Original article

Prevalence of anti-tick-borne encephalitis virus (TBEV) antibodies in Swiss blood donors in 2014-2015

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Background - Disease morbidity of tick-borne encephalitis (TBE) has been increasing over the last decades. Since the 1990s, however, no extensive seroprevalence studies on TBE in humans have been performed in Switzerland. Here we assessed the prevalence of anti-TBE virus (TBEV) antibodies among different groups of the Swiss blood donor population.

Materials and methods - The study was carried out from July 2014 to January 2015. Blood donors participating in the study (n=9,328) were asked to fill in a questionnaire relating to vaccination against or infection with different flaviviruses, and blood samples were collected. All samples were screened for the presence of anti-TBEV IgG antibodies using ELISA testing. Seropositivity rates in different groups of blood donors were compared using Chi square tests with Bonferroni correction.

Results - In 2014 and 2015, 24.6% of healthy Swiss blood donors indicated vaccination against TBE. Among vaccinated blood donors, antibody prevalence was significantly higher in younger (<40y: 85.3%) than older individuals (≥40 to <55y: 80.0%, ≥55y: 76.7%; p=0.005). In non-vaccinated individuals, antibody prevalence was significantly higher in younger (<40y: 10.0%) than older (≥40 to <55y: 4.0%, ≥55y: 3.9%; p<0.005), male (6.8%) than female (3.7%, p<0.0001), and blood donors from endemic (7.0%) than border (6.2%) or non-endemic regions (4.2%, p<0.001). Possible asymptomatic infection, as defined by positive IgG ELISA results in blood donors indicating no vaccination against TBEV, was found in 5.6%.

Discussion - Our data importantly complement the knowledge on TBEV vaccination rates and estimate the frequency of subclinical TBE in Switzerland.

Keywords: *TBEV, seroprevalence, blood donor, asymptomatic, flavivirus.*

INTRODUCTION

Tick-borne encephalitis (TBE) is caused by tick-borne encephalitis virus (TBEV), a member of the genus *flavivirus* of the family *Flaviviridae*. TBEV is mainly transmitted to humans via tick bites. Occasionally, alimentary transmission occurs[1]. Also, a case of transfusion transmitted infection has been reported[2]. The distribution of TBEV correlates with the occurrence of its vector ticks and ranges from Europe to Siberia, Russia and Far-Eastern

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countries. Three subtypes, i.e., European, Siberian, and Far Eastern, have been well-described; in addition, two new subtypes (Himalayan and Baikalian) have been proposed[1].

While TBE is asymptomatic in 70-95% of cases, symptomatic disease may occur as meningeal, encephalitic, poliomyelitic, and myeloradiculitic forms. A large proportion of patients is left with permanent sequel[3-6]. Several effective vaccines are available to prevent TBE, but no curative treatment exists[1].

TBE morbidity has been increasing over the last decades and the disease is continuously spreading to new regions and higher altitudes[1]. This spread is supported by the expansion of the vector tick population promoted by climatic factors[7,8], human social and behavioural changes[9], as well as changes in land use and leisure activities[10].

TBE incidence in European countries was 0.6 and 0.7 *per* 100,000 in 2018 and 2019, respectively[11]. In Switzerland, notification rates reached 4.37 per 100,000 in 2018, 3.03 per 100,000 in 2019 and 5.16 per 100,000 in 2020[12]. Disease incidence is confined to localized endemic areas where TBEV circulates between local tick vector and small mammal reservoir populations[1].

Transmission of different *flaviviruses*, such as West Nile or Dengue virus by blood components is well known[13,14]. For TBEV, transmission by transfusion seems possible especially due to the asymptomatic viraemic phase. Two disease cases in blood component recipients have been described, for which laboratory testing results and risk factor analysis strongly suggested transfusion-transmitted TBE[2].

