scientific reports

Check for updates

OPEN

Tick-borne encephalitis virus seroprevalence and infection incidence in Switzerland, 2020–2021

Arthur Brêchet[1,11], Philipp Kohler[2,11], Tamara Dörr[2], Fabian Grässli[2], Michael Vock[3], Jiří Salát[4,5,6], Daniel Růžek[4,5,6], Andrée Friedl[7], Danielle Vuichard-Gysin[8], Antony Croxatto[1,9], Reto Lienhard[1,9] & Rahel Ackermann-Gäumann[1,9,10]✉

Tick-borne encephalitis virus (TBEV) infection can manifest as disease of variable severity, ranging from subclinical infection to severe disease with neurological involvement and potentially fatal outcome. Although TBE is recognized as a major public health problem in Europe, the true burden of disease is potentially underestimated. Here, we investigated TBEV-specific antibody prevalence, infection incidence, and seroreversion and antibody decline rates in a prospective Swiss healthcare worker (HCW) cohort. We screened serum samples from 1444 HCWs between June and October 2020, and from a subset again between August and September 2021, using a TBEV envelope (E) protein IgG ELISA. Positive samples underwent further analysis with a TBEV non-structural protein 1 (NS1) IgG ELISA, and seroconversions in unvaccinated individuals were confirmed by seroneutralization testing. Questionnaire data were used to determine vaccination status and risk factors. TBEV E protein-specific IgG prevalence was 72.1% (95% CI 68.2–75.7%) in TBEV-vaccinated and 6% (95% CI 4.4–7.8%) in unvaccinated individuals. The estimated annual incidence of infection was 735/100,000. Age was the only factor significantly associated with seroprevalence. The seroreversion rate in unvaccinated individuals was 30.3% within one year, which is almost ten times higher than in vaccinated individuals (3.4%, annual decline rate 8.0%). NS1-specific IgG antibodies were six times more common in vaccinated than unvaccinated HCWs. In conclusion, undetected TBEV infections are common, and infection incidence is much higher than reported clinical cases. Individuals with abortive infections have high antibody decline and seroreversion rates. Whether lifelong protection is conferred and by which immune subsets remain unclear.

Keywords Tick-borne encephalitis, TBE, TBEV, Orthoflavivirus, Prevalence, Incidence, Asymptomatic, Abortive

Abbreviations

DENV	Dengue virus
E protein	Envelope protein
HCW	Healthcare workers
JEV	Japanese encephalitis virus
NS1	Non-structural protein 1

[1]Microbiologie, ADMED Analyses et Diagnostics Médicaux, La Chaux-de-Fonds, Switzerland. [2]Division of Infectious Diseases, Infection Prevention and Travel Medicine, Cantonal Hospital of St. Gallen, St. Gallen, Switzerland. [3]Institute of Mathematical Statistics and Actuarial Science, University of Bern, Bern, Switzerland. [4]Laboratory of Emerging Viral Infections, Veterinary Research Institute, Brno, Czech Republic. [5]Laboratory of Arbovirology, Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic. [6]Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic. [7]Division of Infectious Diseases and Hospital Epidemiology, Kantonsspital Baden, Baden, Switzerland. [8]Thurgau Hospital Group, Division of Infectious Diseases and Hospital Epidemiology, Muensterlingen, Switzerland. [9]Swiss National Reference Center for Tick-Transmitted Diseases, Lausanne, Switzerland. [10]Present address: Interregional Blood Transfusion SRC, Bern, Switzerland. [11]Arthur Brêchet and Philipp Kohler have contributed equally to this work. ✉email: rahel.ackermann@itransfusion.ch

TBE	Tick-borne encephalitis
TBEV	Tick-borne encephalitis virus
WNV	West Nile virus
YFV	Yellow fever virus
ZIKV	Zika virus

Background

Tick-borne Encephalitis (TBE) is a severe disease of the Central Nervous System. It is caused by the tick-borne encephalitis virus (TBEV) and is mainly transmitted to humans via the bite of infected Ixodid ticks, though an estimated 1% of cases occur via alimentary transmission[1]. TBEV is prevalent in Central, Eastern, and Northern Europe, as well as certain parts of Asia, resulting in an annual report of 10,000–15,000 cases[1].

Disease resulting from TBEV infection is classified into two phases: a first viremic phase, which may progress to a second neurologic phase. Disease progression may cease after the first phase, known as the abortive clinical pattern. This form of TBE may be asymptomatic or present as a mild febrile illness, without developing a neurological form[1,2]. However, at least one-third of TBE patients progress to the second phase of the disease[1]. This phase can be further subdivided into meningeal and focal forms, which include meningoencephalitis, meningoencephalomyelitis, and encephaloradiculitis[1].

A nationwide seroprevalence estimate from Switzerland in 2014–2015 found that approximately 5% of the unvaccinated population is seropositive for TBEV[3]. The average annual incidence, based on reporting of diagnosed cases, is only 3–5 cases per 100,000 individuals[4]. This suggests, that a high proportion of infections are not diagnosed. The proportion of individuals experiencing an asymptomatic infection is typically reported to range between 70 to 98% [2]. However, a Czech study suggests that approximately 40% of TBEV infections result in some form of illness[5].

Currently, there is no specific treatment available for TBE. The most important protective measures against TBEV infections are active immunization and prevention of tick bites through personal protection methods[6]. In Switzerland, the vaccination coverage (1 dose) among children aged 16 increased from 10% in 2005–2007 to 55% in 2020–2022 [7]; in adults, the vaccination coverage reached 42% in 2018 (up to 50% in endemic regions)[8].

TBE vaccines contain only trace amounts of the viral non-structural protein 1 (NS1)[9]. Therefore, the detection of NS1-specific antibodies has been suggested as a tool to distinguish the immune response after vaccination and natural infection[10,11]. However, we have recently demonstrated that vaccination induces low titers of NS1-specific antibodies in a dose-dependent manner. Thus, distinguishing between antibodies acquired through infection and those acquired through vaccination may not be unambiguous using NS1-specific IgG testing, and establishing a well-defined cut-off point is critical[12].

The aim of this study was to investigate TBEV seroprevalence, antibody dynamics and infection incidence in a prospective cohort of Swiss healthcare workers (HCW), and to explore associated risk factors.

Materials and methods

Study population

The study was carried out from June 2020 to August 2021 within a prospective HCW cohort (SURPRISE study; SURveillance of infectious diseases among health PRofessionals In SwitzErland) originally conceived to study COVID-19 related topics among employees from nine healthcare networks located in Northern and Eastern Switzerland. The study catchment area is shown in Fig. 1. Eligibility criteria required participants to be aged 16 years or older, with informed consent obtained during online registration. Out of 1,937 HCW consenting to study participation, 1,527 individuals (78.8%) for whom serum samples from time point 1 (June to October 2020) as well as questionnaire data were available, were considered for the analyses.

Ethics and inclusion statement

The study was approved by the ethics committee of Eastern Switzerland (#2020–00,502)[13]. All methods were carried out in accordance with relevant guidelines and regulations. All study data were pseudonymized and REDCap was used for data collection. REDCap is a secure and established web application for clinical studies, compliant with the International Council on Harmonization of Good Clinical Practice (ICH-GCP).

Study objectives

The primary objectives included determining the seroprevalence of envelope (E) protein and NS1-specific antibodies in TBE-vaccinated and unvaccinated individuals, identifying potential risk factors associated with the presence of TBEV-specific antibodies, evaluating seroreversion and decline rates of E protein-specific IgG antibody titers within one year, and estimating annual incidence of infection, both symptomatic and asymptomatic, in a Swiss HCW cohort.

