

ORIGINAL ARTICLE

Cervids as sentinel-species for tick-borne encephalitis virus in Norway - A serological study

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Abstract

Tick-borne encephalitis virus (TBEV) is the causative agent of tick-borne encephalitis (TBE). TBEV is one of the most important neurological pathogens transmitted by tick bites in Europe. The objectives of this study were to investigate the seroprevalence of TBE antibodies in cervids in Norway and the possible emergence of new foci, and furthermore to evaluate if cervids can function as sentinel animals for the distribution of TBEV in the country. Serum samples from 286 moose, 148 roe deer, 140 red deer and 83 reindeer from all over Norway were collected and screened for TBE immunoglobulin G (IgG) antibodies with a modified commercial enzyme-linked immunosorbent assay (ELISA) and confirmed by TBEV serum neutralisation test (SNT). The overall seroprevalence against the TBEV complex in the cervid specimens from Norway was 4.6%. The highest number of seropositive cervids was found in south-eastern Norway, but seropositive cervids were also detected in southern- and central Norway. Antibodies against TBEV detected by SNT were present in 9.4% of the moose samples, 1.4% in red deer, 0.7% in roe deer, and nil in reindeer. The majority of the positive samples in our study originated from areas where human cases of TBE have been reported in Norway. The study is the first comprehensive screening of cervid species in Norway for antibodies to TBEV, and shows that cervids are useful sentinel animals to indicate TBEV occurrence, as supplement to studies in ticks. Furthermore, the results indicate that TBEV might be spreading northwards in Norway. This information may be of relevance for public health considerations and supports previous findings of TBEV in ticks in Norway.

KEYWORDS

cervids, sentinel animals, seroprevalence, tick-borne encephalitis virus

Katrine M. Paulsen and Carlos G. das Neves are contributed equally to this work

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1 | INTRODUCTION

Tick-borne encephalitis virus (TBEV) is a vector borne disease that cause tick-borne encephalitis (TBE) in humans and animals. The virus is widespread throughout Europe and consists of five known subtypes: European, Siberian, Far Eastern, Baikalian and Himalayan (Dai, Shang, Lu, Yang, & Xu, 2018; Dobler, Gniel, Petermann, & Pfeffer, 2012; Kovalev & Mukhacheva, 2017). TBEV is a positive sense single stranded RNA virus belonging to the *Flaviviridae* family, and is a part of a complex of related viruses known as the TBEV complex. In addition to TBEV, this complex includes Louping ill virus, Langat virus, Powassan virus, Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, Spanish sheep encephalomyelitis virus and Greek goat encephalomyelitis virus (Grard et al., 2007). The main vectors for transmission of TBEV in Eurasia are the *Ixodes ricinus* and *Ixodes persulcatus*. It is estimated that TBEV is one of the most important neurological pathogens transmitted by tick bites in Central and Eastern Europe, as well as Russia, with significant impact on the public health (Ruzek et al., 2019). In the past decades, a rapid increase in the incidence of TBE has been observed in many European countries where TBE is endemic, simultaneously with the emergence of new foci (Jaenson, Hjertqvist, Bergstrom, & Lundkvist, 2012; Ruzek et al., 2019; Suss, 2011).

In Norway, *I. ricinus* ticks are mainly distributed along the coastline from Østfold county in the southeast up to near the Arctic Circle (66°33'47.5"N) in Nordland county (Hvidsten et al., 2014; Jenkins et al., 2012; Mehl, 1983; Soleng et al., 2018; Tambs-Lyche, 1943). TBEV has been documented in ticks, where *I. ricinus* is abundant (Andreassen et al., 2012; Paulsen et al., 2015; Soleng et al., 2018). Consistently, the distribution of TBE has also been shown in a blood donor and tick study in Østfold county in eastern Norway. (Larsen et al., 2014). Although studies have found that TBEV in ticks is distributed from southern to northern Norway, the number of human cases of TBE in the country is low, with a total of 139 reported autochthonous cases (incidence ranges from < 0.1–0.4 per 100,000 inhabitants per year) since the first case occurred in 1997. These cases are limited to the southern and south-eastern parts of the country (Norwegian Surveillance System for Communicable Diseases (MSIS), 2019).