In Switzerland, first epidemiological studies in the late 1960s and the early 1970s reported anti-TBEV seroprevalence rates of 0.2 to 0.3% in healthy blood donors, and for the first time described endemic areas[15]. In the 1990s, 0.6% of healthy blood donors tested positive for anti-TBEV antibodies, and a seroprevalence of 2.4% was found in hospitalised encephalitis patients[16]. To our knowledge, no further extensive seroprevalence studies on TBEV in humans have been done ever since.

In this study, we assessed the prevalence of anti-TBEV antibodies among the Swiss blood donor population. We compared seropositivity rates when grouping individuals according to age, gender, or TBEV endemicity (endemic, border, or non-endemic region) in their community of blood donation. The proportion of antibodies resulting from vaccination or natural contact with TBEV were calculated based on questionnaire data and IgG testing results. Our data importantly complement the knowledge on TBEV vaccination rates and give an estimate on the frequency of subclinical TBEV infections in Switzerland. Furthermore, our results emphasize the potential risk of blood component contamination by TBEV.

MATERIALS AND METHODS

Study population

The study was carried out from July 2014 to January 2015 in endemic and non-endemic regions in seven blood transfusion services Bern, Vaud, St. Gallen, Luzern, Basel-Land, Basel-Stadt and Ticino, covering 18 cantons of Switzerland (**Table I**). The sampling regions were selected to include endemic regions (i.e., regions with reported disease cases), border regions (i.e., area surrounding endemic regions with a radius of about 5 km), and non-endemic regions (i.e. regions without any reported disease cases). Communities of blood donations were categorized based on TBE cases reported to the Federal Office for Public Health in 2014. A total of 9,328 samples were collected from healthy, 18 to 75 years old male and female blood donors. Study participation was voluntary.

Sample collection

Ethylene-diaminetetraacetic acid (EDTA) anticoagulated blood samples were collected from the pre-donation bag of blood donors consenting to study participation. Plasma was prepared by centrifugation (5 min at 4,730 rpm) within 24 h after sample collection. Plasma samples were stored at –20°C until analysis; during the testing period, they were thawed and temporarily stored at 4°C.

Questionnaire

Blood donors consenting to study participation were asked to fill in a questionnaire including the following questions: age; sex; previous TBEV infection (yes/no); vaccination against TBE (yes/no); previous infection with other known *flaviviruses* (Dengue virus [DENV], West Nile virus [WNV], Japanese Encephalitis virus [JEV], Yellow Fever virus [YFV], yes/no); vaccination against other *flaviviruses* (JEV, YFV, yes/no).

ELISA screening for anti-TBEV IgG antibodies

All 9,328 samples were screened for the presence of anti-TBEV IgG antibodies using the Siemens Enzygnost Anti-TBE/FSME Virus IgG assay (Siemens, Marburg,

Table I - *Anti-TBEV IgG seropositivity rates of blood donors indicating no vaccination against TBEV and no infection with or vaccination against other flaviviruses*

Endemicity	Canton	N. of blood donation sites	Blood donors indicating no TBEV vaccination	TBEV IgG ELISA positive	Prevalence
Endemic	Aargau	4	88	5	5.7%
	Bern	21	863	32	3.1%
	Fribourg	5	120	15	12.5%
	Luzern	11	385	20	5.2%
	Nidwalden	3	140	10	7.1%
	Obwalden	1	31	2	6.5%
	St. Gallen	1	34	1	2.9%
	Solothurn	1	17	1	5.9%
	Uri	1	14	1	7.1%
	Vaud	5	90	4	4.4%
Border region	Appenzell Ausserrhoden	1	32	1	3.1%
	Bern	2	289	11	3.8%
	Basel Land	1	25	3	12.0%
	Basel Stadt	1	616	46	7.5%
	St. Gallen	4	73	5	6.8%
	Thurgau	1	17	3	17.7%
	Vaud	8	682	38	5.6%
Non-endemic	Appenzell Innerhoden	1	39	4	10.3%
	Appenzell Ausserrhoden	2	45	2	4.4%
	Genève	1	1	0	0.0%
	Graubünden	1	20	2	10.0%
	St. Gallen	7	499	34	6.8%
	Ticino	17	82	32	3.8%
	Vaud	24	1,271	39	3.1%

Germany). Testing was performed automatically according to the manufacturer's instruction on the Quadriga BeFree system and Bep III analysers (Siemens). Grey zone (indetermined) results were regarded as positive.

