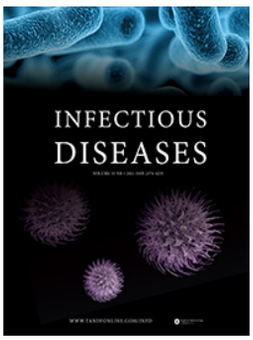

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ORIGINAL ARTICLE

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Low prevalence of tick-borne encephalitis virus antibodies in Norwegian blood donors

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ABSTRACT

Background: Tick-borne encephalitis (TBE) constitutes a public health concern in Europe. Certain coastal municipalities in southern Norway are considered TBE risk areas and in the last two years, there have been increasing numbers of TBE cases. Since the majority of infections are claimed to be asymptomatic, the aim of the current study was to assess the seroprevalence of antibodies to tick-borne encephalitis virus (TBEV) among unvaccinated adults living in a TBE endemic area in Norway.

Methods: One thousand one hundred and twenty-three blood donors living in Vestfold and Telemark county were included and associated sera were analysed for TBEV IgG antibodies. Information regarding tick bites, previous flavivirus exposure and knowledge regarding TBE and TBE prevention were obtained through a questionnaire.

Results: Fifty-eight samples were reactive by ELISA, of which 21 (36.2%) were confirmed by a TBEV-specific serum neutralization test. Of the 21 blood donors with neutralizing TBEV antibodies detected, 17 reported previous TBE vaccination. Thus, only four blood donors (0.4%) had TBEV neutralizing antibodies consistent with previously undergone TBEV infection. Regarding TBE awareness, half of the blood donors were familiar with TBE, but only 35% were aware of a preventive TBE vaccine.

Conclusions: Our study indicates low prevalence of subclinical TBEV infections among blood donors living in Vestfold and Telemark county and there is a lack of awareness among general public.

KEYWORDS
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Introduction

Tick-borne encephalitis (TBE) is one of the most important tick-borne diseases in Europe and Asia [1–4]. The causative agent, tick-borne encephalitis virus (TBEV), is neurotropic and consists of three subtypes described according to their main distribution area: European (TBEV-Eu), Far-Eastern (TBEV-FE) and Siberian subtype (TBEV-Sib) [5]. Three other subtypes of TBEV, TBEV Baikalian (TBEV 886-84), TBEV 178-179 and TBEV Himalayan have also been suggested [6–9]. TBE is a zoonotic disease and transmission to humans is mainly due to tick bites and only a minor extent due to the alimentary route through infected dairy products [1,4,10]. Ticks and small rodents constitute the reservoirs for TBEV. *Ixodes ricinus* is the principal vector for TBEV-Eu and occurs in large parts of Europe. *Ixodes persulcatus*, the vector for the Far-Eastern and Siberian subtypes, occurs in Eastern Europe, Siberia and far east including Japan [2]. Thus, in Europe, human disease caused by TBEV-Eu predominates [2,3,11].

During the last decades, the incidence of TBE in Europa has increased with enlargement of endemic areas and extended season for transmission [2]. TBE epidemiology is multifactorial and influenced by several factors such as tick occurrence, TBEV prevalence in ticks, climatic conditions and human risk behaviour [12]. Within endemic areas there is a characteristic patchy distribution of high-risk foci [2,13]. Worldwide, Russia has the largest proportion of TBE cases [14]. However, TBE also constitutes a major public health concern in central Europe and in the Baltic countries, and in 2018, 3212 TBE cases were reported in the European Union/European Economic Area countries [2,14,15].

Although the majority of infections with TBEV are claimed to be asymptomatic, the virus can cause serve inflammation of the central nervous system (CNS) [1,16–18]. TBE, caused by TBEV-Eu, has a characteristic biphasic course. In the first viraemic phase, patients present with fever and headache as the dominant symptoms [19,20]. Then, after a short asymptomatic period, the second phase with CNS involvement occurs. It presents with meningitis, meningoencephalitis or rarely meningoencephalomyelitis and the severity of the disease increases with age [3,10,19,20]. Unfortunately, no effective antiviral therapy is available and the treatment is solely symptomatic. A post-encephalitis syndrome, impairing quality of life, is reported by more than one-third of the patients [16,19]. The mortality is subtype dependent, with TBEV-Eu regarded as the least virulent with case fatality ratio of 0.5% [3]. Fortunately, the disease is successfully preventable by vaccination. Austria has the highest vaccination coverage in Europe, and it is estimated that over 4000 TBE cases were prevented in Austria between 2000 and 2011 [21,22].

