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Vaccination against tick-borne encephalitis elicits a detectable NS1 IgG antibody response

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ABSTRACT

Vaccine-induced protection against tick-borne encephalitis virus (TBEV) is mediated by antibodies to the viral particle/envelope protein. The detection of non-structural protein 1 (NS1) specific antibodies has been suggested as a marker indicative of natural infections. However, recent work has shown that TBEV vaccines contain traces of NS1, and immunization of mice induced low amounts of NS1-specific antibodies. In this study, we investigated if vaccination induces TBEV NS1-specific antibodies in humans. Healthy army members (n = 898) were asked to fill in a questionnaire relating to flavivirus vaccination or infection, and blood samples were collected. In addition, samples of 71 suspected acute TBE cases were included. All samples were screened for the presence of TBEV NS1-specific IgG antibodies using an in-house developed ELISA. Antibodies were quantified as percent positivity in reference to a positive control. For qualitative evaluation, cut-off for positivity was defined based on the mean OD of the lower 95% of the vaccinated individuals + 3 SD. We found significantly higher NS1-specific IgG antibody titers (i.e., quantitative evaluation) in individuals having received 2, 3, or 4 or more vaccine doses than in non-vaccinated individuals. Similarly, the percentage of individuals with a positive test result (i.e., qualitative evaluation) was higher in individuals vaccinated against tick-borne encephalitis than in unvaccinated study participants. Although NS1-specific IgG titers remained at a relatively low level when compared to TBE patients, a clear distinction was not always possible. Establishing a clear cut-off point in detection systems is critical for NS1-specific antibodies to serve as a marker for distinguishing the immune response after vaccination and infection.

1. Introduction

Despite the availability of effective vaccines, tick-borne encephalitis (TBE) caused by the tick-borne encephalitis virus (TBEV; *Orthoflavivirus encephalitidis*, genus *Orthoflavivirus*, family *Flaviviridae*), remains a major public health problem in much of Europe and Asia (Ruzek et al., 2019). In Europe, TBE is endemic in at least 27 countries, and in some of them, incidence has increased significantly in recent decades (Ruzek et al., 2019). In addition, TBEV is spreading to new areas and forming new endemic foci. For example, TBEV has been newly detected in the United Kingdom or in North Africa (Fares et al., 2021; Mansbridge et al., 2022).

Similar to other orthoflaviviruses, TBEV forms spherical particles approximately 50 nm in diameter with a genome consisting of single-stranded positive sense RNA (Füzik et al., 2018; Pulkkinen et al., 2022; Pulkkinen et al., 2018). The genome encodes a large polyprotein further divided into three structural proteins (envelope protein E, (pre) membrane protein (pr)M, and capsid protein C) and seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) (Lindenbach and Rice, 2003). Of these, the E protein is the major antigenic determinant of viral particles and target for neutralizing antibodies. The detection of E-specific IgM and IgG antibodies represents the gold standard in TBE diagnostics (Ergunay et al., 2016; Taba et al.,

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2017). Also, vaccination against TBE primarily induces antibodies directed against the E protein. In addition to E-specific antibodies, antibodies against NS1 are also formed as a response to TBEV infection (Albinsson et al., 2019; Albinsson et al., 2018). NS1 is a viral non-structural glycoprotein that has essential functions during viral replication. In a TBEV-infected host cell, it is associated with the plasma membrane but is also secreted from the cells to modulate the host immune response (Lindenbach and Rice, 2003). In the infected host, NS1 circulates in the blood during the acute phase of the disease, which in turn triggers an NS1-specific antibody response (Lindenbach and Rice, 2003). Because NS1-specific antibodies are thought to be associated with infection but not vaccination, their measurement is considered an excellent tool for distinguishing between post-infectious and post-vaccine immunity or for identifying breakthroughs after vaccination (Albinsson et al., 2019; Albinsson et al., 2018; Girl et al., 2020; Stiasny et al., 2021). However, our recent work has shown that the two European TBEV vaccines contain, in addition to purified TBEV particles, traces of NS1 that are either associated with the particles or co-purified during the vaccine manufacturing process. Immunization of mice with these vaccines confirmed that one of these vaccines, FSME-Immun, induced not only whole-virus-specific antibodies but also NS1-specific antibodies, although the NS1-specific response was weaker and a higher number of doses was required to induce detectable levels of NS1-specific IgG antibodies compared to antibodies directed against the whole particle (Salat et al., 2020; 2022). Whether vaccination leads to induction of NS1-specific antibodies in humans remained unknown.

