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Immunogenicity against Far Eastern and Siberian subtypes of tick-borne encephalitis (TBE) virus elicited by the currently available vaccines based on the European subtype: Systematic review and meta-analysis

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Abbreviations: C, capside; CEE, Central European encephalitis; CI, confidence interval; d, day; E, envelope; ELISA, enzyme-linked immunosorbent assay; FSME, *Frühsommer-Meningoenzephalitis* [German] (tick-borne encephalitis); GMT, geometric mean titer; HI, hemagglutination inhibition; IFA, indirect immunofluorescence; IgG, Immunoglobulin G; IPVE, Institute of Poliomyelitis and Viral Encephalitis; M, membrane; μNT, microneutralization test; NR, not reported; NS, non-structural; NT, neutralization test; prM, pre-membrane; RCT, randomized controlled trial; RNA, ribonucleic acid; RR, risk ratio; RSSE, Russian spring summer encephalitis virus; SCR, seroconversion rate; SD, standard deviation; SMD, standardized mean difference; SPR, seropositivity rate; TBE, tick-borne encephalitis; TBEV, tick-borne encephalitis virus; TBEV-Eu, European subtype of TBEV; TBEV-FE, Far Eastern subtype of TBEV; TBEV-Sib, Siberian subtype of TBEV; VIEU, Vienna unit; we: week; WHO, World Health Organization; y, year.

Tick-borne encephalitis (TBE) virus, which is usually divided into European, Far Eastern and Siberian subtypes, is a serious public health problem in several European and Asian countries. Vaccination is the most effective measure to prevent TBE; cross-subtype protection elicited by the TBE vaccines is biologically plausible since all TBE virus subtypes are closely related. This manuscript systematically explores available data on the cross-subtype immunogenicity elicited by the currently available Western vaccines based on the European subtype. Completed immunization course of 3 doses of both Western vaccines determined very high seroconversion/seropositivity rates against both Far Eastern and Siberian subtypes among previously flavivirus-naïve subjects. All but one study found no statistically significant difference in titers of neutralizing antibodies against strains belonging to homologous and heterologous subtypes. Pooled analysis of randomized controlled trials on head-to-head comparison of immunogenicity of Western and Russian TBE vaccines did not reveal differences in seroconversion rates against Far Eastern isolates in either hemagglutination inhibition (risk ratio = 0.98, p = 0.83) or enzyme-linked immunosorbent (risk ratio = 0.95, p = 0.44) assays after 2 vaccine doses. This suggests that, in regions where a heterogeneous TBE virus population circulates, vaccines based on the European subtype may be used alongside vaccines based on the Far Eastern subtype. Studies on the field effectiveness of TBE vaccines and investigation of vaccination failures, especially in countries where different subtypes co-circulate, will further elucidate TBE vaccination-induced cross-subtype protection.

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Introduction

Tick-borne encephalitis (TBE) is a serious international public health problem, being endemic to a large geographic area, which extends from North-Eastern France and Scandinavia to North-Eastern China and Northern Japan; moreover, international travel to these regions has increased markedly.[1,2] The causative agent of TBE, the TBE virus (TBEV), belongs to the genus *Flavivirus* of the family *Flaviviridae*.[3] The genome of flaviviruses is constituted by single-stranded positive-sense RNA; the single open reading frame encodes 3 structural (C – capside, prM/M – pre-membrane/membrane and E – envelope) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5).[4] Protein E is the main target of TBEV-neutralizing antibodies[5]; proteins prM, NS1 and NS3 have also been shown to be immunogenic.[6]