In Norway, *I. ricinus* is distributed along the coast as far north as the Arctic Circle [23–26]. Regarding tick borne diseases, disseminated Lyme borreliosis and TBE are mandatory reportable to the Norwegian Surveillance System for Communicable Diseases (MSIS). Lyme borreliosis predominates, with a reported incidence of 5.0–8.2/100,000/year in Norway 2011–2015, contrary to TBE, with a reported incidence of 0.2–0.4/100,000/year in the same period [27]. Although Norway is considered a low-endemic TBE country, parts of the southern coast are endemic [2]. The first published TBE case in Norway was in 1998, and the very first cases were all due to tick bites on Tromøy island in Agder county [28,29]. According to MSIS, 202 TBE cases were reported in Norway in the period 1998–2019 and the majority of cases are domestic infections caused by tick bites in the southern coastal areas, where Vestfold and Telemark county is located [30]. In the last two years, there has been increasing numbers of TBE cases in Vestfold and Telemark county with 2019 as the preliminary peak year [30]. No cases of alimentary TBE have been recorded in Norway, but TBEV RNA has recently been detected in Norwegian unpasteurized cow's milk [31].

Only a few TBEV seroprevalence studies have been conducted in Norway. A retrospective study in 2002 estimated a TBEV IgG seroprevalence of 2.4% in residents of Tromøy, a high-endemic area in Agder county (Figure 1) [28]. Seroprevalences of 0.7% (3/461) and 0% were found among blood donors in the counties of former Østfold and former Sogn og Fjordane, respectively, where no clinical cases ever have been recorded [32,33]. Recently, a survey in Søgne, a district located in Agder county, found a TBEV IgG seroprevalence of 1.4% among unvaccinated adults [34].

The aim of our current study was to determine the seroprevalence of TBEV IgG among healthy unvaccinated blood donors living in an endemic TBE county in Norway. Another objective was to obtain information regarding tick bite exposure and knowledge of TBE and TBE prevention in the same study population.

Materials and methods

Recruitment area

Vestfold and Telemark county is located in the southern part of Norway. TBEV is confirmed to be present in ticks collected along the coastline within the county, and