Another flavivirus, closely related to TBEV, is the louping-ill virus (LIV). TBEV and LIV are maintained by different reservoirs: TBEV mainly by ticks and rodents, LIV by ticks and mountain hare (Gilbert, Jones, Hudson, Gould, & Reid, 2000; Labuda & Randolph, 1999; Norman, Ross, Laurenson, & Hudson, 2004). TBEV is known to cause infections in humans, horses and dogs, whereas LIV is known to cause severe neurological disease in sheep and red grouse (Gordon, Brownlee, Wilson, & Macleod, 1932; Jeffries et al., 2014; Kaiser, 2012; Klaus, Horugel, Hoffmann, & Beer, 2013; Reid, Duncan, Phillips, Moss, & Watson, 1978; Weissenböck, Suchy, & Holzmann, 1998). LIV has not been detected in ticks in Norway previously, and the last reported case of LIV infection in sheep in Norway was in 1991 (Norwegian Veterinary Institute., 2019; Paulsen et al., 2017).

Impacts

- The study is the first comprehensive screening of tick-borne encephalitis (TBE) antibodies in cervid species in Norway.
- The study shows that cervids are useful sentinel animals for distribution of tick-borne encephalitis virus (TBEV) in Norway as a supplement to data on human TBE cases and prevalence of TBEV in ticks.
- This study supports previous findings of TBEV in ticks, which indicate that TBEV is distributed in Norway more widely than suggested by human TBE cases.

However, a previous study in cervids shows that both viruses may circulate in Norway (Ytrehus, Vainio, Dudman, Gilray, & Willoughby, 2013).

Apart from climatic variables and human drivers, many studies have clearly shown the important role of large wildlife species in TBEV epidemiology, as recently summarized by Esser and colleagues (Esser et al., 2019). The use of these cervids as sentinels has been documented in different countries with variable results, but there is a consistent conclusion that these animals represent a relevant epidemiological tool in understanding and mapping the distribution of TBEV, as well as potentially functioning as an early warning system for the presence of these viruses in areas where human cases have not yet been reported.

Deer and moose can serve as transient hosts for TBEV, perhaps with a more relevant role in maintaining tick populations rather than being a relevant reservoir for TBEV (Carpi, Cagnacci, Neteler, & Rizzoli, 2008). The most plausible direct contribution of cervids to TBEV transmission is the non-viremic transmission from infected ticks to naïve ticks co-feeding on the same host (Jaenson et al., 2018; Mlera & Bloom, 2018; Randolph, 2011). Cervid species usually exhibit low or no viremia post TBEV infection, but show a low titre antibody response that can be measured over time (Gerth, Grimshandl, Stage, Doller, & Kunz, 1995; Imhoff et al., 2015). Given that the TBEV prevalence in ticks usually is low (Andreassen et al., 2012; Pettersson, Golovljova, Vene, & Jaenson, 2014), cervid sampling can be an important supporting tool as the TBE antibodies will reflect TBEV circulation. Several studies in wild cervids in different European countries have confirmed the transmission of TBEV within the sampling region, as indicated by records of human TBE. These studies have also helped identify previously unknown foci and confirmed that wildlife mammals can be used as sentinel species for TBEV (Balling, Plessow, Beer, & Pfeffer, 2014; Kiffner, Vor, Hagedorn, Niedrig, & Ruhe, 2012; van der Poel et al., 2005; Skarphedinsson, Jensen, & Kristiansen, 2005).

In Norway, there are four major free-ranging species in the deer family (Cervidae): roe deer (*Capreolus capreolus*), red deer (*Cervus*

elaphus), euroasian reindeer (*Rangifer tarandus tarandus*, both wild and semi-domesticated) and moose (*Alces alces*) (Morellet, Klein, Solberg, & Andersen, 2010). The total number of wild cervids in Norway has been rising during the last decades and was estimated to approximately 450,000 individuals in 2009 (Solberg et al., 2010). Roe deer, red deer, reindeer and moose are all subject to licensed hunting during autumn. In Norway, these species have varying geographical distributions and population densities, as well as different habitat preferences (Apollonio, Andersen, & Putman, 2010). Wild reindeer migrate and feed at high altitudes in the southern part of Norway (Apollonio et al., 2010), mostly above the current altitude limit for tick distribution in Norway (Hvidsten et al., 2015; Larsson,