Sample collection

Blood samples were collected at time point 1 (June to October 2020) and – for a subgroup of individuals – also at time point 2 (August and September 2021) in 10ml tubes (1,5 ml separation gel). Serum was prepared by centrifugation (10 min. at 2,000 rpm) within 24 h after sample collection. Serum samples were stored at -20 °C until analysis; during the testing periods, samples were thawed and temporarily stored at 2–8 °C.

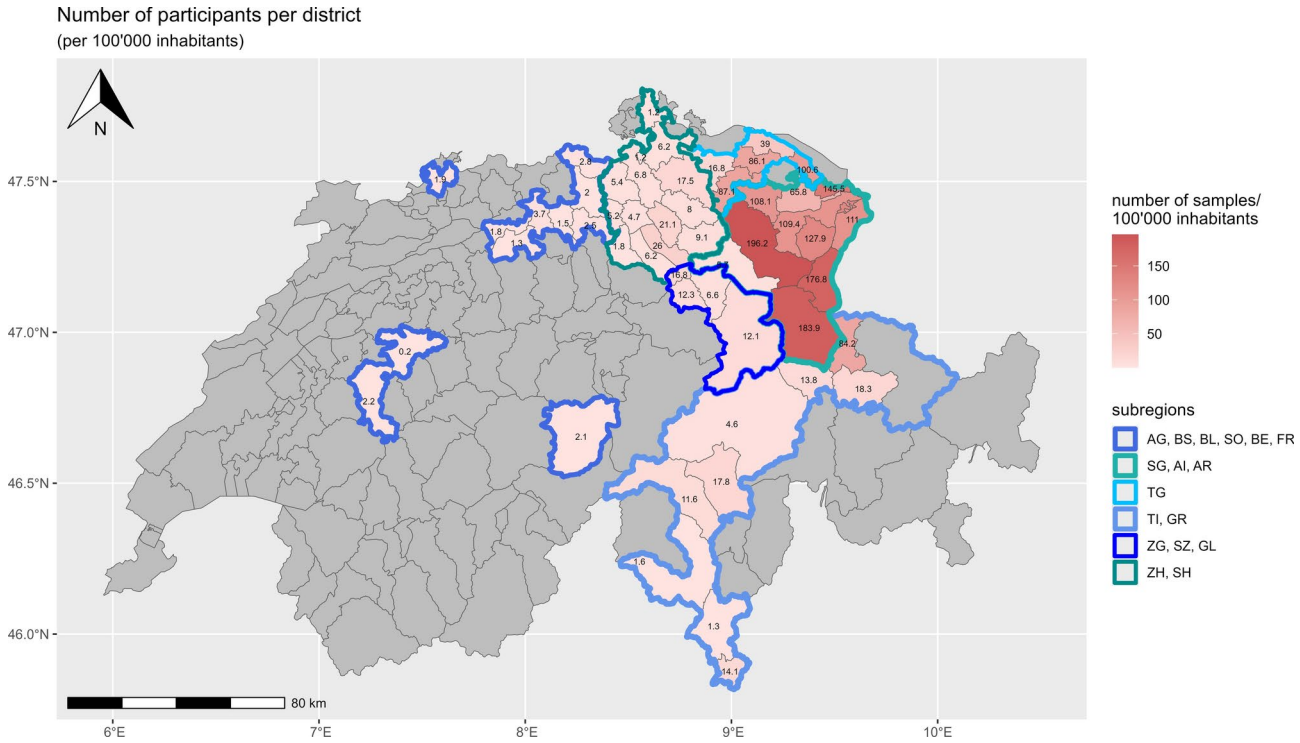

Fig. 1. Study catchment area. The map shows the number of study participants per 100,000 inhabitants per canton. Participants from neighboring countries are not shown (n = 24 for Austria, n = 16 for Germany, and n = 12 for the Principality of Liechtenstein). Abbreviations of cantons: AG Aargau, AI Appenzell Innerrhoden, AR Appenzell Ausserrhoden, BE Bern, BL Basel Landschaft, BS Basel Stadt, FR Fribourg, GL Glarus, GR Graubünden, SG St. Gallen, SH Schaffhausen, SO Solothurn, SZ Schwyz, TG Thurgau, TI Ticino, ZG Zug, ZH Zürich. The map was generated using R (version 4.3.2, https://cran.r-project.org) with the packages readxl, tidyverse, dplyr, janitor, stringr, sf, rgeoboundaries, ggplot2, and ggspatial.

Questionnaire

A multimodular web-based questionnaire was used. Participants received an email invitation to the questionnaire after blood draw for serology at time points 1 and 2. The questionnaire at time point 1 included the following questions: age; sex; place of residence; vaccination against TBE (yes/no). Between time point 1 and time point 2, HCW additionally answered bi-weekly questionnaires on acute symptoms compatible with viral infection, as well as results of SARS-CoV-2 testing. The questionnaire at time point 2 asked the following questions:

- If vaccinated against TBE, how many doses, year of last vaccine dose
- Vaccination against Yellow Fever virus (YFV) or Japanese Encephalitis virus (JEV) (yes vs. no)
- If vaccinated against YFV or JEV, year of last vaccine dose
- Previous infection with other orthoflavivirus (Dengue virus [DENV], West Nile virus [WNV], Zika virus [ZIKV], YFV, JEV) (yes vs. no)
- Tick bites noticed during lifetime (reduced to a binary variable, yes or possible vs. no)
- Consumption of raw goat milk products during lifetime (reduced to a binary variable, yes or possible vs. no).

Participants who seroconverted between time point 1 and 2, and indicated no TBE vaccination were contacted again. They were asked to confirm that they had not received TBE vaccination. Also, symptoms reported during the bi-weekly questionnaire were confirmed.

TBEV envelope (E) protein-specific IgG ELISA

Samples were screened for the presence of TBEV E protein-specific IgG antibodies using the SERION ELISA classic FSME/TBE Virus IgG test (Institut Virion\Serion GmbH, Würzburg, Germany). Testing was performed automatically according to the manufacturer's instructions on the DSX™ Automated ELISA System (DYNEX Technologies, Chantilly, Virginia, USA). Results were expressed as units (U/ml) and classified as negative (< 100 U/ml), borderline (100–150 U/ml), or positive (> 150 U/ml), as specified by the manufacturer.

TBEV NS1 IgG ELISA

The TBEV NS1 IgG ELISA protocol was performed as described previously[12]. Briefly, 96-well polystyrene plates were coated overnight at 4°C with recombinant TBEV NS1 antigen (The Native Antigen Company) at a concentration of 0.25µg/ml in phosphate-buffered saline PBS (pH 7.4). After three wash cycles with PBS, the wells were blocked with the dilution buffer consisting of PBS with 5% bovine serum albumin and 0.5% gelatine

for 2h at room temperature, followed by one wash cycle with PBS and drying for 2h at room temperature. For the analysis, 100µl of the serum diluted 1:100 in dilution buffer were added to each well and incubated for 1h at 37°C. After three washing cycles with washing buffer (PBS & 0.05% Tween 20), 100µl of secondary antibody (Rabbit anti-Human IgG (H + L) -HRP) diluted 1:2000 in dilution buffer were added to each well and incubated for 1h at 37°C. Preceded by three washing cycles with washing buffer, 100µl of substrate tetramethylbenzidine (1-Step™ Turbo TMB-ELISA Substrate Solution, Thermo Fisher Scientific) were added and plates were incubated for 15min in the dark at room temperature. The reaction was stopped by adding 100µl of 1M sulfuric acid. The optical density was measured in an ELISA reader at 450nm, 620nm reference.

