

Seroprevalence of antibodies to tick-borne encephalitis virus and *Anaplasma phagocytophilum* in healthy adults from western Norway

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Abstract

The aim of this study was to assess the seroprevalence of antibodies to tick-borne encephalitis virus (TBEV) and *Anaplasma phagocytophilum* in a healthy adult population from Sogn and Fjordane county in western Norway. Sera from 1, 213 blood donors were analysed for IgG-antibodies to TBEV, and a random subgroup of 301 donors for IgG to *A. phagocytophilum*.

In the TBEV ELISA, five (0.4%) sera were positive. These were all interpreted as “false” positives, as four had received vaccines against flaviviruses, and the remaining was negative for neutralizing antibodies to TBEV.

Antibodies to *A. phagocytophilum* were detected by indirect immunofluorescence in 49 (16.2%) subjects (titer range 80-1280).

The results indicate that TBE currently is not endemic in this part of western Norway. However, there is serological evidence of the existence of human granulocytic anaplasmosis in the population.

Introduction

Located at the western coast of Norway at 61-62 °N and 5-7 °E, the county of Sogn and Fjordane encompasses coastal, fjord and mountainous areas. In the coastal and middle areas, the climate is temperate, with a high yearly rainfall, whereas the eastern part has a more inland-like climate.

Ixodes ricinus, the predominating tick species in Norway, is present along the coast as far as 69 °N. Its latitudinal and altitudinal distribution limits seem to be expanding . It is more abundant along the southernmost coastline. In Sogn and Fjordane county, there are more ticks in the western than in the eastern regions [1].

From 1998 onwards, human cases of tick-borne encephalitis (TBE) acquired along the southern coastline of Norway have been diagnosed [2,3], and tick-borne encephalitis virus (TBEV) has been detected in ticks in this area [4]. So far, no human cases acquired in western Norway have been described. However, a case of TBE-seropositivity in a red deer in the county of Møre and Romsdal, just north of Sogn and Fjordane, has recently been reported [5]. Traavik and co-workers found evidence of tick-borne encephalitis-like virus in this part of Norway in studies of animals and humans in the 1970s [6-8]. However, there is evidence that these findings represented other flaviviruses [9, 10]. As far as we know, no recent survey of TBEV in ticks has been performed in Sogn and Fjordane county.

Granulocytic anaplasmosis, caused by the bacterium *Anaplasma phagocytophilum*, is prevalent in livestock in Norway [11], and probably as much as 300, 000 lambs are infected yearly. After being described as also affecting humans in the United States in 1994 [12], and from 1997 onwards also in Europe [13], two cases from 1998 were reported from southern Norway [14], and serological evidence for human infection has been demonstrated [15]. *A. phagocytophilum* was detected in 8.8% of *I. ricinus* in Sogn and Fjordane in a survey performed in 2011 [16], while an earlier study performed in 2006-7 found a lower prevalence (1.4% and 0.0% in two different locations, respectively) [17].

Among the blood donors included in the present study, 65.7% reported having been bitten by ticks at least once in their lifetime, and 30.0% reported tick bites during the last twelve months [18]. Fewer tick bites were reported from the donors in the eastern than in the western part of the county. The seroprevalence of antibodies to *Borrelia burgdorferi sensu lato* in Enzygnost Lyme link VlsE/IgG, Enzygnost Borreliosis IgM, and Immunitics C6 Lyme ELISA kit, *Borrelia*-EUROLine-RN-AT IgG blot and *Borrelia*-EUROLine-RN-AT IgM blot was 9.6%, 8.2%, 8.4%, 6.4% and 5.7%, respectively [19].

The aim of the present study was to assess the prevalence of antibodies to TBEV and *A. phagocytophilum* in healthy adults from western Norway (blood donors), to obtain an indication of whether diseases caused by these agents currently should be considered in the evaluation of patients after tick bites in this region.

Material and methods

Study population

During the period of January 13th to June 15th 2010, blood donors at the four blood banks in Sogn and Fjordane, Norway, were asked to participate in the Tick-borne Infection Study in Sogn and Fjordane. A total of 1,213 blood donors participated, a response rate of 76%. Characteristics of the participants are presented previously [18]; mean age was 45.8 (range: 19-69) years and 55.2% were men. Sera from a subgroup of 301 subjects, randomly selected, were analysed for antibodies to *A. phagocytophilum*. In this group, the mean age was 45.5 (range: 19-69), and 55.1% were men. Informed consent was obtained from each participant, and the study was approved by the Regional Committee for Medical Research Ethics.