Hvidsten, Stuen, Henningsson, & Wilhelmsson, 2018; Paulsen et al., 2015; Soleng et al., 2018). Roe deer is a browser, meaning that it eats leaves, soft shoots, or fruits of tall, generally woody plants such as shrubs in the lowlands, with preferences for forest clearings and being territorial in the main tick season (Hofmann, 1989). Red deer is an intermediate, opportunistic, mixed feeder, meaning that it would eat both leaves and grass in the lowland, and mainly, in the western part of the country, often in areas of dense forest (Hofmann, 1989). Moose is a browser which preferences dense forests and is often feeding on water plants in lakes and wet areas (Apollonio et al., 2010; Franzmann & Schwartz, 2007), with a wide distribution in Norway both inland and in coastal areas (Solberg et al., 2010).

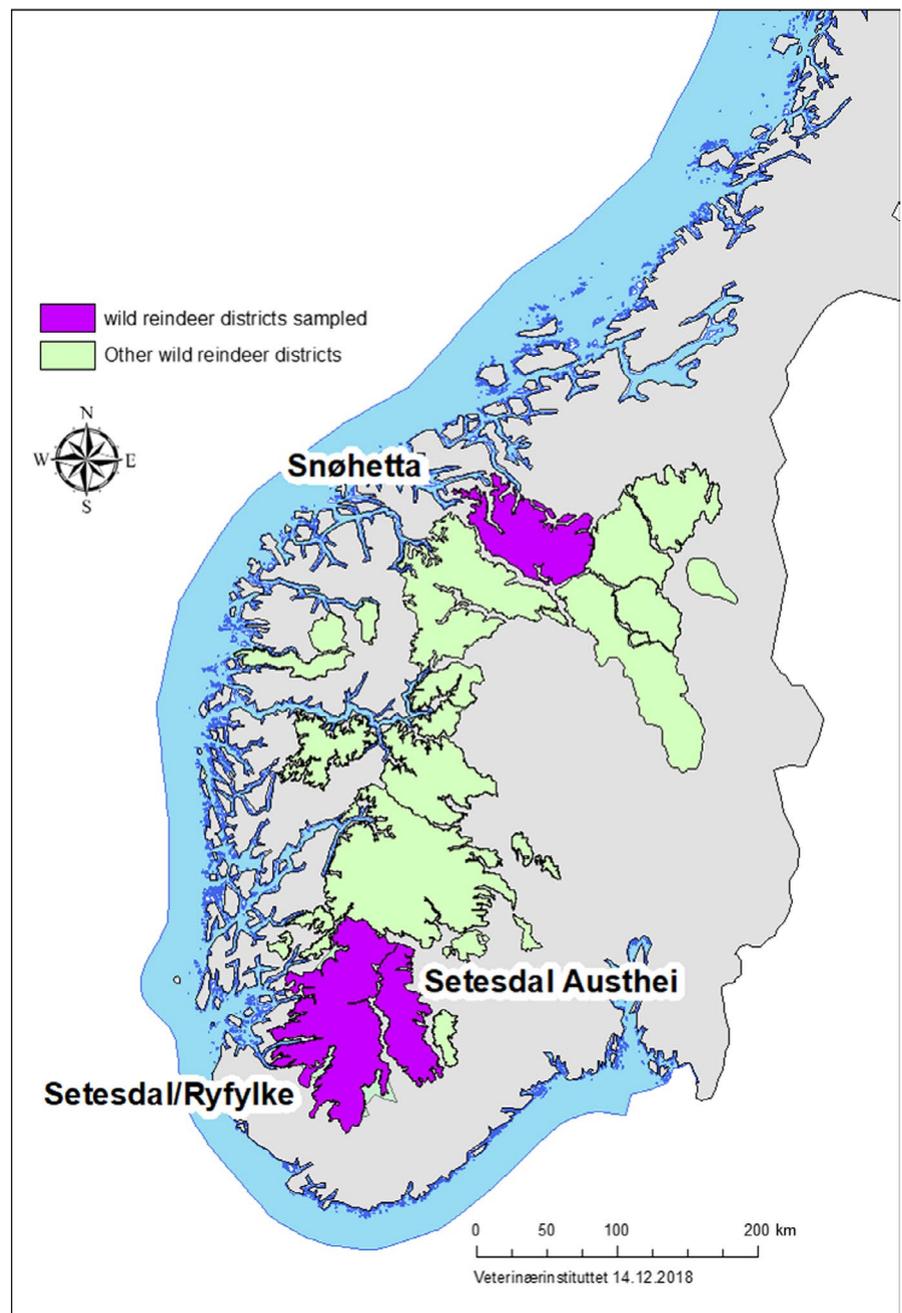


FIGURE 1 Geographical locations of the sampling sites of wild reindeer sera included in the study. The coloured areas in the map indicates the Norwegian wild reindeer management districts (in light green), with those in pink depicting districts with samples included in this study

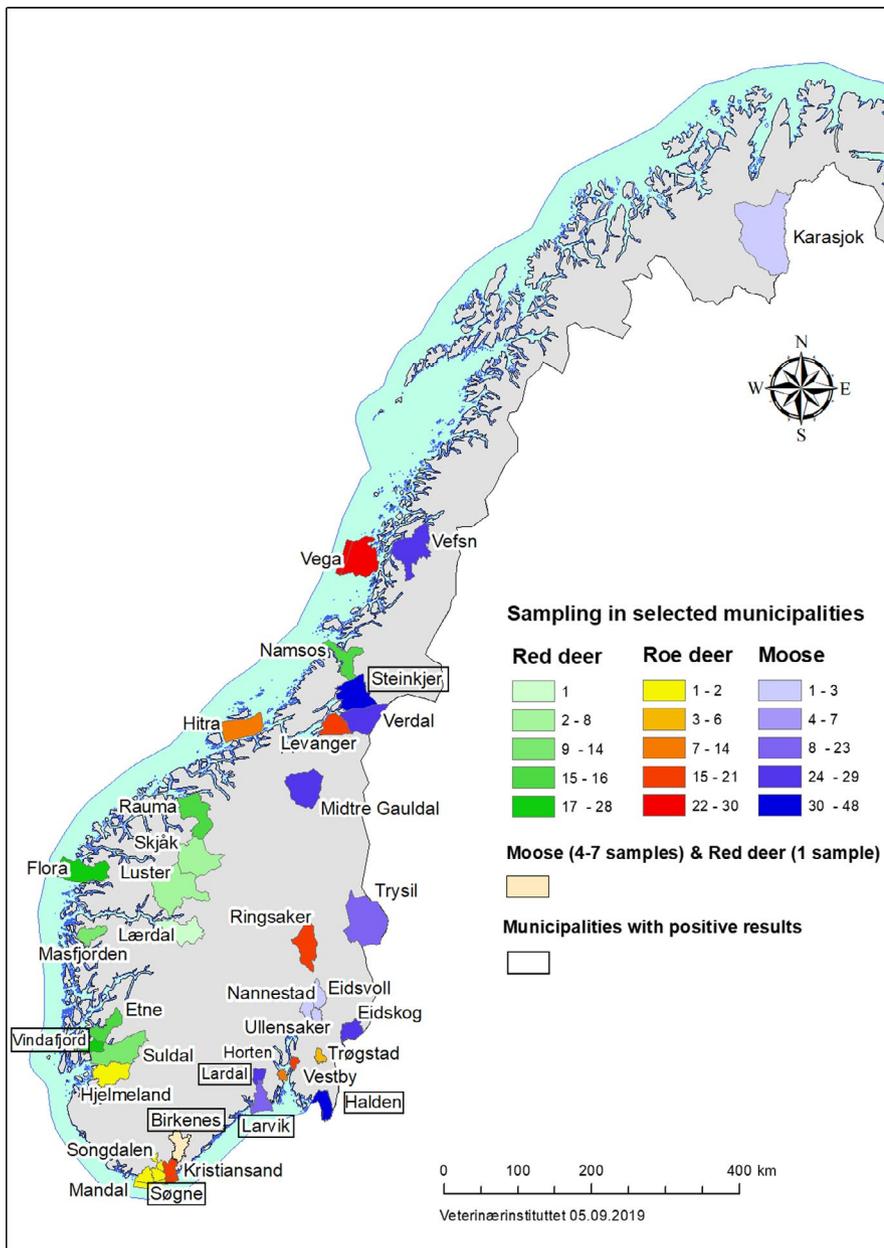


FIGURE 2 Geographical locations of the sampling sites of moose, red deer and roe deer sera included in the study. The coloured areas in the map indicates sampling in a municipality, and municipalities labelled with a square represents municipalities with seropositive samples