NS1-specific IgG antibodies were quantified as percent positivity with reference to a positive control consisting of four pooled serum samples from patients with confirmed TBEV infection (semiquantitative evaluation). For qualitative evaluation (negative/borderline/positive), we calculated the mean percent positivity of the lower 95% of the vaccinated individuals (assuming a seroprevalence of about 5% [3,11]), and set the cut-off for a borderline result at + 2 standard deviations (SD) and the cut-off for a positive result at + 3 SD.

Definition of serconversion and seroreversion

A seroconversion was defined as a positive (i.e., > 150 U/ml) test result and an at least four-fold titre increase in the E protein-specific IgG ELISA in the sample taken on time point 2 from an individual with a negative or borderline test result at time point 1 using the same assay. A seroreversion was defined as a borderline or negative (i.e., ≤ 150 U/ml) test result in the E protein-specific IgG ELISA in the sample taken on time point 2 from an individual with a positive test result (i.e., > 150 U/ml) at time point 1.

TBEV serum neutralization testing

Serum neutralization testing (SNT) was performed on time point 1 and 2 samples from individuals who seroconverted during the study period. SNT was done as described previously[14]: sera were diluted 1:5 in media and inactivated by heat (56°C, 30min.). Then, sera were serially diluted twofold in Leibovitz L-15 medium (Biosera) containing 3% fetal bovine serum, 100U/ml penicillin, 100µg/ml streptomycin, and 1% glutamine (Biosera). In 96-well plates, 50µL of diluted sera were mixed with 50µl virus suspension (1,000 PFU/ml, TBEV strain Hypr). This suspension was incubated for 90min. at 37°C, where after 30,000 porcine kidney stable cells in 100µl were added per well. After five days of incubation at 37°C, the cytopathic effect (CPE) was examined. The highest serum dilution that inhibited CPE was considered the end-point titer. The first well (1:10) was not evaluated because of inconsistent results due to cellular toxicity of some of the sera tested at this dilution. Neutralizing potency was evaluated starting from the second well (1:20). Therefore, serum samples with titers of ≥ 1:20 were considered positive.

Data analyses

The following questions were addressed:

a. what is the overall prevalence of TBEV E protein-specific IgG antibodies in individuals vaccinated or not vaccinated against TBEV at time point 1
b. which factors are associated with the prevalence of TBEV-specific IgG antibodies at time point 1
c. what is the overall prevalence of TBEV NS1 specific IgG antibodies in individuals vaccinated or not vaccinated against TBEV at time point 1
d. what is the proportion of vaccinated or unvaccinated individuals seroreverting during the study period
e. how do quantitative antibody titers evolve over time in vaccinated and unvaccinated individuals
f. what is the incidence of infection in the unvaccinated population during the study period.

The algorithm shown in Fig. 2 was used to select the datasets used to calculate the results for questions a) through f).

Statistical analyses were performed using R version 4.2.1.. Question a) and c) were addressed by a Clopper-Pearson confidence interval based on the results obtained for time point 1 for vaccinated and unvaccinated individuals, respectively. For question b), we fitted logistic regression models; starting from a model including age, sex, and remembered tick bites (yes or possible vs. no), we applied likelihood-ratio tests to test the variables in this initial model and to determine whether adding the region of residence, consumption of raw goat milk products or (for vaccinated individuals) complete (3 or more doses) vs. incomplete vaccination (1, 2, or unknown number of doses received) improved the model. In addition to the fitted logistic regression model, and ignoring all other factors, a Wilcoxon/Mann–Whitney test was used to assess whether quantitative antibody titers were significantly higher in individuals who had received a complete basic vaccination (≥ 3 doses) compared with those who had received one or two doses or could not recall the number of doses received. Question d) was addressed by a Clopper-Pearson confidence interval assessing the proportion of individuals testing positive at time point 1 and negative or borderline at time point 2 for vaccinated and unvaccinated individuals, respectively. For question e), we calculated the ratio of the quantitative TBEV E protein-specific antibody titer at time point 2 to that at time point 1 for each individual and then determined the median of these ratios. Question f) was addressed by a Clopper-Pearson confidence interval based on the seroconversion rate between time points 1 and 2 for unvaccinated individuals.