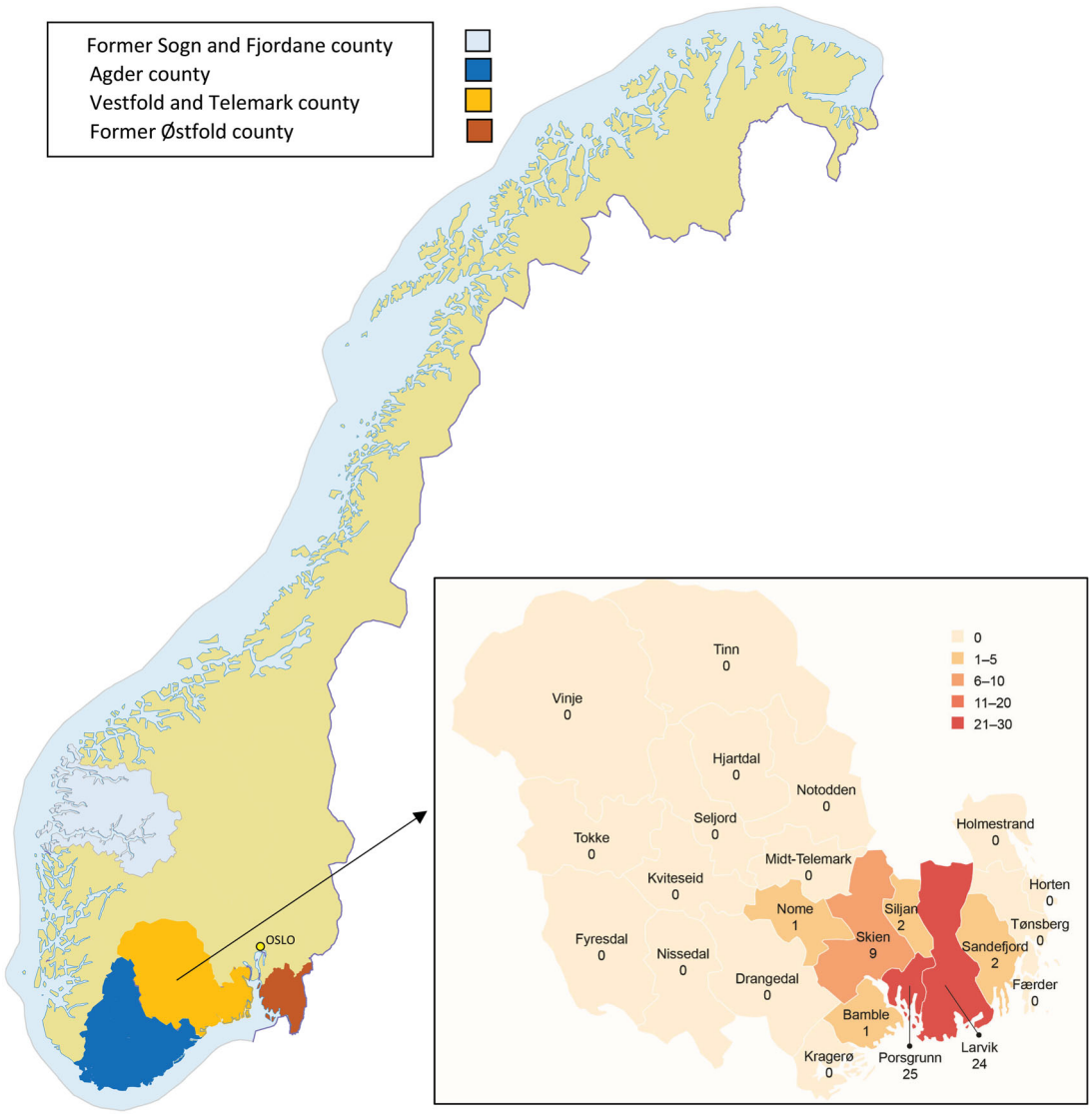

Figure 1. Norway with Agder, Vestfold and Telemark, former Sogn and Fjordane and former Østfold county marked with different colours. The inset is an enlargement of Vestfold and Telemark county with its municipalities. The figures represent reported TBE cases per municipality in the period 1998–2019 according to MSIS.

seven of the 23 municipalities have reported at least one case of assumed locally acquired TBE [30,35]. However, the majority of cases are infected in the two neighbouring municipalities Porsgrunn and Larvik.

Study population

In the period of 25 February 2019 to 29 March 2019, 1136 blood donors aged 18–70 years in Vestfold and Telemark county were invited to participate in the study 'Do blood donors in Vestfold and Telemark have antibodies to tick-borne encephalitis virus?' At the time of inclusion, there were roughly 6750 available blood donors registered in the county and eight blood bank locations. Blood donors were recruited from Tønsberg, Sandefjord, Larvik, Skien, Notodden, Porsgrunn and Kragerø. The location in Tinn did not attend due to its location furthest from the coast (Figure 1). Only three of the 1136 invited blood donors declined to participate in the study. By inclusion, the participants received information about the study, signed a written consent, agreed to donate 10 mL venous blood and filled out a questionnaire. Both the blood samples and the questionnaires were anonymized. The Regional Committee for Medical and Health Research Ethics (REC) in South-Eastern Norway approved the study (REC South-East ref: 2018/2572).

Questionnaire

The participants provided information on gender, age, current residence, frequently used holiday destinations and travel history to endemic TBE areas (central- or

eastern Europe, the Baltics, Finland, north-west Russia, Bornholm and the east or west coast of Sweden). The questionnaire contained questions regarding previous tick bites, specific symptoms after tick bite, medical consultations and antibiotic treatment due to tick bite as well as any tick bite acquired abroad. Previous flavivirus exposure is important to map in order to ensure correct interpretation of the test results. The questionnaire was designed to self-report any previous flavivirus infections (TBE, dengue fever, West Nile fever, hepatitis C) or vaccinations (yellow fever, Japanese encephalitis and TBE). Finally, their knowledge regarding TBE and TBE prevention were obtained.