TBEV is usually divided into 3 subtypes: European (TBEV-Eu), Far Eastern (TBEV-FE) and Siberian (TBEV-Sib); the first subtype is mainly transmitted by *Ixodes ricinus*, while TBEV-FE and TBEV-Sib are chiefly associated with *Ixodes persulcatus*.[1,2,7-9] As has been shown by Ecker et al.,[10] despite the wide geographic distribution of TBEV, the degree of variation in amino acid sequence within subtypes is low and does not exceed 2.2%, while the variation between subtypes is 3.6–5.6%. However, in a more recent publication, Pogodina et al.[11] reported a greater difference in amino acid sequences (up to 9%) and variability of up to 17.3% at the nucleotide level.[11] Moreover, it has been suggested that 2 strains, namely 178–79 and 886-84, isolated in the Irkutsk region in Eastern Siberia, could constitute independent genotypes;[12] subsequent complete genome sequencing has revealed that these strains adjoin TBEV-FE.[13] The existence of more than 3 subtypes was subsequently confirmed by Demina et al.[14], who concluded that subtype 4 is represented by a single strain 178–79, while the fifth subtype includes the strain 886–84 and another 9 isolates forming the so-called "886 group." A detailed analysis of distance and phylogenetic analysis performed by Grard et al.[15] has led to a novel taxonomic proposal of a single species of TBEV which includes 4 distinct types: Louping ill virus (with Spanish, British and Irish subtypes), Western TBEV (corresponding to TBEV-Eu), Turkish sheep encephalitis virus with the Greek goat encephalitis virus subtype, and Eastern TBEV, which comprises TBEV-FE and TBEV-Sib. In the present paper, however, we will adopt the more widely used classification into the 3 main subtypes, namely TBEV-Eu, TBEV-FE and TBEV-Sib.

Active immunization is the most effective means of preventing TBE.[1,2] Four different TBE vaccines are currently on the market: FSME-Immun (Baxter, Austria), Encepur (Novartis vaccines, Germany), EnceVir (the Tomsk branch of the Federal State Unitary Enterprise "*Mikrogen*," Russia) and another Russian vaccine from the Institute of Poliomyelitis and Viral Encephalitis – IPVE vaccine – also called TBE vaccine Moscow.[1,2,7,9] Pediatric formulations of FSME-Immun and Encepur,[1,2] as well the recently licensed pediatric vaccine Kleshch-E-Vak (Tick-E-Vac)[16] produced by the IPVE, are available. A pediatric formulation of EnceVir is under clinical development.[17] Since their introduction, these vaccines have undergone several modifications aimed at improving their immunogenicity, safety and tolerability profiles (e.g., historic versions of FSME-Immun containing thiomersal, an albumin-free formulation known as TicoVac, and a polygeline-stabilized formulation of Encepur); vaccine antigens, however, have not changed. The vaccines are similar, in that they are all based on cell-cultured killed whole TBEV, adjuvanted with Al $(OH)_3$ and produced in accordance with World Health Organization (WHO) Good Manufacturing Practices. On the other hand, these vaccines contain different amounts of antigen and use 4 different strains: Western vaccines are based on TBEV-Eu (Neudörfl strain in FSME-Immun and strain K23 in Encepur), while Russian vaccines are based on TBEV-FE (Sofjin strain in IPVE and Tick-E-Vak vaccines, and strain 205 in EnceVir).[1,2,7,9] Numerous clinical studies have revealed excellent immunogenicity, safety and tolerability profiles of all these vaccines[17-26] and have demonstrated their field effectiveness in regions with a high vaccination coverage.[27,28] A locally used Chinese vaccine based on the Senzhang TBEV-FE strain also exists; this vaccine has successfully passed phase I, II, and III trials,[29] data from which, however, are not available in the international literature.[2,7] Moreover, steady progress in biotechnology has prompted researchers to explore other approaches to obtaining effective TBE vaccines, including RepliVax-TBE candidates,[30] live chimeras,[31] and naked DNA vaccines,[32,33] which are all under development.