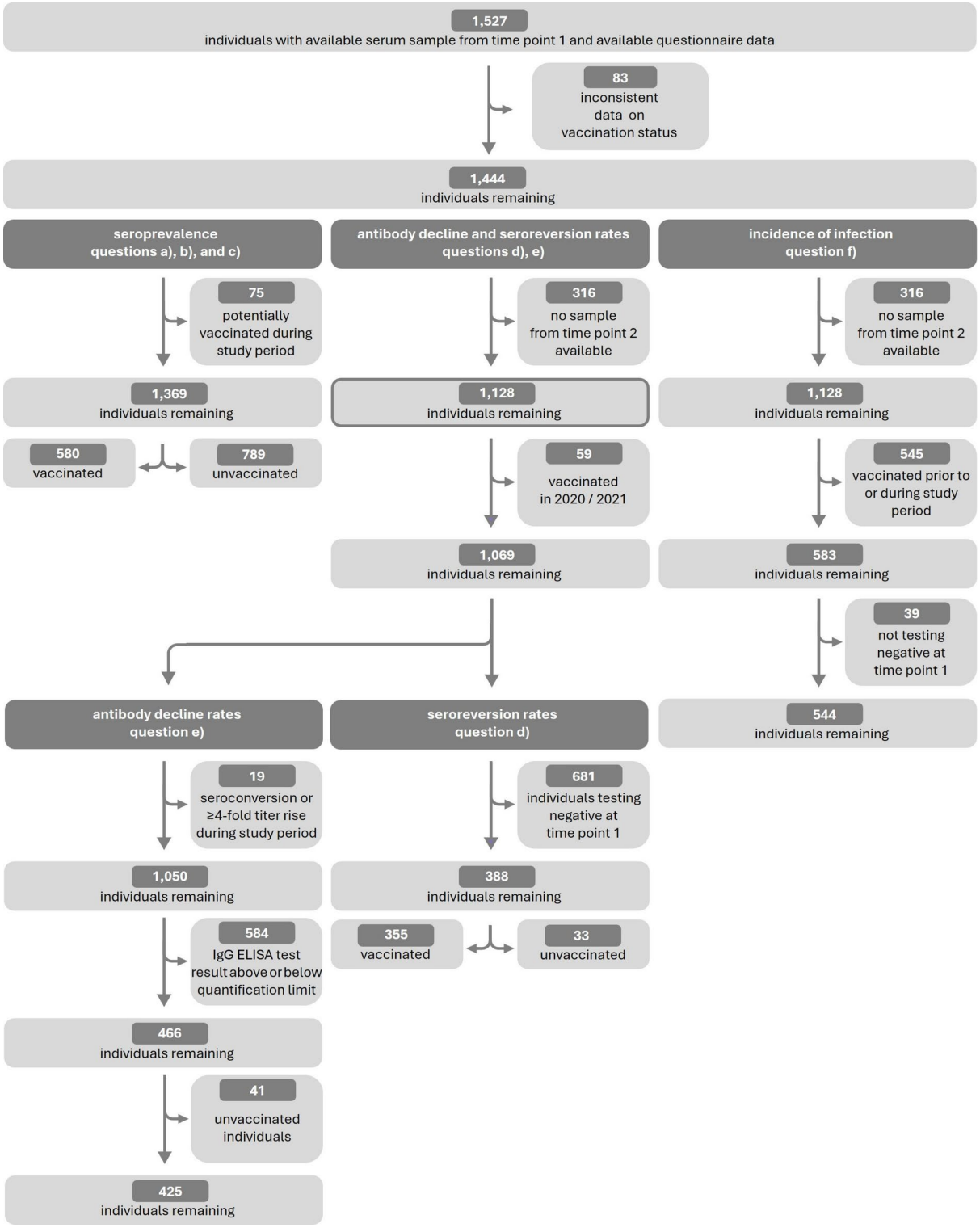

Fig. 2. Data set selection algorithm. For all questions, individuals who provided inconsistent information about their TBE vaccination status were excluded ("yes" at time 1 and "no" at time 2; "no" at time 1 and "yes" at time 2, but reported a year of vaccination prior to 2021 or did not report the year of vaccination). The flowchart shows the criteria used to select the datasets used to calculate the results for questions a) through f). The light grey box with dark border indicates the data set used to create Fig. 4.

			Data set seroprevalence[1]						Data set antibody decline and seroreversion rates[2]						Data set incidence of infection[3]	
	All		All		Vaccinated		Unvaccinated		All		Vaccinated		Unvaccinated		unvaccinated	
Total	1,444		1,369		580		789		1,069		486		583		544	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Age																
< 30	218	15.1	200	14.6	67	11.6	133	16.9	149	13.9	55	11.3	94	16.1	84	15.4
30–50	827	57.3	784	57.3	368	63.4	416	52.7	611	57.2	307	63.2	304	52.2	288	53.0
> 50	399	27.6	385	28.1	145	25.0	240	30.4	309	28.9	124	25.5	185	31.7	172	31.6
Sex																
male	315	21.8	304	22.2	150	25.9	154	19.5	235	22.0	118	24.3	117	20.0	107	19.7
female	1,117	77.4	1,054	77.0	424	73.1	630	79.9	823	77.0	362	74.5	461	79.1	432	79.4
unspecified	12	0.8	11	0.8	6	1.0	5	0.6	11	1.0	6	1.2	5	0.9	5	0.9
Tick bites[4]																
yes/possible	942	65.2	891	65.1	430	74.1	461	58.4	705	66.0	367	75.5	338	58.0	314	57.7
no	502	34.8	478	34.9	150	25.9	328	41.6	364	34.0	119	24.5	245	42.0	230	42.3
Consumption of raw goat milk[5]																
yes/possible	898	62.2	857	62.6	383	66.0	474	60.1	660	61.7	317	65.2	343	58.8	320	58.8
no	546	37.8	512	37.4	197	34.0	315	39.9	409	38.3	169	34.8	240	41.2	224	41.2
Other flavivirus contact[6]																
yes	374	25.9	349	25.5	160	27.6	189	24.0	288	26.9	135	27.8	153	26.2	141	25.9
no	1,070	74.1	1,020	74.5	420	72.4	600	76.0	781	73.1	351	72.2	430	73.8	403	74.1

Table 1. Questionnaire data. [1]Data set used to calculate the results for questions (a, b, and c) focusing on seroprevalence. [2]Data set used to calculate the results for question (d and e) focusing on antibody decline and seroreversion rates. [3]Data set used to calculate the results for question (f) focusing on incidence of infection. [4]Individuals who have ever been bitten by ticks or possibly been bitten (i.e., did not exclude it) vs. individuals who reported to have never had a tick bite. [5]Individuals who have ever consumed or possibly consumed (i.e., did not exclude) raw goat milk or raw goat milk products vs. those who have never consumed these products. [6]Individuals indicating vaccination against YFV or JEV, or infection with DENV, WNV, JEV, YFV, or ZIKV vs. individuals indicating none of them.

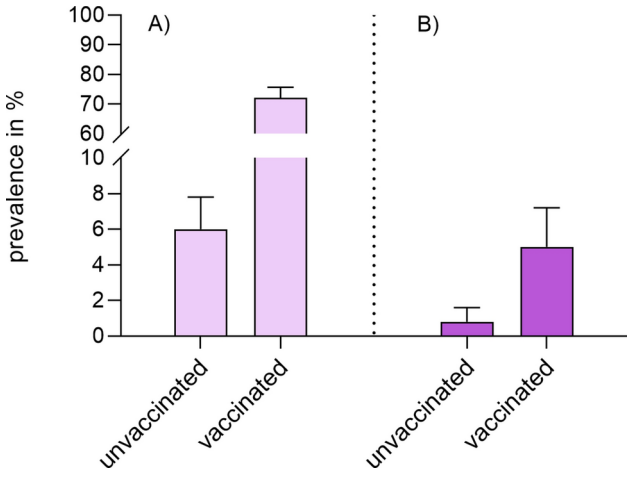

Fig. 3. TBEV antibody prevalence. Prevalence of TBEV E protein-specific antibodies (**A**) and NS1 protein-specific antibodies (**B**) in unvaccinated and vaccinated study participants. Whiskers indicate the upper 95% confidence limits.

Results

Questionnaire data

Out of the 1,527 individuals considered for analysis, 83 were excluded due to inconsistent information about their TBE vaccination status (Fig. 2). The questionnaire responses of the remaining 1,444 HCW are summarized in Table 1.

Prevalence of TBEV E protein-specific IgG antibodies (question a)

At time point 1, 47 out of 789 individuals (6.0%; 95% CI: 4.4–7.8%) who were not vaccinated against TBEV tested positive for TBEV E protein-specific IgG antibodies. Among the 580 vaccinated individuals, 418 (72.1%, 95% CI: 68.2–75.7%) tested positive (Fig. 3A).

Factors affecting the prevalence of TBEV E protein-specific IgG antibodies (question b)

Detailed results of the logistic regression models are shown in Supplementary File 1. None of the investigated risk factors (age, sex, tick bites, place of residence, and consumption of raw goat milk or milk products) were found to be significantly associated with an increase in IgG antibody prevalence in unvaccinated individuals (uncorrected p-values ≥ 0.29).

In vaccinated individuals, higher age was associated with decreased TBEV E protein-specific IgG antibody prevalence (uncorrected $p = 0.046$). The prevalence of antibodies in vaccinated individuals was not significantly affected by sex, exposure to tick bites, place of residence, or consumption of raw goat milk or milk products (p-values ≥ 0.19). Individuals who received a complete basic vaccination (three or more doses) had a significantly higher frequency of positive IgG test results in the fitted logistic regression model compared to those who had received one or two doses or could not recall the number of doses received ($p < 0.0001$, likelihood ratio test). Likewise, ignoring all other factors, the quantitative antibody titers were significantly higher in individuals having received ≥ 3 vaccine doses ($p < 0.0001$, Wilcoxon/Mann–Whitney test).

Prevalence of TBEV NS1 protein-specific IgG antibodies (question c)

For one out of 789 unvaccinated and five out of 580 vaccinated individuals, insufficient sample from time point 1 remained for NS1 IgG antibody testing. Among the 575 vaccinated individuals, 29 (5.0%, 95% CI: 3.4–7.2%) were classified positive. Six out of 788 unvaccinated individuals (0.8%, 95% CI: 0.3–1.6%) tested positive for TBEV NS1-specific IgG antibodies (Fig. 3B).

