer were tested using a panherpesvirus DNA polymerase (DPOL) PCR, revealing 11 and 17 PCR-positive reindeer, respectively. When testing the PCR-positive individuals further using primers targeting the glycoprotein B gene (gB), four semi-domesticated and 15 wild animals tested positive. Amplicon sequences from both genes indicated a high degree of sequence homology among the reindeer, corresponding to a novel gammaherpesvirus species tentatively



named Rangiferine gammaherpesvirus 1, which may be a reindeer-specific gammaherpesvirus (10). The potential clinical impact of gammaherpesvirus infections especially with the viruses in MCFV group in reindeer remains to be elucidated.

The seroprevalence against pestivirus varied from 0% in three herds (Lakselv, Fosen, and Filefjell) to 52 and 37% in Hattfjelldal and Røros, respectively, with an overall mean of 19% ($n = 570$). This is slightly higher than what was found in a larger study conducted in Finnmark [13%, $n = 3,339$; (9)]. No pestivirus has been isolated from wild or semi-domesticated reindeer, but a pestivirus, designated V60, was isolated from a reindeer that suffered from diarrhea and anorexia in a German zoo in 1996 (54). Characterization of the isolate indicated that it was most closely related to Border disease virus type 2 (Pestivirus D; BDV-2) (55–57). An experimental inoculation of reindeer with BVDV caused loose stool with blood and mucus, serous mucopurulent nasal discharge and transient coronitis and laminitis (58).

It is unknown which type of pestivirus that has caused the immune response in this study. Thus, it is difficult to know which impact such infections may have on the reindeer populations. A transient infection in reindeer [e.g., BVDV; (58)] may be subclinical or cause only mild symptoms, whereas persistent infections, similar to mucosal disease (MD) in cattle and border disease (BD) in sheep, may cause severe symptoms. MD in cattle is characterized by high fever, salivation, anorexia and ulcers in the gastro-intestinal tract, whereas BD in small ruminants is characterized by abortion, stillbirths and the birth of small offspring, with abnormal body conformation, tremor and hairy fleece (“hairy shakers”) (59). This study has identified high seroprevalence in reindeer herds in Hattfjelldal and Røros. We have no obvious explanation for the significant increase in seroprevalence recorded for pestivirus during the sampling years. The recorded increase from 4% in 2013 to 43% in 2017 may reflect a real increase of exposure of the herds during this period or represent an arbitrary finding due to the opportunistic sampling regime. However, these findings could be followed up

to further elucidate the role of pestivirus infections in semi-domesticated reindeer.

Interestingly, we detected parapoxvirus-specific DNA in one herd in western Finnmark and one in eastern Finnmark. Parapoxvirus-specific DNA has previously been detected in various tissues in reindeer carcasses from Finnmark with no clinical symptoms of contagious ecthyma (60), but the disease has never been reported in reindeer from this region. ORFV is presumably transmitted from small ruminants and the findings in this and previous studies may indicate that the virus is introduced to reindeer from time to time, maybe without causing clinical symptoms or that such symptoms are not recognized or reported. Lesions in the skin may not affect the health of the animal to a large extent, but when disseminated lesions appear in the oral mucosa it affects the ability of the animal to feed (26) and may cause substantial mortality during severe outbreaks (25).

None of the tested reindeer had antibodies against BTV and SBV. Climatic changes may affect the presence, distribution and biological activity of arthropods, including mosquitos, midges and ticks, in the reindeer herding regions, which may also alter the exposure of reindeer to BTV, SBV and other pathogens in the future. It is thus necessary to monitor reindeer for potential pathogens even though they are not known as being present and causing disease today. Another impact from climate change is increased rain-on-snow events and more frequent freeze-thaw cycles, creating ice-locked pastures for reindeer (61). One mitigating measure to avoid starvation is supplementary feeding. However, feeding may also represent increased stress and animal density and contribute to poor hygiene on feeding spots and in corrals. Virus infections (e.g., CvHV2, pestivirus, parapoxvirus, and others) may cause disease by themselves, but also facilitate secondary bacterial infections by causing lesions in skin and mucosal membranes. One such secondary bacterial infection is oral necrobacillosis caused by *Fusobacterium necrophorum*, that may cause severe outbreaks in feeding corrals (16, 62, 63).

CONCLUDING REMARKS

This study confirms that infections with alphaherpesvirus, gammaherpesvirus (MCFV group), and pestivirus are enzootic in semi-domesticated reindeer, also in herding regions south of Finnmark, whereas reindeer from none of the investigated geographical regions were exposed to BTV and SBV. The potential impact of alphaherpesvirus (i.e., CvHV2) infections has previously been demonstrated, with its role in the pathogenesis of IKC as the most prominent, whereas the role of MCFV and pestivirus infections in reindeer is less investigated. However, the high prevalence of exposure to these viruses, especially in some of the herds, may suggest that they may have an overall impact on reindeer health. It is in fact difficult to identify limited pathogenicity potentials, such as reduced reproductive success and poor fitness of calves, and how these infections may impact or facilitate other pathogens. This study also indicated that, even if disease outbreaks are not reported, parapoxvirus may be circulating among reindeer. Supplementary feeding of

reindeer is rapidly adopted and is increasingly used by reindeer herders. Corraling and feeding may, however, increase the stress load of the animals and lead to increased animal contact and poor hygienic conditions on feeding spots and in corrals, which may facilitate the transmission of pathogens and the severity of infectious diseases. Thus, the enzootic virus infections addressed in this study may become more important in the future management of reindeer health.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because the data on which the article is based on, contains personal data on identifiable reindeer herders and their animals. Requests to access the datasets should be directed to the corresponding author.

ETHICS STATEMENT

Sampling was conducted in cooperation with the herders as a general health surveillance of the herds when animals were gathered and handled for other purposes, and the study was not classified as an animal experiment. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

MT designed the study and drafted the manuscript. JSR, IHN, OCK, and MT conducted field work. EMB, CC, and HL conducted serological screenings. FJAM conducted the statistics. JSR, IHN, EMB, HL, CC, FJAM, and MT evaluated the results. All authors contributed to the manuscript and approved the final version.

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