The aim of the study was to investigate the seroprevalence of TBEV-specific antibodies in cervids in Norway and the possible emergence of new foci. Furthermore, a second aim was to evaluate if cervids in Norway can function as possible sentinel animals for the distribution of TBEV as a supplement to surveillance of TBEV in ticks. This is based on the assumption that cervids as sentinels have: (a) measurable antibody response after infection with TBEV, (b) territory or home range that overlaps the area where ticks are present, (c) sufficient population size and can be easily captured and sampled.

Based on the current geographic distribution of ticks and cervid species, we hypothesize that red deer, roe deer and moose may function as sentinel species, especially along the coastline. We also hypothesize that wild reindeer can function as a relevant sentinel species and as an early-warning system for spread of ticks to higher altitudes.

2 | MATERIALS AND METHODS

2.1 | Sample collection and selection criteria

The Norwegian health monitoring program for deer and muskox (HOP) has been ongoing since 1998 and provides an overview and knowledge of the state of health of Norwegian populations of deer and muskox. In 2013, a broad national sampling was organized and approximately 700 animals were sampled. Criteria for sample selection were: (a) collection in areas with known abundance of cervids, (b) collection during summer months, which coincides with the highest period of tick activity (between April and November), (c) collection of samples of each cervid species in areas with and without reported tick presence. Hunters were asked to collect blood from the thoracic cavity with a plastic Pasteur pipette and transfer it to full blood tubes. The blood samples were sent at ambient

TABLE 1 Seroprevalence of tick-borne encephalitis virus in different cervid species hunted in Norway in 2013

	Total analysed sera	TBEV positive by ELISA (borderline)	Confirmed TBEV positive by serum neutralisation test	Number of municipalities/herding districts with positive animals ^a	Seroprevalence %
Moose	286	29 (3)	27	5/15	9.4
Roe deer	148	0 (1)	1	1/12	0.7
Red deer	140	2 (0)	2	2/11	1.4
Reindeer	83	1 (2)	0	0/3	0
Total	657	32 (6)	30		4.6

^aThe number/y depicts the total number of municipalities or herding districts from which samples were obtained.

temperature to the Norwegian Veterinary Institute (approximately within 1 to 3 days after collection). Upon arrival they were centrifuged at 685 g for 10 min. Serum was transferred to 5-mL tubes, and the samples stored at -40°C until use. Due to the blood sampling from the hunted animals, some samples were haemolysed (<10% of total samples), especially for the roe deer samples.

2.2 | Serological methods

Serum samples from 286 moose, 148 roe deer, 140 red deer, and 83 reindeer (Figure 1, Figure 2, and Tables S1 and S2) were screened for TBE immunoglobulin G (IgG) antibodies with a modified commercial enzyme-linked immunosorbent assay (ELISA, Enzygnost® Anti-TBE virus IgG, Siemens) according to the manufacturer's protocol, as described previously (Ytrehus et al., 2013). The ELISA was modified using peroxidase-labelled affinity purified antibody to deer IgG (H + L) produced in rabbit (TriChem ApS-interkemi). The conjugate was diluted 1:10,000 in IgG Conjugate Buffer Microbiol (Enzygnost® Anti-Rubella Virus IgG, Siemens). Previously confirmed TBE IgG positive and negative roe deer and moose sera by serum neutralisation test (SNT) were used as internal controls.