Here, we analyzed NS1-specific IgG in sera from volunteers immunized against TBE with different numbers of TBE vaccine doses, and compared the titers to those in suspected acute TBE cases. Indeed, the level of NS1-specific antibodies increased with the number of vaccine doses, but remained at a relatively low level when compared to patients. This demonstrates the importance of establishing a clear cut-off point in detection systems to allow a distinction between NS1-specific antibody levels after vaccination and infection.

2. Materials and methods

2.1. Study population

Sampling for the study took place from August 2017 to January 2018 at the Medical Center of the Training Command - Military Academy in Vyškov, Czech Republic, as part of the medical examination of military recruits who came there from all regions of the Czech Republic to undergo basic military training. A total of 898 samples were collected from healthy, 19–51 years of age military recruits (83% male, 17% female). Study participation was voluntary. Sample collection was part of the entry medical procedure, so ethics approval was not required in this case. In addition, we included data from 71 suspected acute TBE cases. These samples had been submitted to the diagnostic laboratory ADMED Microbiologie, Switzerland, and had been tested positive for TBEV whole virus specific IgM and IgG antibodies during routine ELISA diagnostics. For these patients, data on NS1-specific antibody titers had been obtained during a quality enhancement project at our institution. According to national law, the performance and publishing results of such a project can be done without asking the permission of the competent research ethics committee.

2.2. Sample collection

Venous blood (8.5 ml) was collected in a serum separation tube. Serum was prepared by centrifugation (10 min at 1300 g [2900 rpm]) within 5 h after sample collection. Serum samples were stored at − 25 °C until analysis. During the test phase, they were thawed and temporarily stored at 4 °C.

2.3. Questionnaire

Army members consenting to study participation were asked to fill in a questionnaire including the following questions: age; sex; past TBEV infection (yes/no; if yes, year of infection); vaccination against TBE (yes/no/unknown; if yes, number of administered vaccine doses [1, 2, 3, or 4 and more]); past YFV or DENV infection or YFV vaccination (yes/no/unknown; if yes, year of infection/vaccination).

2.4. TBEV NS1 IgG ELISA

The TBEV NS1 IgG ELISA protocol was adapted from (Girl et al., 2020). Polystyrene plates (96-well) (Nunc Immuno Medisorp, Thermo Fisher Scientific, Waltham, Massachusetts, USA) were coated overnight at 4 °C with recombinant TBEV NS1 antigen (The Native Antigen Company, TBEV-NS1–100) at a concentration of 0.25 µg/ml in phosphate-buffered saline PBS (pH 7.4). After three wash cycles with PBS, the wells were blocked with the dilution buffer made from PBS with 5% bovine serum albumin (BSA, SigmaAldrich, St-Louis, Missouri, USA) and 0.5% gelatine (BD, Franklin Lakes, New Jersey, USA) for 2 h at room temperature, followed by one wash cycle with PBS and 2 h drying at room temperature. The plates were stored at − 20 °C.

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

2.5. Calculation of quantitative and qualitative NS1 test results

NS1-specific IgG antibodies were quantified as percent positivity in reference to a positive control consisting of four pooled serum samples from patients with confirmed acute 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 prevalence of undiagnosed infections of about 5% (Ackermann-Gäumann et al., 2023; Bojkiewicz et al., 2022), 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 (Albinsson et al., 2018; Stiasny et al., 2021). The sensitivity and specificity of the assay were defined based on receiver operating characteristic (ROC). We verified the validity of the qualitative evaluation using a different approach, in which the cut-off was defined based on the maximal Youden's index (Habibzadeh et al., 2016).

2.6. TBEV whole virus IgG ELISA

TBEV-specific whole virus IgG antibodies were quantified with the RIDASCREEN® FSME/TBE IgG kit (R-Biopharm, Darmstadt, Germany) according to the manufacturer's instructions. For OD measurements and test evaluation, an automated ELISA system (DYNEX DS2 – 2-plate fully automated ELISA system – with OD read settings) was used.