ELISA confirmatory testing

Since EDTA is toxic to cell cultures required for serum neutralization testing, we were not able to perform a serum neutralization test with our samples. We therefore decided to perform testing with two additional ELISAs for a subset of samples from non-vaccinated individuals, including 394 (i.e., all) testing positive and 341 testing negative in the screening assay: the Euroimmun Anti-FSME/TBE Virus ELISA (IgG) assay (Euroimmun, Lübeck, Germany) and the Euroimmun Anti-FSME/TBE Virus ELISA "Vienna" (IgG) assay (Euroimmun). Samples tested positive or grey zone (indetermined) with two out of three assays were regarded as confirmed positive, and samples tested negative with two out of three assays were regarded as confirmed negative.

Data analyses

Using the questionnaire and anti-TBEV IgG ELISA testing data, we assessed the proportion of blood donors infected with or vaccinated against TBEV. The prevalence of anti-TBEV IgG antibodies was calculated for different groups of vaccinated and non-vaccinated individuals. The following questions were addressed: a) what is the overall prevalence of anti-TBEV antibodies in individuals vaccinated or not vaccinated against TBEV; b) does the prevalence in vaccinated or non-vaccinated individuals differ for endemic, border, and non-endemic regions; c) does the prevalence in vaccinated or non-vaccinated individuals differ for different age groups (<40, ≥40

to <55, ≥55); and d) does the prevalence in vaccinated or non-vaccinated individuals differ for male or female blood donors? Contact to or vaccination against other *flaviviruses* may bias estimation of anti-TBEV antibody prevalence due to the pronounced cross-reactivity in ELISA assays. Therefore, for the primary analysis, blood donors indicating infection with DENV, WNV, JEV or YFV or vaccination against JEV or YFV were excluded. However, all analyses were repeated including blood donors with possible contact to other *flaviviruses*; the respective results are shown in *Online Supplementary* **Table SI** and **SII**.

Statistical analyses were performed using R version 4.0.2[17] (The R Foundation, Vienna, Austria). Given the large sample size, questions a) to d) were addressed by chi-square tests of independence in suitable contingency tables. In order to maintain an overall significance level of 5%, a first Bonferroni correction was used to assign a corrected significance level of 1.25% to each of these four questions. Since questions b), c), and d) consider the vaccinated and non-vaccinated population separately, a second Bonferroni correction was used within each of these questions, further reducing the corrected significance level to 0.625% for the corresponding tests. For ease of interpretation, we provide corrected p-values that can be compared to the usual 5% level.

In order to provide a range of plausible prevalence values for each of the groups compared, exact binomial confidence intervals were calculated. For the determination of the confidence level, another Bonferroni correction was used: If α' denotes the corrected significance level of the corresponding chi-square test, confidence intervals were calculated using a confidence level of $1 - \alpha'/k$, where k is the number of groups compared by the test.

Since assessing the influence of region, age, and gender separately might be misleading, we additionally fitted a logistic regression model including the effect of vaccination, as well as, those of region, age, and gender (where the effects of these three variables were allowed to depend on vaccination status). The p-values resulting from the corresponding Wald tests were all very similar to those obtained from the simpler chi-square tests described above, and all effects had the same direction as before (*data not shown*).

Ethics approval and consent to participate

Participants gave written informed consent to the study protocol approved by the cantonal ethics commissions Bern, Northwest Switzerland, St. Gallen, Thurgau, Ticino and Vaud. The lead ethical committee was in Canton Bern: EK BE 080/14; EK NZ 2014-223; EK SG 14/067; EK TI 2822; EK TG 2014/15; EK VD 245/14.

