

Serological screening for tick-borne encephalitis virus in eight Norwegian herds of semi-domesticated reindeer (*Rangifer tarandus tarandus*)

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Abstract

Tick-borne encephalitis virus (TBEV) is found in *Ixodes ricinus* ticks throughout the area where viable tick populations exist. In Norway, TBEV is found in *I. ricinus* from the south coast until Brønnøy municipality in Nordland County and the range of the vector is expanding due to changes in climate, vegetation, host animals and environmental conditions. TBEV might thus have the potential to establish in new areas when *I. ricinus* expand its geographical distribution. At present, there is little knowledge on the status of the virus in high-altitude areas of inland regions in Norway. It has previously been indicated that reindeer may be an important sentinel species and indicator of the spread of ticks and TBEV in high-altitude regions. In this study, 408 semi-domesticated Eurasian tundra reindeer (*Rangifer tarandus tarandus*) from eight herds, from Tana in Troms and Finnmark County in northern Norway to Filefjell in Innlandet and Viken Counties in southern Norway, were screened for TBEV antibodies using a commercial enzyme-linked immunosorbent assay (ELISA). We found 16 TBEV reactive reindeer samples by ELISA; however, these results could not be confirmed by the serum neutralization test (SNT). This could indicate that a flavivirus and not necessarily TBEV, may be circulating among Norwegian semi-domesticated reindeer. The results also indicate that TBEV was not enzootic in Norwegian semi-domesticated

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reindeer in 2013–2015. This knowledge is important as an information base for future TBEV and flavivirus surveillance in Norway.

KEYWORDS

climate change, flavivirus, *Ixodes ricinus*, sentinels, serology, TBEV, ticks

1 | INTRODUCTION

The burden of vector-borne flaviviruses like tick-borne encephalitis virus (TBEV), West Nile virus, Usutu virus, as well as other vector-borne viruses like picornaviruses, Sindbis virus and Inkoo virus, is increasing in northern Europe (Lim et al., 2018; Shakya et al., 2022; Slunge et al., 2022; Tingström et al., 2016). Ticks and mosquitoes, along with their avian and mammalian hosts, facilitate the enzootic maintenance of these viruses in nature (Esser et al., 2019). The arctic and subarctic regions are facing impacts of climate change faster than the global average (Box et al., 2019). Climate change is upsurging favourable conditions for circulation of these viruses in nature by modifying microclimatic conditions, thus facilitating changes in vegetation and in the population density and geographical distribution of animal hosts. All together, these changes increase the risk of zoonotic infections (Chala & Hamde, 2021; Esser et al., 2019). There is, however, little knowledge on the geographical spread of these flaviviruses in colder arctic regions.

TBEV is a medically important flavivirus that causes encephalitis in humans and animals with possible fatal neurological symptoms (Kaiser, 2002). The severity of the disease is broad, ranging from asymptomatic, to mild fever and headache, and to meningitis or meningoencephalitis (Lindquist & Vapalahti, 2008). Five subtypes of TBEV have been identified: European (TBEV-Eu), Siberian (TBEV-Sib), Far Eastern subtype (TBEV-Fe), Baikalian (TBEV-Bkl) and Himalayan (TBEV-Him) (Dai et al., 2018; Kovalev & Mukhacheva, 2017). The European subtype is present in the coastal areas of Norway with *Ixodes ricinus* ticks as the main vectors (Andreassen et al., 2012; Paulsen et al., 2015; Soleng et al., 2018; Vikse et al., 2020).

Although infected ticks are reported as far north as Brønnøy Municipality in Nordland County, human incidence is limited to the south. This may be related to underdiagnosis or circulation of a milder strain in the northern region (Vikse et al., 2020). In Norway, TBEV foci are identified by the presence of infected cervids such as moose (*Alces alces*), red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) (Paulsen et al., 2020; Ytrehus et al., 2013). These cervids are hence important sentinels to identify TBEV foci in particular areas, and the results can thereafter be coupled with screening of ticks and other animals (Balling et al., 2014; Paulsen et al., 2020; Skarphédinsson et al., 2005; Ytrehus et al., 2013).