2.7. Data analyses

Using questionnaire data, study participants were assigned to one of the following five TBE vaccination status groups: "no vaccination", "vaccinated with 1 dose", "vaccinated with 2 doses", "vaccinated with 3

doses", and "vaccinated with 4 or more doses". Participants indicating past TBE infection (≥7 years), an unknown TBE vaccination status, vaccination against TBE but no information on the number of vaccine doses, vaccination against YFV or infection with YFV or DENV, or an unknown YFV or DENV infection or vaccination status were excluded from further analyses.

Using a Kruskal-Wallis test followed by Dunn's multiple comparison test, we assessed if the quantitative NS1-specific IgG antibody titers significantly differed for individuals having received 0 (non-vaccinated), 1, 2, 3, or 4 or more TBE vaccine doses, or patients with suspected acute TBEV infections, respectively. The percentage of negative, borderline, or positive result for each group was calculated, and 95% confidence intervals were defined with the Wilson Brown test. Using a Chi square test, we assessed whether the six groups differed in their qualitative results (proportion of negative/borderline/positive results). The relationship between the TBEV NS1 IgG ELISA (percent positivity) and the TBEV whole virus IgG ELISA (U/ml) for non-vaccinated and vaccinated individuals was evaluated using Spearman rank correlation analysis. Concordance of qualitative results of the two tests were assessed using Kappa statistics. Cohen's kappa coefficient was calculated using the formula Kappa (κ) = $(P_0 - P_e)/(1 - P_e)$, where P0 is the relative observed agreement, and P_e is the hypothetical probability of random agreement. Kappa values of 0–0.20, 0.21–0.40, 0.41–0.60, 0.61–0.80, and 0.81–1.00 indicate poor, fair, moderate, good, and very good agreement, respectively For all analyses, p values < 0.05 were regarded as statistically significant.

Statistical analyses were performed with GraphPad Prism 9.3.

3. Results

3.1. Questionnaire data

In total, 898 army members voluntarily participated in the study. Participants indicating past (≥7 years) TBE infection (n = 6), an unknown TBE vaccination status (n = 7), vaccination against TBE but no information on the number of vaccine doses (n = 5), vaccination against YFV or infection with YFV or DENV (n = 4), or an unknown YFV or DENV infection or vaccination status (n = 3) were excluded from further analyses, resulting in a sample size of n = 873. From these, 137 (15.7%) were female and 736 (84.3%) were male. Age was not indicated by 22 study participants (2.5%); the mean age of individuals indicating their year of birth in the questionnaire was 23.6 years; two individuals (0.2%) were 50 or more years old. Vaccination against TBEV was indicated by 300 individuals (34.3%). Thereof, 32 indicated vaccination with one dose, 61 with two doses, 158 with three doses, and 49 with four or more doses (Table 1).

3.2. NS1 IgG antibody titers

NS1-specific IgG antibodies were quantified as percent positivity in reference to a positive control (semiquantitative evaluation). Median antibody titers were significantly higher in individuals having received 2 ($p = 0.001$), 3 ($p < 0.0001$), or 4 or more ($p < 0.0001$) vaccine doses than in nonvaccinated individuals, but remained lower than in suspected acute TBE cases (Fig. 1). Qualitative evaluation (negative/borderline/positive) was based on the mean percent positivity of the lower 95% of the vaccinated individuals and adding + 2 SD for a borderline and + 3 SD for a positive result. With this approach, the cut-offs were 33% positivity for a borderline and 40% positivity for a positive result; the assay sensitivity was 83.1% (95% CI: 72.4–90.1%) and assay specificity was 91.2% (95% CI: 89.1–92.9%), as defined by ROC analysis. The percentage of negative, borderline, or positive result for each group is shown in Table 1. Qualitative test results significantly differed ($p < 0.0001$, Chi square test); the percentage of individuals with a positive test result was higher in individuals vaccinated against TBE than in unvaccinated study participants, but lower than in suspected acute TBE cases (Table 1, Fig. 2). Defining the cut-off for positivity with an alternative approach based on a maximal Youden's index essentially presented the same picture. The respective data are shown in Supplementary Material 1. Of note, the proportion of test results interpreted as positive was consistently higher than when defining the cut-off based on the lower 95% of the vaccinated individuals. While assay sensitivity was higher using this approach (97.2%, 95% CI: 90.3–99.5%), specificity was lower (89.8%, 95% CI: 87.6–91.6%).