RESULTS

Questionnaire data

In total, 9,328 blood donors voluntarily participated in the study (3,596 [38.6%] female, 5,732 [61.4%] male). While 2,674 (28.7%) were younger than 40 years of age, 3,685 (39.5%) were between 40 and 54, and 2,969 (31.8%) between 55 and 75 years of age. Vaccination against TBEV was indicated by 2,293 (24.6%) study participants; 1,132 (12.1%) indicated vaccination against and eleven (0.1%) infection with other *flaviviruses*. Thirty-nine blood donors (12 female, 27 male) reported previous TBEV infection, whereof 24 also reported vaccination against TBEV (8 female, 16 male); it is not known, however, if infection occurred before or after vaccination. Finally, 3,082 (33.0%) samples were collected in endemic regions (i.e. regions with reported disease cases), 2,583 (27.7%) in border regions (i.e. area surrounding endemic regions with a radius of about 5 km), and 3,663 (39.3%) in non-endemic regions (i.e. regions without any reported disease cases), as categorized by TBE disease cases reported from the respective municipalities. Among all blood donors reporting previous TBEV infection, 14 donated blood in endemic, 14 in border and 11 in non-endemic regions. The participant's questionnaire responses are summarized in **Table II**.

Anti-TBEV IgG antibody prevalence in blood donors with previous TBEV infection

Thirty-two out of 9,328 blood donors (0.34%; 22 male, 10 female) indicated previous TBEV infection in the absence of vaccination against or infection with other *flaviviruses* (0.40% when including blood donors with possible contact to other *flaviviruses*). Thereof, 19 (59.4%) tested positive in the IgG screening ELISA (12 male, seven female).

Anti-TBEV IgG antibody prevalence in vaccinated blood donors

Out of 2,293 blood donors reporting vaccination against TBEV, 325 also reported contact with other *flaviviruses* (308 vaccinated against YFV, 11 vaccinated against JEV, five vaccinated against YFV and JEV, one with previous DENV infection). Among blood donors indicating vaccination

Table II - *Questionnaire data on gender, age, TBEV infection and vaccination status, and other flavivirus infection and vaccination status*

Blood donors	Total	TBEV vaccination						TBEV infection	
		Yes		Unknown		No			
Group	N.	N.	%	N.	%	N.	%	N.	%
All	9,328	2,293[1]	24.6%	138	1.5%	6,897	73.9%	39	0.4%
Female	3,596	802	22.3%	45	1.3%	2,749	76.4%	12	0.1%
Male	5,732	1,491	26.0%	93	1.6%	4,148	72.4%	27	0.3%
Age <40	2,674	794	29.7%	51	1.9%	1,829	68.4%	9	0.1%
Age ≥40 to <55	3,685	915	24.8%	44	1.2%	2,726	74.0%	16	0.2%
Age ≥55	2,969	584	19.7%	43	1.4%	2,342	78.9%	14	0.2%
Endemic region	3,082	1,156	37.5%	26	0.8%	1,900	61.6%	14	0.2%
Border region	2,583	590	22.8%	66	2.6%	1,972	76.3%	14	0.2%
Non-endemic region	3,663	547	14.9%	46	1.3%	3,070	83.8%	11	0.1%
Other *flavivirus* vaccination	1,132	324	28.6%	15	1.3%	793	70.1%	7	0.1%
Other *flavivirus* infection	11[2]	6	54.5%	1	9.0%	4	36.4%	0	0.0%

[1]2,103 (91.7%) indicated which vaccine and/or how many vaccine doses they had received; [2]n=3 Yellow Fever virus, n=5 Dengue virus, n=3 "*flavivirus*" without specification. Percentages are related to the respective groups. Data on TBEV infection are delineated irrespective of TBEV vaccination status.

against TBEV but no infection with or vaccination against other *flaviviruses*, 1,595/1,968 (81.1%) tested positive in the IgG screening ELISA. Antibody prevalence was significantly higher in the younger than the older (p=0.005, Chi square test with Bonferroni correction) and tended to be higher in female than male (p=0.066) blood donors; antibody prevalence in vaccinated blood donors did not significantly differ depending on TBEV endemicity at the site of blood donation (endemic, border, or non-endemic regions; p=0.56) (**Table III**). Results of the analyses including blood donors with possible contact to other *flaviviruses* are shown in *Online Supplementary* **Table SI** (first section "Blood donors vaccinated against TBEV").