Laboratory method

Blood samples were collected in serum separator tubes with gel in connection with blood donation. After centrifugation, the samples were stored for up to three days in 2–8 °C before divided in aliquots and stored at −70 °C. All samples were analysed for TBEV IgG antibodies using a commercial available ELISA test (Siemens Enzygnost Anti-TBE Virus IgG, Erlangen, Germany) according to the manufacturer's instructions. Sera were classified as negative, borderline and positive according to the instructions of the kit. Positive and borderline values were reanalysed as illustrated in Figure 2. Samples confirmed borderline (titre 6–8 U/mL) or positive (titre ≥9 U/mL) were forwarded to the Center for Virology, Medical University of Vienna, where a TBEV-specific serum neutralization test (NT) was performed as described previously [36]. Briefly, serial twofold dilutions of heat-inactivated serum samples were incubated with TBEV strain Neudoerfl for one hour at 37 °C. BHK cells (BHK-21, ATCC no. CCL-10) were added and incubation was continued for three days. The presence of virus was measured in the supernatants using ELISA. Neutralization titres were defined as the reciprocal of the plasma dilution that gave a 90% reduction in the absorbance readout in the assay compared to the control without antibody. NT titres ≥10 were considered positive.

Statistical analysis

Correlation between TBEV IgG Enzygnost units and NT titre was assessed using Pearson's correlation test. Statistical analysis was performed using SPSS Statistics version 25 (SPSS Inc., Chicago, IL).

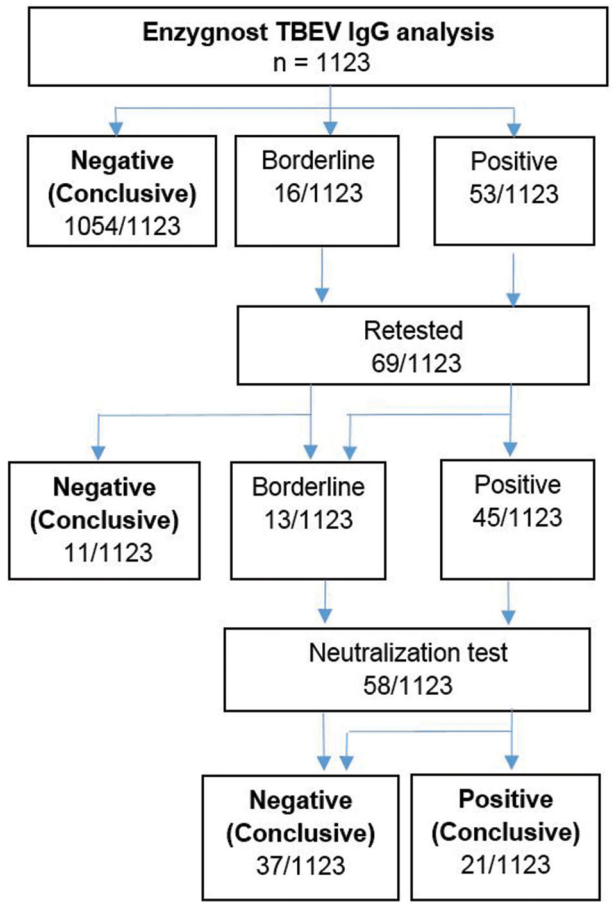

Figure 2. Algorithm of the TBEV IgG analysis of 1123 blood donor sera with associated results.

Results

Study population

A total of 1133 blood donors were enrolled, 10 were excluded either due to major deficiencies in answering the questionnaires ($n = 5$) or a residential address outside the county ($n = 5$). However, blood donors were included despite small deficiencies in the questionnaire response. The study population consisted of 45.2% (508/1123) men and 54.8% (615/1123) women. Regarding residency, 20.6% (231/1123) of the donors live in Porsgrunn and Larvik, which have the highest TBE incidence in the county. In total, 59.8% (672/1123) of the donors are residents in one of the seven municipalities with at least one TBE case reported (Figure 1).