3.3. Correlation between the TBEV NS1 IgG ELISA and the TBEV whole virus IgG ELISA

Quantitative results of the TBEV NS1 IgG (percent positivity) and the TBEV whole virus IgG ELISA (U/ml) of the data set including vaccinated and nonvaccinated individuals showed a rank correlation coefficient (r) of 0.3812 (95% CI 0.3213–0.4381); correlation was statistically significant (two-tailed $p < 0.0001$) (Fig. 3). Qualitative test results showed a slight agreement, with a Kappa coefficient of 0.089 (Table 2).

4. Discussion

Inactivated and purified TBEV whole virus preparations are used as vaccines against TBE (Amicizia et al., 2013; Ruzek et al., 2019). Two TBEV vaccines are available in the European Union and European Economic Area (EU/EEA), FSME-Immun (Pfizer) and Encepur (Bavarian Nordic) (Amicizia et al., 2013; Ruzek et al., 2019). TBEV vaccines were previously thought to contain only structural components of TBEV particles. On the other hand, natural TBEV infection triggered an antibody response not only to the E protein as the major antigen of the virus particle, but also to NS1, which is exposed on the surface of infected cells and is also secreted into the extracellular space (Albinsson et al., 2019; Albinsson et al., 2018; Girl et al., 2020; Mora-Cárdenas et al., 2020). On this basis, it has been suggested that the detection of NS1-specific antibodies could be an excellent tool to distinguish the immune response after vaccination or natural infection. Indeed, serologic studies showed a robust NS1-specific IgG response in individuals who had recovered from TBE, but anti-NS1 IgG was not detected in any or very few individuals who had been vaccinated against TBE (Albinsson et al., 2019; Albinsson et al., 2018; Girl et al., 2020; Mora-Cárdenas et al., 2020). In addition, no substantial NS1-specific priming and anamnestic NS1 antibody

Table 1
Percentage of negative, borderline, or positive results in NS1-specific IgG testing for nonvaccinated individuals, individuals vaccinated with 1, 2, 3, or 4 or more TBE vaccine doses, or suspected acute TBE cases, respectively.

	total	negative			borderline			positive		
group	n =	n =	%	95% CI	n =	%	95% CI	n =	%	95% CI
nonvaccinated	573	558	97.4	95.7–98.4	10	1.7	0.9–3.2	5	0.9	0.4–2.0
1 dose	32	29	90.6	75.8–96.8	1	3.2	0.2–15.7	2	6.3	1.1–20.2
2 doses	61	53	86.9	76.2–93.2	2	3.3	0.6–11.2	6	9.8	4.6–19.9
3 doses	158	145	91.8	86.4–95.1	7	4.4	2.2–8.8	6	3.8	1.7–8.0
≥ 4 doses	49	41	83.7	71.0–91.5	5	10.2	4.4–21.8	3	6.1	2.1–16.5
infected	71	2	2.8	0.5–9.7	10	14.1	7.8–24.0	59	83.1	72.7–90.1

CI, confidence interval calculated using Wilson Brown test. Qualitative test results significantly differed for the different groups ($p < 0.0001$, Chi square test).