Anti-TBEV IgG antibody prevalence in non-vaccinated blood donors

Out of 7,035 blood donors indicating no vaccination against TBEV (6,897) or not answering the question (138), 804 reported vaccination against other *flaviviruses* (779 against YFV, nine against JEV, 16 against YFV and JEV), six reported infection with other *flaviviruses* (three with DENV, one with YFV, two with "*flavivirus*" without specification) and two reported both (one vaccinated against YFV and JEV and infected with DENV, one vaccinated against YFV and infected with "*flavivirus*" without specification). The remaining 6,223 indicated no contact with other *flaviviruses*; thereof, 5.6% (347/6,223)

Table III - *Anti-TBEV IgG antibody prevalence in blood donors indicating vaccination against TBEV but no infection with or vaccination against other flaviviruses*

Blood donors	Blood donors indicating vaccination	TBEV IgG ELISA positive	Prevalence	CI, lower limit	CI, upper limit	p
All	1,968	1,595	81.1%	78.5%	83.4%	
Female	699	589	84.3%	79.8%	88.1%	0.0659
Male	1,269	1,006	79.3%	75.7%	82.5%	
Age <40	682	582	85.3%	80.8%	89.2%	0.0054
Age ≥40 to <55	800	640	80.0%	75.3%	84.2%	
Age ≥55	486	373	76.7%	70.4%	82.4%	
Endemic region	1,031	852	82.6%	78.8%	86.1%	0.5599
Border region	473	383	81.0%	74.9%	86.2%	
Non-endemic region	464	360	77.6%	71.1%	83.2%	

p-values were calculated using Chi square test with Bonferroni correction.

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tested positive for anti-TBEV IgG antibodies. Antibody prevalence was significantly higher in the younger then the older (p<0.0001), significantly higher in male than female (p<0.0001), and significantly higher in blood donors from endemic than border or non-endemic regions (p=<0.001) (**Table IV**). Results of the analyses including blood donors with possible contact to other *flaviviruses* are shown in *Online Supplementary* **Table SI** (second section "Blood donors not vaccinated against TBEV").

Table I summarizes the seropositivity rates among non-vaccinated blood donors according to the sites of their blood donation. For small sample sizes, the calculated prevalence may not reflect the true situation, and statistical significance was therefore not assessed. However, prevalence rates in non-endemic (0.0-10.3%) and border regions (3.1-17.7%) are in a similar range as those in endemic regions (2.9-12.5%). Results of the analyses including blood donors with possible contact to other *flaviviruses* are shown in *Online Supplementary* **Table SII** (section "Non-vaccinated blood donors").

Comparison of anti-TBEV IgG antibody prevalence in vaccinated *vs* non-vaccinated blood donors

Anti-TBEV IgG antibody prevalence was significantly lower in non-vaccinated (347/6,223, 5.6%) than vaccinated (1,595/1,968, 81.1%) blood donors (p<0.0001) (5.9 *vs* 81.3% when not excluding blood donor with possible contact to other *flaviviruses*).

Confirmatory testing

Based on the results of the screening assay, 394 (i.e., all) positive and 341 negative samples were subjected to confirmatory testing using two additional ELISAs (Anti-FSME/TBE Virus ELISA (IgG) assay and Anti-FSME/TBE Virus ELISA "Vienna" (IgG) assay, both from Euroimmun). 377/394 (95.7%) of the positive and 338/341 (99.1%) of the negative screening test results were confirmed when rating samples positive or grey zone (indetermined) in two out of three assays as confirmed positive and samples negative in two out of three assays as confirmed negative.