The question of whether TBE vaccines based on TBEV-Eu strains can also provide protection against strains belonging to TBEV-FE and TBEV-Sib is of crucial interest for several reasons. First, in several regions, comprising a large area from the northern Baltic to the Urals, *Ixodes ricinus* overlaps with *Ixodes persulcatus*;[34] this leads to the co-circulation of the 3 TBEV subtypes in countries such as the Baltic States[35] and in some regions in the European part of Russia.[36] Second, there is documented evidence of the geographical expansion of non-European subtypes, as has been observed in Finland, where 11 TBEV-Sib strains have been isolated.[37] Third, international travel has substantially increased to TBE endemic areas[2] where a vaccinated person may be exposed to a subtype heterologous to the TBEV vaccine subtype. Fourth, in Russia, in addition to the 2 Russian vaccines, both Western vaccines have been registered (FSME-Immun in 1993 and Encepur in 1998), and the question of their ability to provide cross-subtype protection has arisen in Siberia and the Far East, where non-European TBEV subtypes circulate.[38] And finally, a limited protection of all licensed vaccines against some strains belonging to TBEV-Sib has recently been emphasized.[39]

Given the above-mentioned considerations, the present study had 3 main goals: (1) to gather and explore published data on the feasibility of cross-subtype immunogenicity elicited by the currently available Western vaccines; (2) to compare vaccine immunogenicity toward homologous and heterologous subtypes, and (3) to establish whether TBEV-Eu-based Western vaccines are as effective as TBEV-FE-based Russian vaccines against Far Eastern isolates. To address research question 2, we examined studies on vaccine immunogenicity to isolates belonging to both homologous and heterologous subtypes, while with regard to research question 3, we

analyzed only head-to-head randomized controlled trials (RCTs) which investigated the immunogenicity of at least one Western and at least one Russian vaccine against any strain belonging to TBEV-FE. It should be noted that, regarding research questions 2 and 3, the meta-analytical approach was adopted whenever possible. However, if single studies did not report any comparison between vaccines and/or TBEV subtypes/strains, appropriate statistical tests were applied.

Results

Of the 745 citations (739 and 6 retrieved through automatic and manual searches, respectively), 14 papers[17,40-52] met the inclusion criteria (**Fig. 1**). Of these, 4 were single-center RCTs,[17,41,42,49] 4 were derived from a single cohort study in which cross-subtype immunogenicity was measured at different time-points up to 7 y after the primary vaccination course (Leonova GN, personal communication),[45,47,48,50] and 2 papers described the immunological effectiveness of TBE vaccines during a mass immunization program in Sverdlovsk Oblast (Russia);[46,52] the other studies were observational and serological. The characteristics of the studies included are reported in **Table 1**. Eight papers were in Russian[17,41,42,45,46,49,50,52] and the remaining 6 were in English. Most studies (n = 10) were carried out in Russia,[17,41,42,45-50,52] 2 in Japan,[43,44] and the remaining 2 in the European Union.[40,51] The immunogenicity of the Austrian vaccine was evaluated in 7 studies,[17,41-44,49,51] that of the German vaccine in 5,[40,45,47,50,52] and that of both vaccines in 2.[46,48] In 8 studies, participants received 3 doses,[40,43,45-48,51,52] while a 2-dose regimen was investigated in 5 papers.[17,41,42,44,49] Only one study[50] evaluated the immune response to heterologous subtypes in remote periods after the primary vaccination course (up to 7 years) and after a booster administered 7 y after the third dose. Most studies evaluated a conventional vaccination schedule;[40-48,50,52] the rapid schedule of FSME-Immun was examined in 3 studies.[17,49,51] Pediatric formulations were evaluated in 2 Russian papers.[17,52] Throughout the studies, the age of participants ranged from 15 months[52] to 70 y.[49]