Questionnaire

All study participants filled in and returned a questionnaire on the day of blood donation. They were asked to record the number of tick bites ever experienced and tick bites experienced during the last 12 months. The responses for both these questions were given in the categories “none”, “one”, “2-5”, “6-20” and “more than 20”. In addition, participants provided information on gender, age, marital status, education, household income and occupation, pet animals, farm animals, hours spent outdoors during summertime, hunting, orienteering, smoking, symptoms and treatment after tick bites, as well as on a number of subjective health complaints.

The questionnaire included questions on received vaccinations known to induce antibodies reacting in assays for TBEV-antibodies (Yellow fever, Japanese encephalitis, TBE), questions on other

diseases caused by flaviviruses (Dengue fever, West Nile fever, Hepatitis C), and travel to known geographical areas endemic for TBE (central- or eastern Europe, the Baltics, Åland, Bornholm, north-west Russia, the Baltic sea coast of Sweden, the west coast of Sweden, the southern coast of Norway).

Laboratory methods

Blood samples were collected in serum separator tubes with gel, and after centrifugation, sera were frozen in aliquots at -70 °C until testing.

All 1, 213 sera were analysed for IgG-antibodies to TBEV in Serion ELISA *classic* TBE IgG (Institute Virion\Serion GmbH, Würzburg, Germany) according to the manufacturer's instructions. Grey-zone results were repeated. One serum positive in this test was further tested for neutralizing antibodies to TBEV at the Swedish Institute for Communicable Disease Control [20].

A subgroup of 301 sera was examined for IgG to *A. phagocytophilum* by an indirect immunofluorescence assay (IFA) (*Anaplasma phagocytophilum* IFA IgG Kit, Focus Diagnostics, CA, USA). A screening analysis was performed at a serum dilution of 1:80, and sera with a positive reaction in this dilution were further examined in two-fold titrations.

Statistical analysis

Statistical analysis was undertaken using IBM SPSS Statistics version 20 (SPSS Inc., Chicago, IL, USA).

All p values were two-sided and values below 0.05 were considered statistically significant.

Comparisons of categorical variables were performed using chi-squared test.

Results

TBEV

Among the 1, 213 sera tested, five (0.4%) gave positive and one (0.1%) gave grey-zone results in the ELISA. Among these six subjects, five reported having received vaccines that might give positive

reactions in the TBE ELISA; two had received vaccines against yellow fever, one against Japanese encephalitis (in addition to yellow fever), and three against TBE. A positive serum from one subject denying having received any of these vaccines, and also denying symptoms of TBE, was further examined by neutralizing antibodies to TBEV, with negative result.

Table I shows the relationship between reported vaccines and results of the TBE antibody assay. No information is available about the number of vaccine doses or when they were given.

Anaplasma phagocytophilum

The distribution of results for the 301 sera is presented in Table II. Of these, 49 (16.2%) were positive with titer ≥ 80 (range 80-1280).

We found no association between seropositivity and gender, age, geography (location of blood bank), self-reported number of tick bites or presence of antibodies to *B. burgdorferi sensu lato* (data not shown). In this subgroup, 192 (63.8%) reported to ever having been bitten by a tick. Among these, 23 (12.0%) had IgG antibodies to *B. burgdorferi sensu lato*, 32 (16.7%) to *A. phagocytophilum*, and 6 (3.1%) to both agents. The latter group was overrepresented among the 23 persons reporting to ever having consulted a doctor after a tick bite ($p=0.024$), and among the 12 persons having received antibiotic treatment after a tick bite ($p=0.047$, Fisher's exact test).

Discussion

The main findings in this study are that no non-immunized blood donors had specific antibodies to TBEV, and that 16.2% had antibodies to *A. phagocytophilum* at titers ≥ 80 .

Tick-borne encephalitis

Traavik and co-workers reported in the 1970s that antibodies to TBEV were prevalent in animals and humans from the western coast of Norway, and five isolates from ticks were obtained [6-8].

However, the serological methods used at the time, haemagglutination inhibition (HAI) and gel diffusion, were probably not specific for TBEV, and the positive results probably reflect infection with one or more related viruses. They reported a seropositivity rate for humans in HAI of 19.6%. In contrast, in the present study, we found no true positive non-TBE-vaccinated cases, as all but one can be explained by TBE-vaccine or other vaccines giving cross-reacting antibodies (Table I), the only exception was negative in neutralization test. Thus, current ELISA tests seem to be more specific than former tests, and the study results give no evidence for the existence of TBE in humans in Sogn and Fjordane.