DISCUSSION

Between 2002 and 2015, the Federal Office for Public Health in Switzerland recorded 1,892 cases of TBE, corresponding to a notification rate of 1.7 cases/100,000 inhabitants each year[18]. In 2018 and 2020, incidence rates of 4.37 and 5.16/100,000 were reported[12]. In this study, we assessed the prevalence of anti-TBEV antibodies among different groups of the Swiss blood donor population and estimated the proportion of antibodies likely resulting from vaccination or natural contact with TBEV.

TBE vaccination schedules comprise three doses for primary immunization and booster vaccinations for maintaining protection; Switzerland applies extended booster intervals of ten years directly after complete primary immunization[19]. Vaccination recommendations are given by the Federal Office for Public Health and adopted at the cantonal level. Since 2006, when recommendations included about 3% of all municipalities, the recommendations have periodically been updated and in 2019 expanded to include all Swiss cantons with

Table IV - *Anti-TBEV IgG antibody prevalence blood donors indicating no vaccination against TBEV and no infection with or vaccination against other flaviviruses*

Blood donors	Blood donors indicating no vaccination	TBEV IgG ELISA positive	Prevalence	CI, lower limit	CI, upper limit	p
All	6,223	347	5.6%	4.8%	6.4%	
Female	2,437	89	3.7%	2.6%	4.9%	<0.0001
Male	3,786	258	6.8%	5.7%	8.1%	
Age <40	1,680	168	10.0%	7.9%	12.4%	<0.0001
Age ≥40 to <55	2,441	97	4.0%	2.9%	5.3%	
Age ≥55	2,102	82	3.9%	2.7%	5.4%	
Endemic region	1,782	127	7.0%	5.4%	9.2%	0.0005
Border region	1,734	107	6.2%	4.5%	8.2%	
Non-endemic region	2,707	113	4.2%	3.1%	5.5%	

p-values were calculated using Chi square test with Bonferroni correction.

the exception of Geneva and Ticino[20]. However, as there is no national immunization registry, the overall level of vaccine coverage in Switzerland is not precisely known. In 2007, 17% of Swiss people had received at least one dose of TBEV vaccine[21]. In 2014, 42% of 16-years old Swiss people had received one dose of TBEV vaccine, with pronounced regional variations[18]. In the present study from 2014-2015, 24.6% of blood donors aged 18-75 reported vaccination with one or more vaccination doses (**Table II**). Three years later, a nationwide coverage of 41.7% for one dose and 32.9% for a primary series of three doses was reported, based on evaluation of vaccination records from randomly selected participants from different age groups[20]. This increase in vaccination coverage suggests some impact of extended vaccination recommendations and cost coverage by compulsory health insurance. In other endemic European countries, vaccination rates are between 0 and 88% (Austria almost 88% for at least one dose and 58% maintain a regular vaccination schedule; Czech Republic 23%, Germany 13-27%; Slovenia 12.4%; Sweden 11%: Estonia 10%; Hungary 5-15%; Poland 0.34%; Slovakia 0.25% in adults and 0.4% in children; reviewed in[22]).

In our study, 19.0% of samples from blood donors reporting previous TBEV vaccination and excluding contact to other *flaviviruses* tested negative in the IgG screening ELISA. TBEV antibody titers induced by vaccination decline over time but persist between 3 to 10 years at least[23,24]. As we did not evaluate any data on the time elapsed since the last vaccination, a proportion of negative ELISA tests might result from waning antibody titers in blood donors having received their TBEV vaccination several years ago. Also, the number of administered doses was not evaluated. As indicated in the package inserts, seroconversion rates as defined by ELISA testing range between 50% after the first and 99% after the third dose of primary immunization. Therefore, a proportion of negative ELISA tests despite indicated vaccination might be the result of an incomplete primary immunization. In confirmatory testing, 95.7% of positive and 99.1% of negative results were confirmed using two additional ELISAs, wherefore the observed discrepancy between indicated vaccination and detectability of IgG antibodies may not be completely attributable to technical aspects of ELISA testing. Recall bias can be a problem in studies involving questionnaire data. We attempted to minimize this bias by asking study participant to report information on the name of the vaccine and the number of vaccine doses they had received. This information cannot usually be provided without referring to the vaccination card. The respective data were provided by 2,103 out of 2,293 (91.7%) of blood donors indicating vaccination against TBEV.