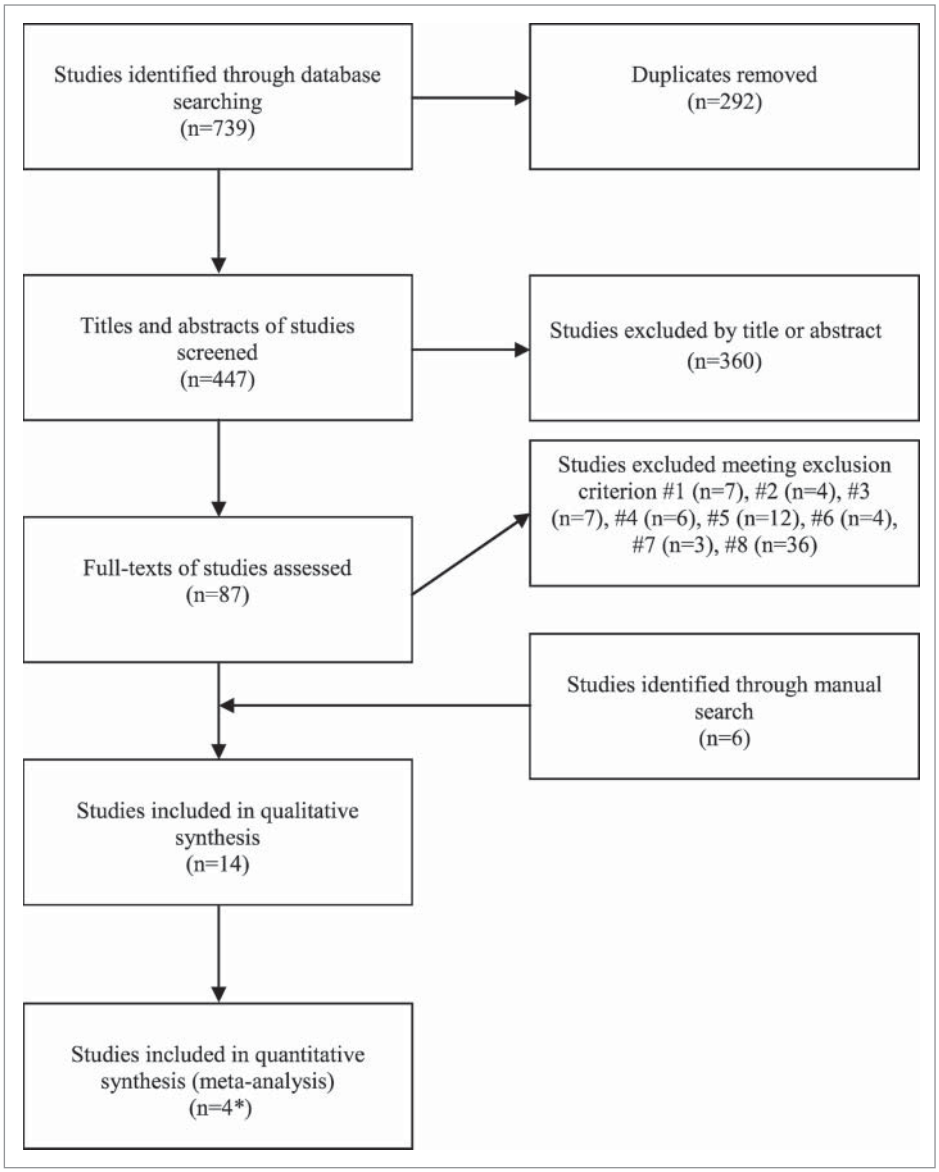

Figure 1. Flowchart of the study selection process. Since 2 studies[41,42] used a commercial hemagglutination inhibition (HI) kit based on strain 139, 1 study[17] used an enzyme-linked immunosorbent assay (ELISA) kit based on strain 205 and another study[49] used both commercial kits, 2 separate meta-analyses on the basis of serological assay were performed.

The immune response against subtypes heterologous to vaccine subtype was most frequently measured by means of a neutralization test (NT) (7 studies)[40,43,44,47,48,50,51] and heterologous ELISA IgG (enzyme-linked immunosorbent assay) (7 studies),[17,46-50,52] followed by hemagglutination inhibition (HI) (4 studies)[41,42,45,49] and indirect immunofluorescence assay (IFA) (1 study).[45] Six papers[41,45,47-50] described vaccination-induced immunogenicity measured by more than one assay. ELISA IgG was performed by means of a commercially available kit (Joint Stock Company Vector-Best, Novosibirsk, Russia), which is based on strain 205 belonging to TBEV-FE. HI was also performed by means of a commercial kit (NPO Virion branch of NPO Mikrogen Tomsk, Russia) based on TBEV-FE strain 139.