To confirm the TBE ELISA results, all positive and borderline serum samples were re-tested by a TBEV-specific SNT at the Center for Virology of the Medical University of Vienna, as described previously (Stiasny, Holzmann, & Heinz, 2009). Briefly, serial dilutions of heat-inactivated samples were incubated with TBEV (strain Neudoerfl) for 1h at 37°C. Baby hamster kidney (BHK-21) cells were added and incubated for three days. The presence of virus in the cell culture supernatant was assessed by ELISA. The virus neutralisation titre was defined as the reciprocal of the sample dilution that showed a 90% reduction in the absorbance readout compared to the control without antibody. Samples with titres equal to ten and higher were defined as TBE seropositive.

All positive and borderline serum samples by TBE ELISA were also analysed for IgG antibodies to LIV using haemagglutination inhibition test (HI) and SNT at Moredun Research Institute in Scotland as described previously (Clarke & Casals, 1958; Grist, 1966). The HI test for antibody to LIV was performed using gander erythrocytes, as described by Clarke and Casals (1958), and modified to use tissue culture-grown virus and a microtiter plate. This assay format is

validated at Moredun Research Institute in routine-diagnostic use for many species, including deer (Ytrehus et al., 2013). Nonspecific inhibitors and goose erythrocyte agglutinins were removed by kaolin and goose erythrocyte absorption. Positive and negative controls (ovine sera) were included in each test batch to confirm assay performance. Samples giving a result of HI at a titre of greater than 20 were considered positive. Samples with a titre of 10 were considered inconclusive, and titres of <10 were considered negative.

For confirmation of the LIV HI results and for comparison to TBEV titres, all positive and borderline samples from the TBE ELISA screening test were re-tested by LIV SNT using the constant virus varying serum method (Grist, 1966). The test was modified to be performed in 96-well plates using BHK-21 cells with the LIV strain L31 using 30–300 median tissue culture infective dose (TCID₅₀) per well. Virus controls, known positive and negative serum controls, toxicity controls, and uninfected control wells were run in each test. Serum samples with a titre higher or equal to 4 were interpreted as IgG positives against LIV by SNT.

The combination of the four serological tests was used to determine if a sample contained antibodies homologue to the TBEV-complex antigens. Specifically, the TBE ELISA was performed to screen the serum samples followed by validation by TBEV SNT. Due to the history of LIV in Norway the samples were also analysed by LIV HI and LIV SNT to assess possible cross-reactions between viruses within the complex. The titres of TBEV SNT and LIV SNT were compared and evaluated.

2.3 | Statistics

Statistical analysis was carried out using Stata/SE 14 for Windows (Stata Corp.). We used the Spearman correlation (ρ) to assess the relationships between SNTs. The squared value ρ^2 can be interpreted in terms of predictive power (explained variability) of one SNT ranks by the other SNT ranks. p -value was considered significant if below .05 (Thrusfield, 2007).

3 | RESULTS

A total of 657 cervid specimens from Norway were analysed for the presence of IgG antibodies against TBEV. The collection sites for

serum from wild reindeer, red deer, roe deer and moose are shown in Figures 1 and 2.

In total, 38 samples were positive by TBEV ELISA. The overall seroprevalence of antibodies against the TBEV complex in the cervid specimens from Norway confirmed by TBEV SNT was 4.6% (30/657 TBEV seropositive cervids). The highest number of TBEV seropositive cervids was detected in the county of Vestfold (Larvik and Lardal municipalities) in south-eastern Norway. Seropositive cervids were also detected in the counties of Aust-Agder (Birkenes municipality) and Vest-Agder (Søgne municipality) located in southern Norway, Østfold (Halden municipality) in south-eastern Norway, Rogaland (Vindafjord municipality) in western Norway and Trøndelag (Steinkjer municipality) in central Norway (Figure 2 and Tables S1 and S2). No antibodies against TBEV were confirmed in any of the wild reindeer samples (0/83). Antibodies against TBEV detected by SNT were present in 9.4% (27/286) of the analysed moose sera, 1.4% (2/140) in red deer and 0.7% (1/148) in roe deer. The majority (27/30) of the positive serum samples originated from moose (Table 1).

All TBE IgG positive and borderline samples from the ELISA were also examined for the presence of antibodies to LIV by HI and SNT. Seroreactivity to LIV was detected in 30 of the 38 ELISA positive TBEV samples by LIV HI-test, and in 32 of the same 38 samples by LIV SNT.