TBEV E protein-specific IgG antibody decline and seroreversion rates (questions d and e)

Among the 355 vaccinated individuals testing positive at time point 1, 12 (3.4%, 95% CI: 1.8–5.8%) seroreverted during the study period of one year. Among the 33 unvaccinated individuals positive at time point 1, 10 (30.3%, 95% CI: 15.6–48.7%) seroreverted (Fig. 4).

For vaccinated individuals, the median of the ratio of TBEV E protein-specific antibodies at time point 2 vs. time point 1 was 0.92 (95% CI: 0.91–0.95; lower quartile: 0.80, upper quartile: 1.06), corresponding to a reduction rate of 8.0% within one year, respectively. For unvaccinated individuals, the annual reduction rate was not calculated due to the small sample size. Vaccination against YFV or JEV during the study period did not produce a positive result in the TBEV E protein-specific ELISA and therefore did not affect our evaluation of antibody dynamics over time (Supplementary file 2).

Incidence of TBEV-infections in unvaccinated individuals during study period (question f)

Out of the 544 unvaccinated individuals, 4 seroconverted from negative to positive between time point 1 and 2, as defined by TBEV E protein-specific IgG ELISA testing (individuals 1–4). All of them had at least a four-fold titer increase in IgG ELISA testing and a two- to three-fold titer increase in virus-specific neutralization testing and were therefore considered as true seroconversions and (probable) TBEV infections during the study period (Table 2). The probability of an unvaccinated individual to seroconvert during the study period was 0.735% (95%CI: 0.2–1.9%). This translates to an annual incidence of infection of 735/100,000 in the unvaccinated study population. Two individuals seroconverted from borderline to positive (individuals 5 and 6). These individuals had less than a fourfold increase in ELISA titer and no increase in serum neutralization titer and were therefore considered to be artefacts rather than true seroconversions (Table 2).

Discussion

Although TBE is widely recognized as a major public health problem in Europe, surveillance is generally sporadic rather than systematic, and the true burden of disease is potentially underestimated[15]. Our results indicate that undiagnosed infections are widespread and that the true incidence of TBEV infection is significantly higher than the number of reported clinical cases of TBE. In addition, we found that NS1-specific antibodies were six times more common in vaccinated than unvaccinated individuals, suggesting that these antibodies may not be a completely reliable marker for distinguishing the immune response following vaccination from infection, especially in epidemiologic contexts.

We found an overall prevalence of 6% of TBEV E protein-specific IgG antibodies in unvaccinated individuals, indicating that a history of undiagnosed TBEV infection is relatively common in our study population. This percentage is similar to previous studies in Switzerland and Poland, where 5.6% [3] and 5% [16] of blood donors reported no TBE vaccination tested seropositive. Our estimate of the annual incidence of infection (symptomatic or abortive, i.e., asymptomatic or presenting as a mild febrile illness without developing a neurological form) in unvaccinated individuals was 735/100,000, which is approximately 150–250 times higher than the incidence of clinically manifest, reported TBE cases in Switzerland[4]. This is in line with a Swedish study, which found that 96.9% of infections are not diagnosed and therefore not notified[10], and that TBE burden is significantly underestimated. Of the four seroconversions observed within one year in our unvaccinated study population, one individual had confirmed, diagnosed TBE, while the others were undiagnosed. Of the latter, two had an episode of subfebrile illness and one had an episode of mild neurological symptoms. While these symptoms may have been related to other illnesses during the study period, it is also possible that undiagnosed TBEV infections may present with mild symptoms rather than being completely asymptomatic. The discrepancy between ELISA (negative) and serum neutralization test (positive) results at time point 1 for individuals 3 and 4 may be explained

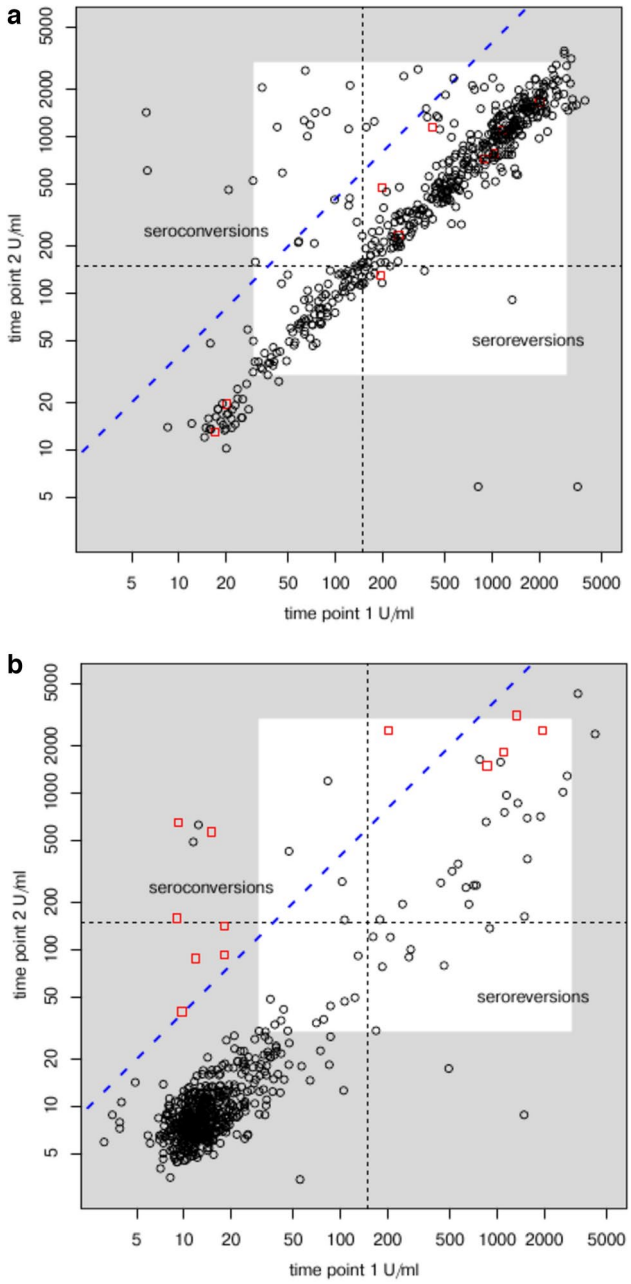

Fig. 4. TBEV E protein-specific antibody titer plots of vaccinated (**A**) and unvaccinated individuals (**B**). The black dashed lines indicate the cut-off for a positive test result (150 U/ml). Seroconversions appear in the upper left section delimited by the black dashed lines, seroreversions in the lower right section. Red squares indicate individuals who reported vaccination during 2020 or 2021. The points to the left above the blue dot and dash line are those with a ≥ fourfold titer increase from time point 1 to time point 2. The quantification limit of the ELISA (30–3000 U/ml) is shown as a white square. The dataset used to create this plot appears as a light grey box with dark border in Fig. 2 (n = 1,128, thereof 533 vaccinated and 595 unvaccinated at time point 1).

by slightly reduced sensitivity of the SERION ELISA classic FSME/TBE Virus IgG test, compared to the SNT[17]. The low SNT titers at time point 1 suggest prior exposure for these individuals, whereas the significant increase in antibody titers at time point 2 (> threefold titer increase in neutralizing antibody titers, > fourfold titer increase in ELISA titers) suggests a booster response as a result of a new virus exposure.