Most frequently, immunogenicity was assessed within one month post-vaccination. Overall, 12 heterologous strains were

Table 1. Characteristics of studies included.

Study first author, year, location [ref]	Vaccine used	N of subjects on enrollment	Age	N of doses (schedule)	Serum collection after vaccination	TBEV subtypes and strains tested	Immunological assay used	Immunological outcomes reported	Notes
Klockmann et al., 1991, Germany [40]	Encepur	17	20-50 y	3 (0-1-11 mo)	4 we after 3rd dose	TBEV-FE: Sofjin TBEV-Eu: 10 strains	NT	Minimum, median, maximum titers; GMT	Protection against Louping ill virus was also tested. TBEV-Eu strains tested: K23, IX10, Hypr, Trpisovsky, Petracova, 274/II, Gbelce, Cg1, Dobrostan, Absettarov
Vorobyeva et al., 1996, Russia [41]	1. FSME-Immun; 2. IPVE	1. 50 2. 50	18-23 y	2 (0-2 mo)	1 mo after 2nd dose	TBEV-FE: 139 TBEV-Eu: Neudörfl	HI, ELISA IgG	SCR in HI (≥1:10) and ELISA IgG (≥1:32 VIEU at serum dilution 1:50)	Results of serological tests are reported for only 30 subjects immunized with FSME-Immun and 35 subjects in IPVE group. Immune response against strain 139 was measured by HI commercial kit, while that against strain Neudörfl was measured by commercial ELISA IgG kit.
Pavlova et al., 1999, Russia [42]	1. FSME-Immun 2. IPVE	1. 107 2. 116	7-17 y	2 (0-4 mo)	4 we after 2nd dose	TBEV-FE: 139	HI	SCR (4-fold increase), $\log_{10}$ GMT	The 2nd was administered to 95 subjects in FSME-Immun group and to 106 subjects in IPVE group. HI was performed by using only 147 paired serum samples, 2 of which were seropositive on enrollment and were excluded. Immune response against strain 139 was measured by HI commercial kit.
Chiba et al., 1999, Japan [43]	FSME-Immun	10	24-53 y	3 (0-4we-1y)	4 we after each dose	TBEV-FE: Oshima 5-10, Sofjin TBEV-Eu: Hochosterwitz	NT	SPR (≥1:20), individual titers and GMT	2 subjects were seropositive to Japanese encephalitis virus and therefore excluded. Only 4 subjects received 3 doses.
Hayasaka et al., 2001, Japan [44]	FSME-Immun	11	23-53 y	2 (0-4we)	4 we after 2nd dose	TBEV-FE: KH98-5, VL99-m11 TBEV-Sib: IR99-2f7 TBEV-Eu: Hochosterwitz	NT	SPR (≥1:20), individual titers	-