A strong correlation was found between TBEV and LIV SNTs using the Spearman correlation ($\rho = .75$) (p -value $> .001$). Detailed information on individual results are summarized in Tables S1 and S2.

4 | DISCUSSION

The present study represents the first comprehensive screening of cervid species in Norway for viruses in the TBEV complex. We identified TBEV complex neutralizing antibodies in moose and in small numbers in roe deer and red deer. The majority of the positive serum samples from cervids included in this study originated from south-eastern Norway. This is in the area where human TBE cases have been reported in Norway according to the Norwegian Surveillance System for Communicable Diseases (MSIS). TBE positive samples were furthermore detected in the counties of Østfold, Rogaland and Trøndelag, which is located outside the area of reported human cases. This supports previous findings of TBE antibodies in blood donors, and in TBEV in ticks and unpasteurized cow milk (Larsen et al., 2014; Paulsen et al., 2015, 2019; Soleng et al., 2018).

The presence of TBE antibodies in moose has only been studied in Sweden in the early 1960s (Svedmyr, Zeipel, Borg, & Hansen, 1965) and more recently in Norway (Yttrhus et al., 2013) and Finland (Tonteri, Jokelainen, Matala, Pusenius, & Vapalahti, 2016). Given that the distribution of moose is mostly restricted to north-eastern Europe (Scandinavia, Finland Latvia, Estonia and Poland) with some additional animals in the Czech Republic, Ukraine and Belarus, it is not surprising that the number of studies in this species is limited (Imhoff et al., 2015). It is often difficult to compare studies using

different methodologies and sampling techniques. The previous Swedish and Norwegian studies seem to be based on animals taken almost exclusively from endemic areas, which might help explain the high prevalences found in those studies (Svedmyr et al., 1965; Yttrhus et al., 2013). We therefore believe the best source for comparison comes from the Finnish study. Tonteri and colleagues tested animals from both endemic and non-endemic areas, and found a low prevalence of 0.74%, whereas our results reveal a prevalence of 9.4%.

The positive moose sample from the municipality of Steinkjer in central Norway represents the northernmost detection of a large TBEV seropositive animal in Norway. No human cases have been reported in this area. Moreover, Steinkjer is located too far away from TBEV endemic areas to attribute migration of mammals from endemic areas (Norwegian Surveillance System for Communicable Diseases (MSIS), 2019). This, in accordance with previous findings in ticks and cow's milk in non-endemic areas (Paulsen et al., 2015, 2019; Soleng et al., 2018), seems to indicate that TBEV is spreading northwards, which may be of relevance for public health considerations. One must also take into consideration the role of migrating birds in the distribution TBEV in Norway (Hasle, 2013; Hasle et al., 2009; Waldenstrom et al., 2007). Moose preference for foraging in wet/lake areas may also contribute to the higher prevalence observed, as several studies (including in Scandinavia) have clearly identified waterbodies and well-connected forests of oak, birch or pine, as relevant factors for tick abundance (Zeimes, Olsson, Hjertqvist, & Vanwambeke, 2014). Since moose is more sparsely distributed along the western Norwegian coast than in inland areas, it would be interesting to obtain samples in the western parts of the country in the future.

We found TBEV complex neutralizing antibodies in two red deer and one roe deer. One red deer and one roe deer that were positive originated from endemic areas with well-documented human TBE cases (Norwegian Surveillance System for Communicable Diseases (MSIS), 2019). This study identified one seropositive red deer along the western coast of Norway, an area where TBEV has been documented in ticks (Paulsen et al., 2015). There are, however, few studies of TBEV complex in red deer, making it difficult to conclude if these results result from an "off-target" sampling or if red deer are in fact not as susceptible as other cervids to TBEV.