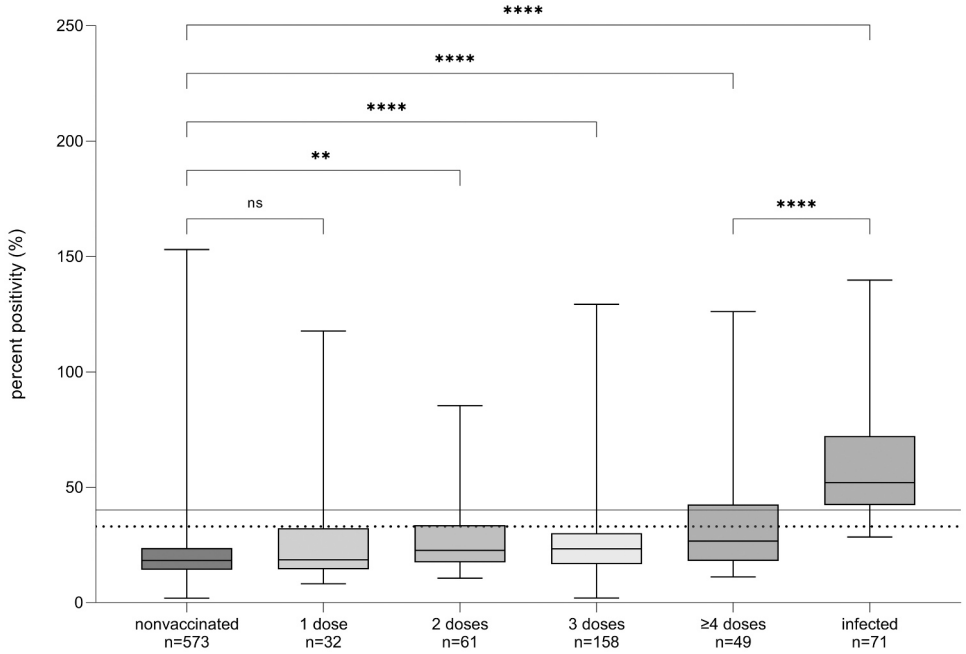

Fig. 1. NS1-specific IgG antibody titers in nonvaccinated individuals, individuals vaccinated with 1, 2, 3, or 4 or more TBE vaccine doses, and suspected acute TBE cases. Results are shown as percent positivity in reference to a high-positive control. The line indicates the median result of each group. Significance was determined with Kruskal-Wallis test followed by Dunn's multiple comparison test. ns, not significant. ** , $p = 0.001$, *** , $p < 0.001$. **** , $p < 0.0001$. Dashed and solid lines indicate borderline and positivity cut-offs, respectively.

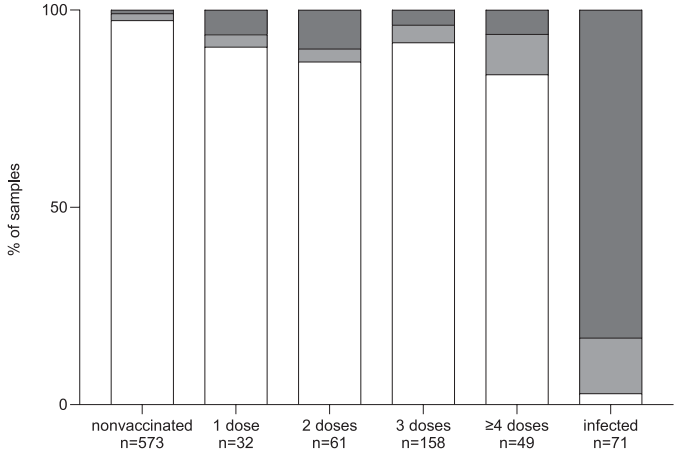

Fig. 2. Qualitative NS1-specific antibody test results (negative/borderline/positive) for nonvaccinated individuals, individuals vaccinated with 1, 2, 3, or 4 or more TBE vaccine doses, and suspected acute TBE cases. Cut-offs were calculated based on the mean OD of the lower 95% of the vaccinated individuals and setting the cut-off for a borderline result at + 2 SD and the cut-off for a positive result at + 3 SD. Qualitative test results significantly differed for the different groups ($p < 0.0001$, Chi square test).

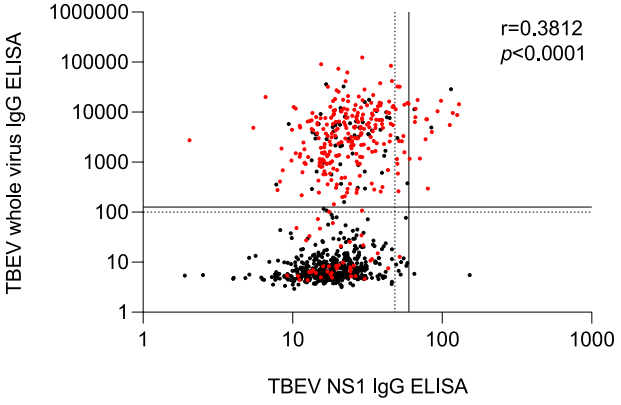

Fig. 3. Correlation between the TBEV NS1 IgG ELISA and the TBEV whole virus IgG ELISA for nonvaccinated and vaccinated individuals. Red dots indicate results of vaccinated, and black dots indicate results of unvaccinated individuals. Axes are in a logarithmic scale. Dashed and solid lines indicate borderline and positivity cut-offs, respectively. r, Spearman's rank correlation coefficient.