Pavlenko et al., 2005, Russia [45]*	Encepur	145	17-68 y	3 (0-4we-1y)	1 mo after 1st dose, 1 and 11 mo after 2nd dose and 1 mo after 3rd dose	TBEV-FE: P-73, P-202, P-69, 139	HI, IFA	SPR (unclear threshold titer, probably ≥1:10), GMT	28 subjects had IgG antibodies to TBEV on enrollment and were excluded. Immune response against strains P-73, P-202 and P-69 was measured in both HI and IFA. Immune response against strain 139 was measured by HI commercial kit.
Prochorova et al., 2006, Russia [46]	1. Encepur 2. FSME-Immun 3. IPVE 4. EnceVir	1. 121 2. 125 3. 123 4. 103	1. 7-8 y 2. 12-13 y 3. 12-13 y 4. 18-20 y	3 (conventional schedule)	1 mo after each dose	TBEV-FE: 205	ELISA IgG	SPR (≥1:100)	2, 7, 4, 28 subjects in FSME-Immun, Encepur, IPVE, EnceVir groups, respectively, had IgG antibodies to TBEV on enrollment and were excluded. ELISA IgG was performed by using commercial kit produced by "Vektor-Best" and is based on strain 205.
Leonova et al., 2007, Russia [47]*	Encepur	44	26-68 y	3 (0-4we-1y)	1 y after 2nd dose and 1 mo after 3rd dose	TBEV-FE: P-73, P-69, P-202, 205	NT, ELISA IgG	SPR in NT (≥1:10) and ELISA IgG (according to instructions, probably ≥1:100), GMT	Immune response against strains P-73, P-69 and P-202 was measured by NT, while that against strain 205 was measured by ELISA IgG commercial kit.
Leonova et al., 2009, Russia [48]*	1. Encepur 2. FSME-Immun 3. IPVE 4. EnceVir	1. 6/11 2. 51/32 3. 30/17 4. 17/56	1. 43.0±6.3 y§ 2. 33.4±2.9 y§ 3. 43.4±4.9 y§ 4. 45.6±3.2y§	3 (0-4we-1y)	a. 2-5 mo after 3rd dose b. 2 y after 3rd dose	TBEV-FE: P-73, 205	NT, ELISA IgG, IFA	SCR in NT (≥1:10), ELISA IgG (according to instructions, probably ≥1:100), GMT	Immune response against strain P-73 was measured by NT, while that against strain 205 was measured by ELISA IgG commercial kit. Results of IFA are reported in a pooled form, indistinctly for subjects who received different vaccines (comprising Russian ones), and were not recorded.
Shutova et al., 2009, Russia [49]	1. FSME-Immun; 2. EnceVir	1. 50 2. 50	18-70 y	2 (0-14 d)	14 d after 1st dose and 14, 30 d after 2nd dose	TBEV-FE: 205 and 139	HI, ELISA IgG	SCR (4-fold increase), seroprotection rate (unclear threshold titer, probably ≥1:100), log_{10} GMT	11 subjects in FSME-Immun group and 21 in EnceVir group had anti-TBEV antibodies and were excluded. Immune response against strain 139 was measured by HI commercial kit, while that against strain 205 was measured by commercial ELISA IgG kit ("Vektor-Best"). Seroprotection rate was

(*Continued on next page*)

Table 1. Characteristics of studies included. *(Continued)*

Study first author, year, location [ref]	Vaccine used	N of subjects on enrollment	Age	N of doses (schedule)	Serum collection after vaccination	TBEV subtypes and strains tested	Immunological assay used	Immunological outcomes reported	Notes
									reported only 1 mo post-dose 2.
Pavlenko et al., 2011, Russia [50]*	Encepur	20	NR	3+1 (0-4we-1y-7y)	1 mo, 3 y, 5 y and 7 y after 3rd dose and 1 mo after a booster	TBEV-FE: P-73, 205 TBEV-Sib: Kolarovo-2008	NT, ELISA IgG	SCR in NT (≥1:10), ELISA IgG (according to instructions, probably ≥1:100), GMT	Booster dose was administered to 15 subjects. Immune response against strain P-73 was measured by NT, while that against strain 205 was measured by ELISA IgG commercial kit.
Orlinger et al., 2011, Belgium [51]	FSME-Immun	41	16-65 y	3 (0, 12±2 d, ≈12 mo)	21st d after the 3rd dose	TBEV-FE: Sofjin, Oshima 5-10; TBEV-Sib: Vasilchenko TBEV-Eu: Neudörfl, K23	μNT	SPR (probably ≥1:16), mean μNT titer	Tested strains were all hybrid viruses in which the prM and E sequences of the West Nile virus strain NY99 were exchanged with the corresponding TBEV sequences. Protection against Omsk hemorrhagic fever virus was also tested.
Ankudinova et al., Russia, 2013 [52]	Encepur Children	140	15-40 mo	3 (0, 29±1 d, 300 d)	2 we and 9 mo after the 2nd dose and 3 we after the 3rd dose	TBEV-FE: 205	ELISA IgG	SCR (4-fold increase), seroprotection rate (≥1:100), GMT	4 children had IgG antibodies to TBEV on enrollment; 68 children did not undergo blood collection and/or 2nd and/or 3rd dose, and were excluded.
Feldblium et al., 2013, Russia [17]	1. FSME-Immun Junior; 2. EnceVir children formulation	1. 44 2. 42	3-17 y	2 (0-14 d)	14 d after 1st dose and 1 and 6 mo after 2nd dose	TBEV-FE: 205	ELISA IgG	SCR (4-fold increase), GMTs	ELISA IgG was performed by using commercial kit produced by "Vektor-Best" and is based on strain 205.