We found an annual decline rate of TBEV E protein-specific IgG antibodies of 8.0% and an annual seroreversion rate of 3.4% in vaccinated individuals. Others have reported an average annual decline rate of 6–7% [18] or 14.6% after a complete series of primary immunization[19]. While several studies found higher decline rates in the early years than in the later years after vaccination[20,21], others found no significant association between time since last vaccine dose and seropositivity[22]. However, longevity of protection probably depends more on the number of previous doses than on the interval to the last dose[20,21,23]. Interestingly, we found the annual seroreversion rate

	Time point 1			Time point 2								
Individual	IgG E protein ELISA[1]	IgG NS1 ELISA[2]	SNT[3]	IgG E protein ELISA[1]	IgG NS1 ELISA[2]	SNT[3]	Age[4]	Sex[4]	Tick bites[4]	Raw milk[4]	Remarks[4]	Classification
1	12.4 (neg)	17.6 (neg)	< 1:20 (neg)	627.5 (pos)	113.2 (pos)	1:320 (pos)	42	female	yes	no	confirmed and notified TBE case	confirmed TBEV infection during study period
2	46.9 (neg)	17.6 (neg)	< 1:20 (neg)	425.6 (pos)	29.2 (neg)	1:80 (pos)	42	female	no	yes	one-week episode of headache, neck pain, and restricted movement of left arm reported during study period, which occurred after working in the vineyards the week before	probable TBEV infection during study period
3	83 (neg)	29.2 (neg)	1:40 (pos)	1197.1 (pos)	25.3 (neg)	1:320 (pos)	31	male	no	no	one episode of subfebrile temperature, nausea, and myalgia reported during study period, COVID PCR negative	probable TBEV infection during study period
4	11.5 (neg)	34.2 (neg)	1:20 (pos)	487.9 (pos)	24.5 (neg)	1:320 (pos)	35	female	no	no	one episode of subfebrile temperature, headache, and tiredness reported during study period, COVID PCR negative	probable TBEV infection during study period
5	106.2 (?)	51.2 (pos)	1:80 (pos)	155.1 (pos)	18.2 (neg)	1:80 (pos)	56	female	yes	yes	one episode of subfebrile temperature upon COVID vaccination	artefact, no TBEV infection during study period
6	102.5 (?)	18.2 (neg)	1:40 (pos)	272.0 (pos)	23.7 (neg)	1:40 (pos)	21	female	no	no	–	artefact, no TBEV infection during study period

Table 2. Information on individuals with a TBEV E protein-specific antibody seroconversion during the study period. [1] SERION ELISA classic FSME/TBE Virus IgG test; results are given in U/ml; neg = negative, < 100 U/ml; pos = positive, > 150 U/ml; ? = borderline, 100–150 U/ml. [2] in-house-developed protocol; antibodies were quantified as percent positivity in reference to a positive control (percent positivity); results are given as percent positivity in reference to a positive control; neg = negative, < 35.4; pos = positive, ≥ 45.8; ? = borderline, 35.4–45.8. [3] in-house developed protocol, SNT = serum neutralization test. The highest serum dilution that inhibited cytopathic effect was considered the end-point titre; serum samples with titres of ≥ 1:20 were considered positive. [4] questionnaire data.

to be almost ten times higher in unvaccinated (30.3%) than in vaccinated (3.4%) individuals. This seems to be in contrast to the described more durable seroresponse in naturally infected compared to vaccinated individuals[24]. However, data on the durability on antibody responses typically come from symptomatic TBE patients. As discussed above, we estimate that the proportion of abortive TBEV infections is high. Thus, our findings may be explained by a less durable humoral immune response in abortive compared to symptomatic TBEV infections. As suggested by others[25], our results indicate that antibodies do not remain detectable for life after infection, but are likely to reflect the past epidemiologic situation. These findings underscore the challenges of accurately assessing the true burden of TBEV infection based on seroprevalence data alone, which is likely to result in an underestimate. Furthermore, it remains to be determined whether protection is lifelong after abortive infections, taking into account both humoral and cellular immune responses.

In vaccinated individuals, we found an overall prevalence of 72.1% of TBEV E protein-specific IgG antibodies. Antibody titers were significantly higher in individuals who had received a complete basic vaccination compared with those who had received one or two doses or could not recall the number of doses received. These findings are consistent with reduced seroconversion rates after receiving only one dose of vaccine, compared with a complete primary immunization series[26,27]. Negative test results despite TBE vaccination may also be due to the decline in antibody titers over time, as discussed above. However, there is increasing evidence that TBE vaccine effectiveness remains high (>90%) for at least 10 years after completion of the primary series[28,29], despite the marked decline in both total IgG and neutralizing antibody titers over time. This indicates that antibody responses do not always clearly correlate with, and may underestimate, protection, suggesting important roles for other immune populations in maintaining long-term protection[6].

In our analysis of risk factors, only age was significantly associated with the prevalence of TBEV E protein-specific IgG antibodies in vaccinated individuals, whereas no significant association was found between seroprevalence and sex, tick bites, place of residence, and consumption of raw goat milk or mild products was found in either vaccinated or unvaccinated individuals. Older individuals were significantly more likely to be seronegative, consistent with the general decline of immune functions[30] that is manifested by lower antibody seroconversion rates, reduced titers, and reduced long-term seropositivity in older individuals[6]. Although clinical TBE is reported more frequently in males than in females[31], we did not identify sex as a risk factor for seropositivity (in either vaccinated or unvaccinated individuals). This discrepancy could be due to the selected population in our study, which consisted of predominantly female HCW, resulting in a relatively low power for detecting a sex effect or mirroring the fact that classical male professions at high TBE risk such as farmers, forest workers, or hunters were not included in our study. The absence of any effect of goat's milk consumption is not unexpected, as this is a rare route of infection[2]. Similarly, although tick bites are a well-established epidemiologic factor important for virus transmission, self-reported tick bite recognition was not significantly associated with increased TBEV seroprevalence. This could partly be due to the high proportion of unrecognized tick bites, especially among individuals who do not actively check for ticks after outdoor exposure. This limitation highlights the challenge of using self-reported data to assess tick-related risks. TBEV prevalence in ticks in Switzerland varies widely depending on the location, typically being less than 1% in questing ticks, but reaching up to 14.3% in some cases[32]. However, no significant differences in TBEV seroprevalence were found between subregions of the study.

Due to our approach in defining the cut-offs for the semi-qualitative evaluation of the NS1 IgG ELISA, where we set the cut-off at +/- 3 SD of the lower 95% of vaccinated individuals, the prevalence found for vaccinated individuals is biased and the results may not be compared to similar studies in other countries such as those from Germany[11] and Sweden[10]. Notably, however, the prevalence in unvaccinated individuals was 0.8%, which is approximately sixfold lower than in vaccinated individuals. Although it is possible that vaccinated individuals have a higher likelihood of natural exposure to TBEV—possibly leading to their decision to be vaccinated—we believe that this alone does not fully explain the observed difference in NS1-specific antibody prevalence between vaccinated and unvaccinated individuals. Our findings are in line with the previous observation, that vaccination against TBEV elicits a detectable NS1 IgG antibody response, and that NS1-specific antibodies may not be a completely reliable marker for distinguishing the immune response after vaccination versus infection[12]. NS1-specific antibody tests require carefully defined cut-offs and results must be interpreted in the context of other test results and epidemiological or clinical information. Furthermore, depending on the geographical area, testing for NS1-specific antibodies may require the use of antigens derived from different TBEV subtypes[33]. However, this is not relevant in our study, as only the European subtype of TBEV is present in Switzerland. Interestingly, of the four individuals who seroconverted for E protein-specific antibodies during the study period, only the one individual with diagnosed TBE also developed positive NS1-specific IgG titers, whereas the three undiagnosed individuals did not. Thus, it remains to be determined whether levels of NS1-specific antibodies are lower in asymptomatic compared to symptomatic infections, as described for JEV[34], or whether they are detectable only for a short time, as described for DENV[35]. Another possibility is that NS1-specific antibodies are elicited only upon primary contact, which may explain the negative test result in the two individuals with pre-existing low SNT titers at time point 1. In any case, our results suggest that seroprevalence estimates based on NS1-specific antibodies may underestimate the frequency of natural TBEV infections. Given the small sample size in our study, further research is needed to confirm and extend these observations.