Tick bites and symptoms

Previous tick-bite was reported by 55.9% (628/1123) of the donors (Table 1). Skin rash was the predominant

Table 1. Self-reported number of tick bites and symptoms following a tick bite during lifetime among 1123 blood donors in Vestfold and Telemark county.

	n	%
Total tick bites ever experienced (n = 1123)		
None	332	29.6
Unaware of	163	14.5
1–10 tick bites	542	48.3
11–50 tick bites	79	7.0
51–100 tick bites	5	0.4
No. not specified	2	0.2
Any tick bite (sum)	628	55.9
Symptoms experienced after tick bite (n = 618)[a]		
No symptoms	470	76.1
Skin rash	137	22.2
Fever	2	0.3
Headache	7	1.1
Joint pain or swollen joints	6	1.0
Palsy in face	2	0.3

[a]Numbers do not add to 628 due to missing data.

accompanying symptom, while the reported occurrence of fever, headache, joint complaints and palsy in the face was low. Of those who reported a history of tick bite, 20.7% (130/628) have consulted a physician and 15.1% (95/629) have received antibiotic treatment, the latter most likely due to suspected Lyme borreliosis.

Flavivirus exposure

Vaccination against yellow fever, TBEV and Japanese encephalitis was reported by 7.0% (79/1222), 3.7% (42/1122) and 1.3% (15/1122) of the donors, respectively. Unfortunately, it was not possible to decide whether vaccination against TBE was performed according to manufacturers' guidelines. Regarding previous flavivirus infections, 98.7% (1108/1123) of the donors responded with four cases of TBE, three cases of dengue fever and two cases of hepatitis C reported, all by separate donors.

TBE awareness

According to the questionnaires, 49.9% (557/1116) of the donors were familiar with TBE, whereas 35.5% (398/1122) were aware of a preventative TBE vaccine.

Laboratory results

After the first TBEV IgG ELISA analysis, 93.9% (1054/1123) of the donors were concluded to be TBEV IgG negative (Figure 2). Upon reanalysis, additionally 11, all of them initially borderline, were also TBEV IgG negative. The remaining 58 reactive ELISA samples (13 borderline and 45 positive) were forwarded to the Center for Virology of the Medical University of Vienna, in order to perform NT. Twenty-one of the 45 positive TBEV IgG ELISA samples neutralized TBEV, and all borderline ELISA samples were found to be non-neutralizing. There was a significant correlation between the TBEV IgG Enzygnost units and the NT titres ($p<.001$). Of the 21 blood donors with neutralizing TBEV antibodies detected, 17 reported a previous TBE vaccination. Thus, only 0.4% (4/1123) of the enrolled blood donors had neutralizing antibodies against TBEV, consistent with a previously undergone TBEV infection. They were all men residing in municipalities with confirmed TBE cases. Although three reported previous tick bites, none of them reported a history of or symptoms suggestive of TBE. Neither reported tick bites acquired abroad, but three reported hiking in countries where TBE is more prevalent than in Norway (Finland, Sweden and central Europe). Thirty-seven donors had a reactive TBEV IgG ELISA not confirmed by NT, 15 of these donors reported vaccination against TBE. An overview of reported previous flavivirus exposure is presented in Table 2.

Table 2. Self-reported flavivirus exposure earlier in life among blood donors with a reactive TBEV IgG ELISA (borderline and positive) and a negative NT ($n = 37$).

		Enzygnost TBEV IgG	
	n	Borderline	Positive
Vaccination received			
Tick-borne encephalitis	15	5	10
Yellow fever	1	0	1
Japanese encephalitis	2	1	1[a]
Flavivirus infections			
Tick-borne encephalitis	0	–	–
Dengue virus	2	0	2
West Nile fever	0	–	–

[a]This donor had also received vaccination against yellow fever.

Discussion

This is the first TBEV IgG prevalence study performed on blood donors living in an endemic TBE area in Norway. Blood donors, in this context, represent the healthiest group of the adult population between the ages of 18 and 70, but they are only a small proportion of the region's total population. Approximately, 60% of the donors were residents of a municipality with at least one TBE case reported. However, the coastal areas are popular resorts and recreational areas and the travel distances are small. Thus, a greater proportion of the donors might have been in TBE risk areas. Previous tick bites were reported by approximately 56% of the participants. Four donors reported a history of TBE, but based on an overall assessment of their questionnaires, all were concluded as cases of misreporting. Only 0.4% of

the blood donors had neutralizing TBEV antibodies, consistent with a prior TBEV infection. None of them reported a history of TBE, but all were settled in TBE risk areas and three donors also confirmed previous tick bites. In addition, all but one donor reported travel to other possible TBE risk areas. Naturally infected subjects exhibited stronger immune response in the form of higher and long-persistent TBEV IgG antibody titres than obtained after immunization [37,38]. Therefore, and due to low TBE incidence in the county, false-negative TBEV-infected blood donors in our material seems unlikely.