Notes: *The same cohort of vaccinees was followed-up and cross-subtype immunogenicity was measured at different time-points by means of different assays (Leonova GN, personal communication). §Combined age of 2 independent cohorts. d, day; ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; IFA, indirect immunofluorescence assay; GMT, geometric mean titer; mo, month; NR, not reported; NT, neutralization test; SCR, seroconversion rate; SPR, seropositivity rate; TBEV, tick-borne encephalitis virus; TBEV-Eu, European subtype of TBEV; TBEV-FE, Far Easter subtype of TBEV; TBEV-Sib, Siberian subtype of TBEV; VIEU, Vienna unit; we, week; y, year.

Table 2. Immune response against heterologous subtypes after 2 doses of Western TBE vaccines.

Assay	Vaccine	Time after 2nd dose, months	Strain	GMT	Sero-outcome % (N/Total)	Ref
NT	FSME-Immun	1	Oshima 5–10*	40.0[§¶]	87.5 (7/8)[¶]	[43]
			Sofjin*	47.6[§¶]	100 (8/8)[¶]	
			KH98–5*	37.6[§]	100 (11/11)	[44]
			VL99-m11*	62.2[§]	100 (11/11)	
			IR99–2f7**	58.4[§]	100 (11/11)	
	Encepur	12	P-73*	19.7	56.8 (25/44)	[47]
			P-202*	15	52.3 (23/44)	
			P-69*	11.3	27.3 (12/44)	
ELISA IgG	FSME-Immun	0.5	205*	309	71.8 (28/39)	[49]
		1		NR	91.6 (109/119)	[46]
				524.8	97.4 (38/39)	[49]
				692.4	72.7 (32/44)	[17]
		6		919.4	100 (44/44)	
	Encepur	0.5		66	72.1 (49/68)/35.3 (24/68)[^]	[52]
		1		NR	39.8 (47/118)	[46]
		9		3	20.6 (14/68)/4.4 (3/68)[^]	[52]
		12		NR	40.9 (18/44)	[47]
HI	FSME-Immun	0.5	139*	15.1	79.5 (31/39)	[49]
		1		79.4	100 (75/75)	[42]
				NR	83.3 (25/30)	[41]
				15.8	84.6 (33/39)	[49]
	Encepur	1	139*	8	62.9[#]	[45]
			P-73*	32	94.3[#]	
			P-202*	37	92.4[#]	
			P-69*	15	71.4[#]	
		11	139*	9	NR[¨]	
			P-73*	12	82.4[#]	
			P-202*	11.3	84.6[#]	
			P-69*	11.3	NR[¨]	
IFA	Encepur	1	P-73*	60	NR[¨]	
			P-202*	52	NR[¨]	
			P-69*	42	NR[¨]	
		11	P-73*	19.7	NR[¨]	
			P-202*	16	NR[¨]	
			P-69*	10.6	NR[¨]	

Notes:
*Far Eastern subtype.
**Siberian subtype.
[§]Calculated values.
[¶]Two subjects had anti-Japanese encephalitis virus antibodies and were excluded; the authors reported GMT of 44 and 43 against Oshima 5–10 and Sofjin strains, respectively.
[^]Subjects with IgG titer ≥1:100/subjects with a 4-fold increase in IgG titers. On enrollment, 117 subjects were TBEV-naïve; however, it is unclear how many serum samples were collected at each time-point.
[¨]only figures are available. ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; IFA, indirect immunofluorescence assay; GMT, geometric mean titer; NR, not reported; NT, neutralization test.

tested; of these, 9 belonged to TBEV-FE (KH98-5, Oshima 5–10, P-69, P-73, P-202, Sofjin, VL99-m11, 139 and 205) and 3 to TBEV-Sib (IR99-2f7, Kolarovo-2008 and Vasilchenko). All but one strain (Oshima 5–10) were isolated in the Asian part of Russia.