Our negative findings are in accordance with the known current distribution of TBE in Norway [2]. From the southernmost coastline in Norway, Skarpaas and co-workers found that three of 126 (2.4%) inhabitants of Tromøy in Aust-Agder county were seropositive [21]. A recent study from the south-eastern county of Østfold found a seroprevalence in non-immunized blood donors of 0.65% [22], indicating that TBEV exists in this region, close to endemic regions of Norway and Sweden, although no human cases of TBE have been notified so far. A study from Sweden found a seropositivity rate of 4-22% in non-immunised participants, depending on the area investigated [23]. The same study reported that in 362 orienteers from the county of Stockholm, 1% of the individuals were seropositive. Among the 65 subjects reporting to ever having been orienteers in our material, none were positive.

Anaplasma phagocytophilum

A cut-off in the IFA test of 1:64 or 1:80 is widely used for epidemiological purposes. However, as discussed by several authors [24, 25], this may be set too low, as a significant proportion of adults and children without clinical evidence of human granulocytic anaplasmosis (HGA) will test positive for *A. phagocytophilum* antibodies when these cut-offs are used. Thus, Walder and co-workers in Austria chose a cut off of 1:128, the 98th percentile of a control population with low likelihood of having had HGA [25].

In addition to uncertainty of the proper cut-off, serological cross-reactions may complicate the judgement of IFA results. False positive results may be due to serological cross-reactions, e.g. due to other *Anaplasma* or *Ehrlichia* spp., Epstein-Barr virus infection, Lyme borreliosis or autoimmune disorders [26].

Surveys of antibodies to *A. phagocytophilum* among blood donors using the IFA have found seroprevalences of 11.3% (18/159) among *Borrelia* blot negative donors in Westchester county, New York, using a cut-off of ≥ 80 [24], 0.5% (5/992) in Wisconsin and 3.5% (35/992) in Connecticut, USA (cut-off ≥ 64) [27], and 9.0% (32/357) in Tyrol, Austria (cut-off ≥ 128) [25]. In Denmark, Skarphedinsson and co-workers [28] found that 2 of 100 blood donors from Odense, Denmark and 5 of 100 blood donors from Iceland were positive (cut off ≥ 64).

In a survey of blood samples from patients with physician-diagnosed Lyme borreliosis in the county of Telemark in southern Norway, 10.2% (6/58) were positive in IFA (≥ 80 , range 1:80 – 1:160) [15]. Dumler and co-workers found that 11.4% (21/185) of inhabitants at the island of Koster at the western coast of Sweden were positive (≥ 80) [29], whereas Wittesjö and co-workers found a seroprevalence of up to 28% in inhabitants in Aspö island at the Baltic sea coast of Sweden [30]. In Denmark, 21.0% (63/300) of sera from patients clinically suspected of having Lyme borreliosis were positive for antibodies to *A. phagocytophilum* [28].

Compared to these studies, the seroprevalence of 16.2 % in our material was relatively high. As discussed above, this may represent an over-estimate, but the selected cut-off allows for the comparison.

The overrepresentation of subjects with IgG antibodies both to *A. phagocytophilum* and *B. burgdorferi* sensu lato among those having sought a doctor after a tick bite and among those having received antibiotic treatment, is an interesting observation. However, this should be interpreted with caution because of low numbers.

In conclusion, this study found no evidence of TBE as an endemic disease in the county of Sogn and Fjordane in western Norway. There was, however, serological evidence for the existence of human granulocytic anaplasmosis, indicating that clinicians should be aware of this condition in the diagnostic considerations after tick bites in this geographic area.

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Table I. Results of anti-TBE IgG ELISA in relation to reported vaccinations in the Tick-borne Infections Study in Sogn and Fjordane, 2010 ($n= 1,213$)

Vaccination received	n^a	anti-TBE IgG result		
		-	+/-	+
Yellow fever	52	50	0	2 ^b
Japanese encephalitis	5	4	0	1 ^b
Tick-borne encephalitis	9	6	1	2
None of the above	1149	1148	0	1 ^c

^a Numbers do not add to 1,213 as some had received multiple vaccinations and some data were missing.

^b One subject positive in anti-TBE IgG had received vaccinations for both yellow fever and Japanese encephalitis.

^c Negative in TBE neutralization test.

Table II. Distribution of results in indirect immunfluorescence for *Anaplasma phagocytophilum* IgG in the Tick-borne Infections Study in Sogn and Fjordane, 2010 ($n = 301$)

Titer	<i>n</i> (%)
<80	252 (83.7)
80	18 (6.0)
160	20 (6.6)
320	8 (2.7)
640	2 (0.7)
1280	1 (0.3)