Surprisingly, we did not find evidence of a higher TBEV IgG seroprevalence among blood donors in Vestfold and Telemark county than in Østfold county, a non-endemic area with no clinical TBE cases ever reported [30,32]. However, different TBEV-specific neutralization assays were applied for confirmation and there might be differences in both sensitivity and specificity among different NT protocols [39]. Although Larsen et al. detected TBEV RNA in locally collected ticks, Østfold county borders to Western Götaland, a well-known TBE endemic region in Sweden, so these TBE cases might also have been infected elsewhere [32,40].

Results of seroprevalence studies are highly dependent on the test population and assay technology applied. In Europe, the TBEV IgG seroprevalence rates in the general population are estimated to range between 0 and up to 5%, while seroprevalence rates conducted on high-risk populations in endemic countries, such as forest workers or residents of high-endemic areas, is substantially higher [41,42]. In Norway, Thortveit et al. and Skarpaas et al. found seroprevalence rates of 1.4% and 2.4% among adults residing in Agder county [28,34]. In both studies, the TBEV IgG results were based on the ELISA methodology and might therefore be overestimated. Neutralization assays are the most type-specific serological tests and are recommended for confirmation of TBEV IgG ELISA results, especially in surveys conducted in non-endemic TBE areas [38,43]. Also in this study, a higher proportion of the blood donors would be positive according to the ELISA results without the use of a confirmatory NT.

Interference caused by flavivirus cross-reactive antibodies, due to common antigenic sites within the E protein, is well documented with the ELISA method among several flaviviruses that infect humans, like Japanese encephalitis virus, dengue virus and yellow fever virus [38,43–45]. A comparative study of different commercial TBEV IgG-ELISA kits, including Enzygnost, revealed particularly specificity problems with dengue virus IgG [45]. In the current study, exposure to other flaviviruses, either through vaccination or undergone infections, were obtained. Two of the three donors with a history of dengue fever had a reactive TBEV IgG ELISA due to cross-reactivity. In addition, at least two other donors had a reactive ELISA due to vaccination against Japanese encephalitis and/or yellow fever. Thus, at least four cases of flavivirus cross-reactive antibodies were observed. Skarpaas et al. did not assess false-positive TBEV IgG ELISA results due to any flavivirus exposure, while Thortveit et al. obtained information about TBE and/or yellow fever vaccinations. According to the literature, and our observations, a history of dengue fever is an important flavivirus exposure to identify. Louping-ill virus (LIV) is another flavivirus transmitted by *I. ricinus* ticks and is antigenically closely related to TBEV [44]. LIV can cause encephalomyelitis of sheep and is mainly restricted to the British Isles. However, LIV infections in sheep have been reported in Norway although the last case was in 1991 [46,47]. LIV is a rare cause of human disease and no human cases have ever been reported in Norway [48]. Thus, at present, cross-reactivity due to LIV antibodies is not a current issue.

Of the 42 donors, who reported vaccination against TBE, only 40% had neutralizing TBEV antibodies detected. In the absence of previous flavivirus exposure, the TBEV IgG level determined by ELISA usually correlates with the presence of neutralizing antibodies after TBEV vaccination [43]. According to Lindblom et al., age and the number of vaccine doses are the two most important factors determining the immunological response to TBE vaccination. The TBEV antibody titre, measured by ELISA methods, declined linearly with increased age to each vaccine dose given [49]. Unfortunately, it was not possible to determine whether the participants were vaccinated according to given guidelines. Therefore, no causal relationships were further investigated. In addition, self-reporting is fraught with the possibility of error and it was not possible to verify the blood donors' vaccination histories.

Based on this study, there is only weak evidence of subclinical TBEV infections among blood donors living in Vestfold and Telemark county. Thus, the national vaccination recommendations appear adequate as only those at risk, and not the general population, should consider vaccination against TBE. Noteworthy, only about half of the participants were familiar with TBE and only 35% were aware of a preventative TBE vaccine.

Our study indicates low prevalence of TBEV infections among blood donors living in Vestfold and Telemark

county. However, the awareness of TBE and TBE prevention seems insufficient and there is an urgent need for public information.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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