Reindeer is a key cervid species with broad social and ecological value in Norway and are mostly herded by indigenous Sami herders (Riseth et al., 2019). Reindeer are adapted to colder climates than other deer species, having a wider distribution from

Impacts

- This study contributes to a better understanding of the TBEV-status in areas of Norway where ticks are present, but not abundant.
- This study supports previous findings for TBEV in ticks in Norway, that is, that TBEV is present in coastal areas and not in inland areas.
- We recommended further research to identify potential flaviviruses circulating in reindeer in the future.

the coast to higher altitudes, both in the Taiga and Tundra areas (Nowak, 1999). Reindeer have been documented to support enzootic maintenance of viruses like herpesvirus, pestivirus and Hepatitis E virus (Rinaldo et al., 2021; Romano et al., 2021; Sacristán et al., 2021; Tryland et al., 2021). Recently, antibodies against Inkoo virus and Inkoo virus-specific RNA were detected in reindeer for the first time in Norway which indicates that reindeer are exposed to a wide range of viruses (Shakya et al., 2022). The aim of the present study was to use semi-domesticated reindeer from eight regions as sentinels to evaluate TBEV distribution from northern to southern Norway.

2 | MATERIALS AND METHODS

2.1 | Sample collection

Serum samples ($n=480$) from semi-domesticated reindeer were collected (Tryland et al., 2021) from eight different reindeer herding regions: Tana, Lakselv, Tromsø, Lødingen, Hattfjell, Fosen, Røros and Filefjell during the winter seasons (October–April) of 2013, 2014 and 2015 (Table 1, Figure 1). The serum samples were stored at -80°C until further analysis.

2.2 | Serological methods

The serum samples were screened for TBEV immunoglobulin G (IgG) antibodies by a modified commercial enzyme-linked immunosorbent assay (ELISA), Enzygnost® Anti-TBE virus IgG, (Siemens) at The Norwegian Institute of Public Health, Oslo. The ELISA method was

TABLE 1 Seroprevalence of tick-borne encephalitis virus or a similar flavivirus by ELISA in Eurasian tundra reindeer (*Rangifer tarandus tarandus*) from eight different herding districts in.

4County	Location	TBEV positive (borderline)/total tested			
		2013	2014	2015	Total
Troms and Finnmark	Tana	0 (0)/20	0 (0)/20	2 (0)/20	2 (0)/60
Troms and Finnmark	Lakselv	0 (1)/20	0 (1)/20	0 (1)/20	0 (3)/60
Troms and Finnmark	Tromsø	0 (0)/21	0 (1)/20	0 (0)/20	0 (1)/61
Nordland	Lødingen	0 (0)/22	2 (1)/20	–	2 (1)/42
Nordland	Hattfjelldal	0 (0)/30	0 (0)/20	0 (1)/20	0 (1)/70
Trøndelag	Fosen	0 (0)/20	0 (0)/20	0 (0)/20	0 (0)/60
Trøndelag	Røros	0 (1)/22	1 (1)/20	2 (1)/20	3 (3)/62
Innlandet and Viken	Filefjell	0 (0)/25	0 (0)/20	0 (0)/20	0 (0)/65
	Total	0 (2)/180	3 (4)/160	4 (3)/140	7 (9)/480

FIGURE 1 Map of Norway illustrating reindeer sampling locations. Map created using the Free and Open Source QGIS. Map data: © OpenStreetMap-Mitwirkende, SRTM | Map position: © OpenTopoMap 129 (CC-BY-SA). Reindeer pastures data source: NIBIO.



modified using peroxidase-labelled affinity purified antibody to deer IgG (H+L) in rabbit (TriChem ApS-interkemi) and adapted for deer samples as described previously (Paulsen et al., 2020). Previously confirmed TBE IgG positive and negative moose and roe deer serum samples by SNT were used as internal controls (Paulsen et al., 2020). Positive TBE ELISA results were re-tested by a TBEV-specific SNT

at the Medical University of Vienna, Centre for Virology (Austria) as described previously (Malafa et al., 2020; Stiasny et al., 2009). The virus neutralization titre was defined as the reciprocal of the serum dilution that gave 90% reduction in the absorbance readout compared with the control without antibody. An SNT titre ≥ 10 was considered positive.