response were observed in vaccine breakthroughs (Stiasny et al., 2021). However, our recent findings showed that TBEV vaccines contain not only structural TBEV proteins but also trace amounts of NS1 that are co-purified during the purification process and/or remain associated with the viral particles (Salat et al., 2020; Salat et al., 2022). This raised new questions about whether individuals, who have received multiple doses of the vaccines are still negative for NS1-specific IgG and whether attempts to distinguish between vaccination and infection based solely on detection of NS1 antibodies could be compromised by the potential vaccine-induced NS1 antibody response.

To address this question, we analyzed the levels of NS1-specific IgG in serum samples from 300 vaccinated individuals, who were divided into groups based on the number of vaccine doses received. 573 nonvaccinated individuals were used as negative controls, and results were

Table 2
Relationship of qualitative test results between the TBEV NS1 IgG ELISA and the TBEV whole virus IgG ELISA for nonvaccinated and vaccinated individuals.

		TBEV whole virus IgG ELISA			
		negative	borderline	positive	total
TBEV NS1 IgG ELISA	negative	540	4	282	826
	borderline	9	0	16	25
	positive	2	0	20	22
	total	551	4	318	873

compared to those of 71 suspected acute TBE cases. Overall, NS1 IgG levels were low in the vast majority of vacinee's samples, but individuals who had received two or more doses of the vaccine had significantly higher NS1 IgG levels (quantitative evaluation) than nonvaccinated individuals ($p < 0.001$ for two doses, $p < 0.0001$ for 3 and 4 or more doses). In addition, the percentage of borderline and positive NS1 IgG samples (qualitative evaluation) increased statistically significantly with the number of doses of vaccine received ($p < 0.0001$) (Fig. 1, Fig. 2, Table 1). Furthermore, qualitative test results showed agreement (Kappa coefficient 0.089), and quantitative NS1-specific and whole virus-specific IgG levels were significantly correlated ($r = 0.3812$, $p < 0.0001$) (Fig. 3, Table 2). All of this clearly indicates that TBEV vaccination does indeed elicit an NS1-specific IgG response and that the levels increase with the number of vaccine doses. When compared to suspected acute TBE cases, median NS1-specific antibody titers in vaccinated individuals were low (Fig. 1, Table 1). Nevertheless, false-positive results are possible, and distinguishing between antibodies acquired through infection and those acquired through vaccination is not unambiguous using NS1-specific IgG testing.

One of the possible limitations of our study is the lack of information on which vaccine was used to immunize the subjects studied. We have previously shown that despite the mass spectrometry-detectable NS1 concentration in the Encepur vaccine, this vaccine failed to induce NS1 IgG responses in mice that received up to 6 doses of the vaccine (Salat et al., 2020; Salat et al., 2022). On the other hand, vaccination with FSME-Immun induced a robust NS1-IgG response in mice (Salat et al., 2020; Salat et al., 2022). On this basis, the FSME-Immun vaccine, but not the Encepur vaccine, could be expected to induce NS1-specific immune responses in humans as well, but this needs to be investigated in future studies.

Our results seem to be in contrast to previous studies in which no substantial NS1 IgG response was observed in vaccinated subjects (Albinsson et al., 2019; Albinsson et al., 2018; Girl et al., 2020; Mora-Cárdenas et al., 2020; Stiasny et al., 2021). However, this is most likely due to differences in the cut-offs for the assays used in the different studies and their sensitivity. Despite the marked increase in NS1-IgG levels with the number of vaccine doses, the levels of these antibodies remained low even in individuals who had received 4 or more doses of the vaccine. The low NS1-specific IgG levels are not surprising, as the amounts of NS1 contained in the vaccines tend to be very low and experiments in mice have shown that multiple doses of the vaccine are required to produce detectable NS1 IgG levels (Salat et al., 2022).