A major strength of our study is the prospective design, which includes analysis of two different time points, allowing us to observe changes over time. However, there are limitations that should be considered when interpreting our study results. Our study population consists of health care workers, and may therefore not be representative of the general population. Due to age restrictions, children and some of the older population were excluded from this study. Also, false-positive ELISA results due to antibodies against other orthoflaviviruses are possible. In fact, 25.5% of all study participants reported vaccination against or infections with other orthoflaviviruses. While we can exclude an effect on our evaluation of antibody dynamics over time (vaccination

against YFV or JEV in 2020 or 2021 did not induce seroconversion in the TBEV E protein-specific ELISA test), our prevalence estimate may be confounded by other orthoflavivirus contacts. However, this effect is likely to be minimal, as a previous Swiss study found only 0.2–0.3% differences in TBEV seroprevalence estimates related to other orthoflavivirus contacts[3]. The TBEV E protein-specific ELISA test used in this study (SERION ELISA classic FSME/TBE Virus IgG test) has a high specificity at the expense of a slightly reduced sensitivity[17]. Consequently, this test may have sensitivity limitations for the detection of low-titer antibodies, which may result in an overestimation of seroreversion rates. Another limitation of our study is the small sample size for calculating the seroreversion rate in unvaccinated individuals, which needs to be confirmed with a larger sample size. Finally, the follow-up period of one year does not capture long-term antibody dynamics.

Conclusion

This study contributes to our understanding of the burden of TBE in Switzerland. Undiagnosed infections are common, and the incidence of TBEV infection is substantially higher than the number of reported clinical cases of TBE. Seroreversion rates in individuals with abortive infections are high; whether protection after such infections is lifelong and by which immune subsets it is mediated remain to be defined.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Received: 9 November 2024; Accepted: 28 February 2025
Published online: 11 March 2025

References

1. Ruzek, D. et al. Tick-borne encephalitis in Europe and Russia: Review of pathogenesis, clinical features, therapy, and vaccines. *Antiviral Res.* **164**, 23–51 (2019).
2. Bogovic, P. & Strle, F. Tick-borne encephalitis: A review of epidemiology, clinical characteristics, and management. *World J Clin Cases.* **3**(5), 430–441 (2015).
3. Ackermann-Gaumann, R. et al. Prevalence of anti-tick-borne encephalitis virus (TBEV) antibodies in Swiss blood donors in 2014–2015. *Blood Transfus.* **21**(2), 100–109 (2023).
4. BAG. Zahlen zu Infektionskrankheiten Zeckenenzephalitis FSME: Swiss Federal Office of Public Health; [Available from: https://www.bag.admin.ch/bag/de/home/zahlen-und-statistiken/zahlen-zu-infektionskrankheiten.exturl.html/aHR0cHM6Ly9tZWxkZXN5c3RlbWUuYmFnYXBwcy5jaC9pbmZyZX/BvcnRpbmcvZGF0ZW5kZXRhaWxzL2QvZnNtZS5odG1sP3dlYmdy/YWI9aWdub3Jl.html.
5. Lunácková, J., Chmelík, V., Sípová, I., Zampachová, E. & Becvárová, J. Epidemiologic monitoring of tick-borne encephalitis in Rimov in Southern Bohemia. *Epidemiol. Mikrobiol. Imunol.* **52**(2), 51–58 (2003).
6. Ackermann-Gaumann, R., Lang, P. & Zens, K. D. Defining the "Correlate(s) of Protection" to tick-borne encephalitis vaccination and infection - key points and outstanding questions. *Front. Immunol.* **15**, 1352720 (2024).
7. Federal Office for Public Health Switzerland. Cantonal vaccination monitoring. Available online: https://www.bag.admin.ch/bag/de/home/gesund-leben/gesundheitsfoerderung-und-praevention/impfungen-prophylaxe/informationen-fachleute-gesundheitspersonal/durchimpfung.html. Accessed on 06.02.2024. 2022.
8. Baroutsou, V., Zens, K. D., Sinniger, P., Fehr, J. & Lang, P. Analysis of Tick-borne Encephalitis vaccination coverage and compliance in adults in Switzerland, 2018. *Vaccine* **38**(49), 7825–7833 (2020).
9. Salat, J. et al. Tick-borne encephalitis virus vaccines contain non-structural protein 1 antigen and may elicit NS1-specific antibody responses in vaccinated individuals. *Vaccines* **8**(1), 81 (2020).
10. Albinsson, B. et al. Seroprevalence of tick-borne encephalitis virus and vaccination coverage of tick-borne encephalitis, Sweden, 2018 to 2019. *Eurosurveillance* **29**(2), 2300221 (2024).
11. Euringer, K. et al. Tick-borne encephalitis virus IgG antibody surveillance: vaccination- and infection-induced seroprevalences, south-western Germany, 2021. *Eurosurveillance* https://doi.org/10.2807/1560-7917.ES.2023.28.12.2200408 (2023).
12. Ackermann-Gaumann, R. et al. Vaccination against tick-borne encephalitis elicits a detectable NS1 IgG antibody response. *J Virol Methods.* **322**, 114831 (2023).
13. Kohler, P. et al. Impact of baseline SARS-CoV-2 antibody status on syndromic surveillance and the risk of subsequent COVID-19-a prospective multicenter cohort study. *BMC Med.* **19**(1), 270 (2021).
14. Zimna, M., Brzuska, G., Salat, J., Ruzek, D. & Krol, E. Influence of adjuvant type and route of administration on the immunogenicity of Leishmania-derived tick-borne encephalitis virus-like particles - A recombinant vaccine candidate. *Antiviral Res.* **228**, 105941 (2024).
15. Erber W, Schmitt HJ, Jankovic TV. TBE-epidemiology by country—an overview. Chapter 12a. In: Dobler G, Erber W, Bröker M, Schmitt HJ, editors. The TBE Book. Singapore: Global Health Press; 2023.
16. Bojkiewicz, E. et al. The prevalence of asymptomatic infections with tick-borne encephalitis virus and attitude towards tick-borne encephalitis vaccine in the endemic area of northeastern Poland. *Vaccines* **10**(8), 1294. https://doi.org/10.3390/vaccines10081294 (2022).
17. Ackermann-Gaumann, R., Tritten, M. L., Hassan, M. & Lienhard, R. Comparison of three commercial IgG and IgM ELISA kits for the detection of tick-borne encephalitis virus antibodies. *Ticks Tick Borne Dis.* **9**(4), 956–962 (2018).
18. Rendi-Wagner, P. et al. Persistence of protective immunity following vaccination against tick-borne encephalitis–longer than expected?. *Vaccine.* **22**(21–22), 2743–2749 (2004).
19. Rendi-Wagner, P. et al. Immunogenicity and safety of a booster vaccination against tick-borne encephalitis more than 3 years following the last immunisation. *Vaccine* **23**(4), 427–434 (2004).
20. Plentz, A., Jilg, W., Schwarz, T. F., Kuhr, H. B. & Zent, O. Long-term persistence of tick-borne encephalitis antibodies in adults 5 years after booster vaccination with Encepur Adults. *Vaccine* **27**(6), 853–856 (2009).
21. Zent, O. et al. TBE booster immunization according to the rapid immunization schedule: are 3-year booster intervals really necessary?. *Vaccine* **23**(3), 312–315 (2004).
22. Dorko, E. et al. Effectiveness of primary vaccination against tick-borne encephalitis in employees of the armed forces. *Cent Eur J Public Health* **26**(Suppl), S42–S46 (2018).
23. Askling, H. H., Vene, S., Rombo, L. & Lindquist, L. Immunogenicity of delayed TBE-vaccine booster. *Vaccine* **30**(3), 499–502 (2012).