3 | RESULTS AND DISCUSSION

A total of 229 adult and 251 calf («calves of the year») reindeer serum samples, from eight different reindeer herding districts distributed geographically from north to south of Norway, were screened for antibodies against TBEV using ELISA and SNT. Seven serum samples (1.5%) were classified as positive and nine (1.9%) as borderline for TBEV by the ELISA (Table 1). The reindeer classified as positive were sampled in Tana ($n=2$; 2015), Lødingen ($n=2$; 2014) and Røros ($n=1$; 2014 and $n=2$; 2015) (Table 1). Reindeer classified as borderline were detected in the other herding districts, except for Fosen and Filefjell. None of the samples were positive by the SNT (Table S1); hence, the TBEV infection in reindeer could not be confirmed.

The lack of coherence between the ELISA and the SNT in this study might be due to serological cross-reactions among similar flaviviruses based on close antigenic similarity (Krzysiak et al., 2021; Lim et al., 2018; Marvik et al., 2021; Paulsen et al., 2020). A previous study also showed that TBEV ELISA reactive reindeer were negative in the SNT (Paulsen et al., 2020). Thus, these ELISA results are probably reflecting an exposure of reindeer to a virus closely related to TBEV, which could be tick-borne or mosquito-borne in origin. Louping ill virus (LIV), a tick-borne flavivirus closely related to TBEV, has been found in sheep in Norway in the 1980s, and antibodies against LIV have been found in willow ptarmigan (*Lagopus lagopus lagopus*) and deer species like red deer, but not in reindeer (Paulsen et al., 2020; Ulvund, 1987; Ytrehus et al., 2013). Mosquito-borne flaviviruses like West Nile virus (WNV) and Lammi virus (LAMV) show serological cross reactivity with TBEV (Huhtamo et al., 2009; Tonteri et al., 2016). Although WNV have been found to infect deer and cause clinical disease, this is probably rare (Palmer et al., 2004; Tonteri et al., 2016). In the study by Tonteri et al. (2016), 1371 cervids (moose, white-tailed deer and roe deer) were tested for TBEV in Finland, of which 10 were positive for TBEV and two of these were positive for LAMV. In addition, two TBEV negative were positive for LAMV. None of the deer were infected by WNV (Tonteri et al., 2016). Based on these findings, LAMV is shown to circulate in deer species. This could be due to cross reaction or double infection LAMV is detected in mosquitoes from Finland (Huhtamo et al., 2009) and as the cervids are known to be exposed to both tick and mosquito vectors, Tonteri et al. (2016) deemed it necessary to include the examination of both WNV and LAMV in their study. Notably, a subset of the deer samples tested were seropositive for LAMV and TBEV (Tonteri et al., 2016). In our study, the positive TBEV ELISA results that were negative in SNT (Table 1) may suggest reactivity with other flaviviruses as a result of exposure to mosquito bites.

Previous studies have shown that an unidentified flavivirus closely related to TBEV and LIV, may be circulating far north along the western coast of Norway (Ytrehus et al., 2013). Unlike ticks, mosquitoes are found all over Norway, including the inland regions (Shakya et al., 2022). Sequencing of RNA from reindeer nasal