Sera of suspected acute TBE patients in general yielded higher NS1-specific IgG antibody titers as compared to those obtained in vaccinated individuals (Fig. 1, Table 1). Nevertheless, 2.8% (95% CI: 0.5–9.7%) were classified as negative and 14.1% (95% CI: 7.8–24.0%) were classified as borderline. It has previously been described that, while typically well-detectable after infection (Albinsson et al., 2019; Albinsson et al., 2018; Girl et al., 2020; Mora-Cárdenas et al., 2020), NS1-specific antibody titers may be lower than whole virus-specific antibodies (Stiasny et al., 2021) and may become detectable later after onset of symptoms (Mora-Cárdenas et al., 2020; Stiasny et al., 2021). Thus, the negative and borderline results for suspected acute TBE patients in our study may be explained by both technical (cut-off definition, sampling time point) as well as physiological (truly lower antibody titers) factors.

The persistence of TBEV NS1-specific antibodies is not known yet. While some reports suggest IgG persistence for up to 28 years (Girl et al., 2020), data from other flaviviruses imply a shorter period of time during which NS1-specific IgG antibodies remain detectable (approximately 2–4 years) (Konishi and Kitai, 2009; Konishi and Suzuki, 2002).

We have defined cut-offs for borderline and positive qualitative test results considering the results of vaccinated individuals. Nevertheless, the proportion of positive test results was significantly higher in vaccinated than nonvaccinated individuals ($p < 0.0001$) (Table 1). Defining cut-off based on the maximum Youden's index (Habibzadeh et al., 2016) would have generated higher proportions of positive results (Supplementary Material 1). We consider it appropriate to base cut-off determination on test results from vaccinated individuals, especially as this allows for a slight improvement of test specificity as compared to the approach based on the maximum Youden's index. Assay specificity is of high importance for a diagnostic test that may be used to support diagnosis of vaccination breakthrough infections. Nevertheless, false-positive results are possible and NS1-specific antibodies may not be a completely reliable marker for distinguishing the immune response after vaccination versus infection. Analyzing IgM might additionally improve specificity of NS1-speficic antibody testing. Due to the shorter seroreversion time of IgM, the probability of false-positive results arising from antibody persistence after vaccination is significantly reduced for IgM as compared to IgG.

While quantitative test results of NS1-specific and whole virus-specific IgG antibodies significantly correlated ($r = 0.3812$, $p < 0.0001$), qualitative test results only showed a slight agreement (Kappa coefficient 0.089). These findings are in agreement with another study describing a low correlation of NS1-specific and whole virus IgG antibody titers (Mora-Cárdenas et al., 2020).

We and others have previously shown that the presence of NS1-specific IgG antibodies plays a partially protective role against TBE (Aleshin et al., 2005; Jacobs et al., 1994; Khoretonenko et al., 2003; Kuzmenko et al., 2017; Salat et al., 2020; Timofeev et al., 2004; Volpina et al., 2005), and there is speculation that NS1 may represent a promising immunogen for the next generation of flavivirus vaccines (Carpio and Barrett, 2021). However, it is unlikely that NS1 IgG induced by whole virus TBE vaccines plays a significant role in protection because only small amounts of these antibodies are produced. On the other hand, it has been suggested that NS1 from some flaviviruses might also induce the production of autoantibodies reactive against several self-antigens in brain and muscle (Cavazzoni et al., 2021; Robbiani and Růžek, 2021) or can lead to vascular injury (Sun et al., 2015). Whether this is also true for NS1 of TBEV remains to be investigated. However, given the weak NS1 IgG response observed in TBE vaccinated individuals, the induction of such immunopathological responses seems highly unlikely.

5. Conclusions

Taken together, our results demonstrate that vaccination with inactivated TBEV vaccines induces NS1-specific antibodies, although at low titers. Establishing a clear cut-off point in detection systems is critical for NS1-specific antibodies to serve as a marker for distinguishing the immune response after vaccination versus infection.

CRediT authorship contribution statement

Rahel Ackermann-Gäumann: Data curation, Formal analysis, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Arthur Brêchet:** Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. **Jan Smetana:** Data curation, Resources, Investigation, Supervision, Writing – review & editing. **Jiri Salat:** Formal analysis, Writing – review & editing. **Reto Lienhard:** Methodology, Supervision, Validation, Writing – review & editing. **Antony Croxatto:** Methodology, Supervision, Validation, Writing – review & editing. **Petra Polcarova:** Data curation, Resources, Investigation, Writing – review & editing. **Roman Chlibek:** Resources, Investigation, Supervision, Writing – review & editing. **Daniel Ruzek:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jviromet.2023.114831.

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