24. Baldovin, T. et al. Persistence of immunity to tick-borne encephalitis after vaccination and natural infection. *J. Med. Virol.* **84**(8), 1274–1278 (2012).
25. Kriz, B. et al. Results of the screening of tick-borne encephalitis virus antibodies in human sera from eight districts collected two decades apart. *Vector Borne Zoonotic Dis.* **15**(8), 489–493 (2015).
26. Galgani, I. et al. Systematic literature review comparing rapid 3-dose administration of the GSK tick-borne encephalitis vaccine with other primary immunization schedules. *Exp. Rev. Vaccines* **16**(9), 919–932 (2017).
27. Loew-Baselli, A. et al. Prevention of tick-borne encephalitis by FSME-IMMUN vaccines: review of a clinical development programme. *Vaccine* **29**(43), 7307–7319 (2011).
28. Steffen, R., Erber, W. & Schmitt, H. J. Can the booster interval for the tick-borne encephalitis (TBE) vaccine "FSME-IMMUN" be prolonged? - A systematic review. *Ticks Tick Borne Dis.* **12**(5), 101779 (2021).
29. Zens, K. D. et al. Retrospective, matched case-control analysis of tickborne encephalitis vaccine effectiveness by booster interval, Switzerland 2006–2020. *BMJ Open.* **12**(4), e061228 (2022).
30. Weiskopf, D., Weinberger, B. & Grubeck-Loebenstein, B. The aging of the immune system. *Transpl Int.* **22**(11), 1041–1050 (2009).
31. Switzerland FOfPH. Vaccination protects against TBE: reporting data Switzerland, 2002 - 2015. BAG Bulletin. 2016;41:622–6.
32. Zens KD. TBE in Switzerland and Liechtenstein. Chapter 13. . In: Dobler G, Erber W, Bröcker M, Chitimia-Dobler L, Schmitt HJ, editors. The TBE Book. 7 ed. Singapore: Global Health Press; 2024.
33. Freimane Z, Dobler G, Chitimia-Dobler L, Karelis G, Girl P, Kuzmane S, et al. Development and validation of a novel enzyme-linked immunosorbent assay for the differentiation of tick-borne encephalitis infections caused by different virus subtypes. Infection. 2024.
34. Konishi, E., Kitai, Y. & Kondo, T. Utilization of complement-dependent cytotoxicity to measure low levels of antibodies: application to nonstructural protein 1 in a model of Japanese encephalitis virus. *Clin. Vaccine Immunol.* **15**(1), 88–94 (2008).
35. Chao, D. Y., Galula, J. U., Shen, W. F., Davis, B. S. & Chang, G. J. Nonstructural protein 1-specific immunoglobulin M and G antibody capture enzyme-linked immunosorbent assays in diagnosis of flaviviral infections in humans. *J. Clin. Microbiol.* **53**(2), 557–566 (2015).

Acknowledgements

The authors thank the participants of the SURPRISE study and the members of the study group (in alphabetical order): Ulrike Besold, MD (Geriatric Clinic St. Gallen); Angela Brucher, MD (Psychiatry Services South, St. Gallen); Andrée Friedl, MD (Cantonal Hospital Baden); Stephan Goppel, MD (Psychiatry Services North, St. Gallen); Fabian Grässli, MSc (Cantonal Hospital St. Gallen); Christian R. Kahlert, MD (Children's Hospital of Eastern Switzerland, St. Gallen); Joelle Keller (Hirslanden Clinic Zurich); Simone Kessler (Cantonal Hospital St. Gallen); Philipp Kohler, MD, MSc (Cantonal Hospital St. Gallen); Stefan P. Kuster, MD, MSc (Cantonal Hospital St. Gallen); Eva Lemmenmeier, MD (Clienia Littenheid); Dorette Meier Kleeb, MD (Cantonal Hospital Baden); Elisabeth Möller (Clienia Littenheid); J. Carsten Möller, MD (Clinic Zihlschlacht); Maja F. Müller (Hirslanden Clinic Zurich); Vaxhid Musa (Cantonal Hospital St. Gallen); Manuela Ortner (Rheintal Werdenberg Sarganserland Hospital Group, Grabs); Philip Rieder, PhD (Hirslanden Clinic Zurich); Lorenz Risch, MD, PhD (Laboratory Risch Buchs); Markus Ruetti, MD (Fuerstenland Toggenburg Hospital Group Wil); Matthias Schlegel, MD (Cantonal Hospital St. Gallen); Hans-Ruedi Schmid, PhD (Cantonal Hospital Baden); Reto Stocker, MD (Hirslanden Clinic Zurich); Pietro Vernazza, MD (Cantonal Hospital St. Gallen); Matthias von Kietzell, MD (Clinic Stephanshorn St. Gallen); Danielle Vuichard-Gysin, MD, MSc (Thurgau Hospital Group Muensterlingen); and Benedikt Wiggli, MD (Cantonal Hospital Baden).

Author contributions

AB: method establishment, realization of laboratory experiments, data collection, reviewing and editing PK: conceptualization and design, data collection, reviewing and editing TD: data collection, figure generation, reviewing FG: data collection MV: data analysis, reviewing and editing JS: data collection, data analysis, reviewing and editing DR: data analysis, reviewing and editing AF: data collection, reviewing and editing DV-G: data collection, reviewing and editing AC: method establishment, data analysis, reviewing and editing RL: method establishment, data analysis, writing of original draft, reviewing and editing RA-G: conceptualization and design, method establishment, realization of laboratory experiments, data collection, data analysis, writing of original draft, reviewing and editing.

Funding

This work was supported by the Swiss National Sciences Foundation (grant number PZ00P3_179919 to PK). JS was supported by grant project no. NU21-05–00143 from the Ministry of Health of the Czech Republic. The funding organizations had no role in the study design, data collection, data analysis, interpretation of data, writing of the manuscript, or decision to submit the manuscript for publication.

Declarations

Competing interests

RA-G reports honoraria for lectures and/or research grants from Pfizer and Bavarian Nordic, which do, however, not relate to the present work. RL reports honoraria for presentations and discussions on the impact and prevention of different tick-borne diseases and the epidemiology of Lyme disease, which do also not relate to the present work. The other authors report no conflicting interests.

Ethical approval

The study was approved by the ethics committee of Eastern Switzerland (#2020–00502). All study data were pseudonymized and REDCap was used for data collection. REDCap is a secure and established web application for clinical studies, compliant with the International Council on Harmonization of Good Clinical Practice (ICH-GCP).

Consent for publication
Not applicable.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-92560-1.

Correspondence and requests for materials should be addressed to R.A.-G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.