and/or rectal swabs have identified viral sequences representing the *Flaviviridae* family in reindeer in Norway, which is hosted mainly by arthropod vectors like ticks and mosquitoes (Romano et al., 2021). Furthermore, the mosquito-borne virus INKV was detected in mosquitoes collected from Røros and INKV-specific antibodies were detected in reindeer from all the reindeer herding areas included in this study (Shakya et al., 2022). In the present study, the TBE ELISA-positive samples came from six out of eight locations from north to south of Norway where viable mosquito populations have been reported by Sánchez Romano et al. (2021) and Shakya et al. (2022) (Figure 1, Table 1). Most of the positive samples came from Røros in Trøndelag County, followed by Lødingen in Nordland County and Lakselv in Troms and Finnmark County (Table 1). These findings support that reindeer in these areas are exposed to mosquito-bites. Therefore, we report that Norwegian semi-domesticated reindeer are in some areas, exposed to a TBEV-related flavivirus, that might be a mosquito-borne flavivirus (Table 1). This highlights the need for additional study to determine the prevalence of LAMV and other flavivirus infections among mosquitoes and cervids in Norway.

Reindeer herding covers large geographic areas, typically in mountain tundra regions of Norway, with long winter migration to pastures in inland regions (Riseth et al., 2019; Tryland et al., 2021). However, in a few areas, like in Nordland County, reindeer herds are more oriented to the coastal regions using snow free areas for pasture during the winter (Riseth et al., 2019). The inland regions are dryer, colder and generally geographically elevated compared with the coastal regions (Hanssen-Bauer et al., 2017), and hence sustainable tick populations cannot thrive (De Pelsmaeker et al., 2021; Quiller et al., 2014). Therefore, TBEV infection in tick populations is reported mostly from coastal regions of Norway (Andreassen et al., 2012; Soleng et al., 2018; Vikse et al., 2020). TBEV seropositive moose, roe deer and red deer have been detected in the southern and western parts of Norway (Paulsen et al., 2020). Reindeer, have, however, to our knowledge, never been reported seropositive for TBEV, which might be due to absence of TBEV

TABLE 2 Distribution by gender and age of reindeer classified as Positive and Borderline for anti-TBEV antibodies using a commercial ELISA (Enzygnost® Anti-TBE virus IgG).

	Number of reactive samples for TBEV ELISA test		
	Borderline	Positive	
Adult female	7/189	3	4
Calf ^a female	5/102	3	2
Adult male	0/40	0	0
Calf ^a male	2/135	2	0
Unknown	2/14	1	1
Total	16/480	9	7

^aCalf of the year (born in April–May, sampled in October–April).

infected ticks in the reindeer pasture areas. However, climate change is expected to modify existing environmental conditions in northern and inland regions of Norway in the future (Hanssen-Bauer et al., 2017). This might promote circulation of flaviviruses in new environments by supporting life cycles of arthropod hosts such as ticks and mosquitoes. Therefore, surveillance of flavivirus circulation should be continued and should include key indicator species such as reindeer.

The seroprevalence for TBEV was higher in female reindeer (4.1%) compared with males (1.1%) (Table 2). Similar to this study, a higher number of INKV-seropositive female reindeer were reported in an earlier study, based on the same sample collection (Shakya et al., 2022). The sample collection is, however, biased, due to a restricted availability of bulls during the sampling period. Thus, only 40 adult males were included compared with 189 adult females, which may have had impact on the results.

This study is an important baseline study for future research involving reindeer and ticks in Norway. General knowledge on climate change effects and adaptation strategies has increased significantly in recent years, but there is still a substantial information gap regarding the influence of climate change on zoonotic diseases. Changes in climate may give opportunities for vectors and flaviviruses to occur in new areas of Norway. It is, therefore, important to continue the surveillance of both vectors and viruses.

AUTHOR CONTRIBUTIONS

MT, JSR and IHN collected reindeer sera samples, AL did ELISA analysis, interpretation and drafted the manuscript. ÅKA designed and initiated the study, supervision, data analysis, interpretation, critically revising the manuscript and proofreading; RV and AS contributed by supervision, writing, revising the manuscript and proofreading and KMP contributed to ELISA assay, revising and proofreading the manuscript. KS performed the SNT analysis and revised the manuscript draft. All authors revised and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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