The TBE seropositive red deer from the western coast of Norway, had a high LIV SNT titre (20 for TBEV and 128 for LIV). Interestingly, this red deer was hunted in Vindafjord, which is located in western Norway, close to the area with reported LIV infections in sheep in the 1980s and early 90s (Norwegian Veterinary Institute., 2019; Ulvund, Vik, & Krogsrud, 1983). This could indicate that LIV might circulate in western Norway. Yttrhus et al. (2013) found antibodies against TBEV and LIV in Farsund in southern Norway, supporting the conclusion of a possible co-circulation, which has also been demonstrated in Bornholm in Denmark (Jensen, Skarphedinsson, & Semenov, 2004; Yttrhus et al., 2013). There have been no reports of clinical LIV cases among sheep in Norway since it was last diagnosed in 1991 (Gao et al., 1993; Norwegian Veterinary Institute., 2019;

Ulvund et al., 1983). In our opinion, it would seem implausible that a virus known to cause neurological disease in sheep could be circulating in one of the highest sheep density areas in Norway without any clinical reports for more than twenty years. In addition, 7,615 *I. ricinus* ticks have been analysed for LIV in Norway, and all were found to be negative (Paulsen et al., 2017). It is recommended to confirm the ELISA results by SNT, since TBEV and LIV are genetically closely related and antibodies to either virus may cross-react in the test, as seem to be the case in our study (Calisher et al., 1989; Klaus, Ziegler, Kalthoff, Hoffmann, & Beer, 2014).

Roe deer is one of the most surveyed species of cervids for TBEV in Europe. In many countries across Europe, roe deer is a key host for ticks, and due to the high animal densities and broad geographic spread, a good indicator for the occurrence of human TBEV infections. A recent study on roe deer in Denmark revealed an overall seropositivity against TBEV complex viruses of approximately 7% (Andersen et al., 2019). This study found positive animals in known endemic areas but also helped to map new risk areas for TBE. Other recent studies in roe deer have revealed varying prevalences: in Germany, 10% (Balling et al., 2014), in the Netherlands, 2% (Jahfari et al., 2017), in Austria, 2.4% (Duscher, Wetscher, Baumgartner, & Walder, 2015) and in Belgium 5.1% (Tavernier et al., 2015). In our study, one sample (0.7%) was TBE positive. Observations from other countries reveal relatively higher prevalences in roe deer. However, in our study, only 32 of 148 samples were collected in areas with reported human TBE cases. Further studies with a greater sampling size in endemic areas should be conducted to clarify to what extent roe deer can function as a sentinel species in Norway.

All wild reindeer tested in our survey were found to be negative to both TBEV and LIV by neutralizing assays. The likely absence of TBEV in these animals may be of special relevance in understanding the epidemiology of tick-borne diseases in a climate change perspective. Wild reindeer in southern and central Norway tend to range at higher altitudes, away from the coastline. Several studies have shown the negative effect of increasing altitude on all tick stages due to the effect of temperature, which limits questing periods and development rates in ticks (Jouda, Perret, & Gern, 2004a, 2004b; Perret, Guigoz, Rais, & Gern, 2000; Randolph, 2004). A shift in the altitudinal distribution of *I. ricinus* has been documented in Scotland, suggesting that the abundance of ticks at higher altitudes will increase as a response to climate change (Gilbert, 2010). In this perspective, wild reindeer can represent a unique sentinel species to understand the changes in tick distribution and abundance at high altitudes.

5 | CONCLUSION

The present study represents the first comprehensive screening of cervid species in Norway for TBE antibodies and provides updated information on the distribution of TBEV and indicates that TBEV is spreading northwards in Norway. In many ways similar to other screenings across Europe, our results indicate that cervids are useful

as sentinel animals for distribution of TBEV, in addition to studies in ticks.

This study supports previous findings of TBEV in ticks, which indicates that TBEV is distributed in Norway more widely than suggested by human TBE cases. There is a growing interest in the use of wild animals as sentinel species for understanding the epidemiology of emerging diseases and detecting them as early as possible. This approach, in line with the ONE HEALTH concept, has clear benefits in terms of both public and animal health, and warrants further studies on wildlife sentinels and reservoirs. Moose because of their wide distribution in Norway, habitat and foraging preferences, may constitute an important "candidate" for sentinel species. Wild reindeer ranging at high altitudes in southern Norway may have an important function as an early-warning system for spread of ticks in altitude as a result, among other factors, of climatic changes. Finally, the possibility of other flaviviruses closely related to TBEV circulating in Norway should also be further investigated. The information from this study is highly relevant for public health considerations.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICAL APPROVAL

The work presented in this manuscript required no specific ethical approvals.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

TableS1-S2

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