Vaccination against TBEV was indicated by 26.0% of all male and 22.3% of all female blood donors (**Table II**). As discussed in Baroutsou *et al.*[20], vaccinations provided during military service compulsory for young men might enhance vaccine uptake. Also, men more likely practice professions at risk for tick bites such as foresters, which might increase the proportion of vaccinated men. Antibodies were detectable in 84.3% of female but only 79.3% of male blood donors (p=0.066) indicating vaccination against TBEV and excluding contact with other *flaviviruses* (**Table III**). This elevated humoral immunity in females compared to males has previously been described and is phylogenetically well conserved[25]. Indicated vaccination rates declined with age (**Table II**); among those indicating vaccination against TBEV and excluding contact with other *flaviviruses*, antibody prevalence was significantly higher in the younger than in the elderly (p=0.005; **Table III**), which is in agreement with higher age being known to significantly reduce seropersistence of antibodies upon TBEV vaccination[24]. Finally, vaccination was more frequently indicated by blood donors in endemic than in border ornon-endemic regions (**Table II**), but seroconversion rates did not significantly differ depending on TBEV endemicity at the site of blood donation (p=0.56) (**Table III**). Our observations agree with the highly variable vaccination coverage throughout Switzerland described in later years, highlighting the impact of heterogeneous vaccination recommendations and implementations at the cantonal level of Switzerland[20].

Previous TBEV infection in the absence of vaccination against or infection with other *flaviviruses* was reported by 0.34% of all study participants; however, only 59.4% tested positive in the IgG screening ELISA. Anti-TBEV neutralizing antibody titers are much higher among individuals who developed disease than among those who were vaccinated, and they neither show an age-dependent decrease nor do titers significantly decrease with time elapsed since disease[26].

In the 1960s and early 1970s, a seroprevalence of 0.2 to 0.3% was reported among healthy blood donors[15]. In 1994, the anti-TBEV antibody prevalence was 0.6% among blood donors, whereas hospitalised patients showed a seroprevalence of 2.4%[16]. In our study in 2014 and 2015, antibodies against TBEV were detected in 5.6% of blood donors indicating no vaccination against TBEV and no vaccination against or infection with other *flaviviruses*, which is a clear increase over time. In 2018, Baroutsou *et al.* estimated a TBE incidence of 6.83/100,000 among non-vaccinated, and an incidence of 4.37/100,000 among all (i.e., vaccinated and unvaccinated) individuals[20]. In Europe, annual notification rates ranged from 0.41 to 0.65/100,000 during 2012 to 2016[27]. We observed a higher antibody prevalence in non-TBEV-vaccinated blood donors from endemic than from border and non-endemic regions (p<0.001) (**Table IV**). The relatively high seroprevalence of anti-TBEV antibodies in so-called non-endemic regions challenges the small-scale (cantonal level) classification of areas with vaccination recommendations. For instance, vaccination is still not recommended for people living in the canton of Ticino[20], despite an anti-TBEV antibody seroprevalence of 3.8% among blood donors from this canton (**Table I**). Even though areas endemic for TBE are focally distributed, the whole population of Switzerland should be regarded as at risk, due to higher mobility adding to heightened exposure. Anti-TBEV antibodies were more prevalent in younger than elder blood donors indicating no vaccination against TBEV in the absence of vaccination against or infection with other *flaviviruses* (p<0.001) (**Table IV**). This might be due to higher tick exposure rates in the younger population. Finally, anti-TBEV antibody prevalence was higher in non-TBEV-vaccinated male than female blood donors (p<0.0001). Clinical TBE is more frequently reported in males than females[1,18]; based on our data, asymptomatic or subclinical disease might be more frequent in male than female individuals, as well. Physiological differences including crosstalk between sex hormones and immune effectors are supposed to be main drivers of gender differences in infectious disease susceptibility[28]. We thus hypothesize that the higher anti-TBEV antibody seroprevalence in men than women in our study is unlikely attributable to elevated exposure to tick bites in men. Rather, physiological aspects influence both symptomatic and asymptomatic infections.

Whereas 0.4% of blood donors enrolled in this study indicated previous TBE infection, antibodies were detectable in as much as 5.7% of all study participants. This emphasizes the risk of transfusion transmission of TBE from asymptomatic, viraemic blood donors. TBEV has been listed by Stramer and colleagues as an infectious disease agent with actual or potential risk of transfusion transmission[14]. The fundamental risk factor for acquiring any tick-borne infection is contact with a tick vector. Thus, exposure to ticks and related tick bites, has been suggested as a potential risk factor on which the eligibility for blood donations is based[29]. The fact that only 0.4% of blood donors were aware and notified their previous TBE infection, but 5.7% had detectable antibodies against TBEV demonstrates that the compliance of the general blood donation questionnaire is probably not so high. Unfortunately, the current knowledge on the transmissibility of TBEV is generally not known and therefore additional investigations should be performed. An important point to enhance the safety of blood products could be to increase of the TBEV vaccination of blood donors, especially in high endemic regions.

Although specific details concerning transfusion-transmitted TBEV infections are limited, the case of a donor becoming ill after donating blood and the respective two recipients became febrile shortly after transfusion is well documented[2]. A key factor in transmissibility of TBEVs is their relative capacity to survive in stored blood products, which is supported by their intracellular location; specific information for TBEV to our knowledge is not available so far. However, in Switzerland, leukoreduction is performed for all red blood cell concentrates, pathogen reduction is mandatory for platelets since 2011, and about 50% of transfusion plasma is pathogen-reduced.

The present study also highlights the fact that the blood donor population can serve as "sentinel" for the general population or specific cohorts of individuals[30] for epidemiological investigations. In general, blood donors are highly motivated and willing to participate

in similar epidemiological studies and therefore it is quite easy to conduct and manage such surveys.

CONCLUSIONS

In summary, in 2014 and 2015, 24.6% of a healthy Swiss blood donor population indicated vaccination against TBE. Antibody prevalence was significantly higher in vaccinated younger (<40y: 85.3%) than older individuals (≥40 to <55y: 80.0%, ≥55y: 76.7%; p=0.005). In non-vaccinated individuals, the antibody prevalence was significantly higher in younger (<40y: 10.0%) than older individuals (≥40 to <55y: 4.0%, ≥55y: 3.9%; p<0.005), in male (6.8%) than female (3.7%, p<0.0001), and in blood donors from endemic (7.0%) rather than border (6.2%) or non-endemic regions (4.2%, p<0.001), underlining the importance of human physiological and behavioural aspects. A possible asymptomatic infection, as defined by positive IgG ELISA testing in blood donors indicating no vaccination against TBEV, was found in 5.6%, which is a clear increase to respective prevalence rates below 1% in the 1990s. Although seroprevalence was higher in endemic than in non-endemic areas, the whole population of Switzerland should be regarded as at risk for TBEV infection.

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AUTHORSHIP CONTRIBUTION

CN, CE and RA were involved in the development and the planning of the study and developed the questionnaire. All three also contributed important intellectual contents to this manuscript. RA and CN wrote the first draft of the manuscript. MV performed all statistical calculations. PG, CT and SL organized and planned the laboratory work to be performed. MB, AB, SF, JT and TWE were responsible for the organization in the respective blood transfusion services. All Authors edited the manuscript.

The Authors declare no conflicts of interest.

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