Immune response elicited by FSME-immun and Encepur against TBEV-FE and TBEV-Sib strains

Ten studies[17,41-47,49,52] reported data on immunogenicity after 2 doses (**Table 2**). Generally, 2 doses of both vaccines elicited considerable humoral immune responses against heterologous subtypes. However, as demonstrated by both seroconversion/seropositivity rates (SCRs/SPRs) and the reciprocal of geometric mean titers (GMTs), immune response against single strains varied. For instance, Leonova et al.[47] found statistically lower NT SPRs on using strain P-69 than on using P-73 and P-202 Far Eastern isolates.

Most subjects who received a full vaccination course of 3 doses were seropositive against all heterologous strains of both subtypes (**Table 3**). NT was most frequently used; indeed, one paper[48] reported a higher sensitivity of NT than ELISA, although SCRs determined by the 2 assays generally matched. As in the case of post-dose 2, there was some degree of variation in the level of antibody titers against single heterologous strains. This again involved strain P-69; only one third of vaccinees proved seropositive and GMT levels were relatively low (1:28).[47]

Table 3. Immune response against heterologous subtypes after 3 doses of Western TBE vaccines.

Assay	Vaccine	Time after 3rd dose, months	Strain	GMT	Sero-outcome % (N/Total)	Ref
NT	FSME-Immun	0.75	Sofjin*	319[§]	100 (41/41)	[51]
			Oshima 5–10*	409[§]	100 (41/41)	
			Vasilchenko**	224[§]	100 (41/41)	
		1	Oshima 5–10*	80.0[¶^]	100 (3/3)[^]	[43]
			Sofjin*	63.5[¶^]	100 (3/3)[^]	
		2–5	P-73*	112	88.2 (45/51)	[48]
		24	P-73*	34	78.1 (25/32)	
	Encepur	1	Sofjin*	56	100 (17/17)[#]	[40]
			P-73*	128	95.5 (42/44)	[47]
			P-202*	34	97.7 (43/44)	
			P-69*	28	63.6 (28/44)	
			Kolarovo-2008**	208	100 (20/20)	[50]
		2–5	P-73*	91	100 (6/6)	[48]
		24		42	100 (11/11)	
		36		49	100 (19/19)	[50]
		60		45	100 (14/14)	
		84		39	100 (15/15)	
		36	Kolarovo-2008**	140	100 (19/19)	
		60		140	100 (14/14)	
		84		112	100 (15/15)	
ELISA IgG	FSME-Immun	1	205*	NR	96.6 (115/119)	[46]
		2–5		NR	86.3 (44/51)	[48]
		24		NR	75.0 (24/32)	
	Encepur	0.75	205*	592	92.6 (63/68)/79.4 (54/68)[¨]	[52]
		1		NR	94.9 (112/118)	[46]
				NR	93.2 (41/44)	[47]
		2–5		NR	83.3 (5/6)	[48]
		24		NR	100 (11/11)	
		36		1940	100 (19/19)	[50]
		60		1380	100 (14/14)	
		84		1120	93.3 (14/15)	
HI	Encepur	1	139*	39	98.8[¶¶]	[45]
			P-73*	45	100[¶¶]	
			P-202*	39	100[¶¶]	
			P-69*	15	100[¶¶]	
IFA	Encepur	1	P-73*	49	100[¶¶]	
			P-202*	45	98.8[¶¶]	
			P-69*	37	98.8[¶¶]	

Notes:
*Far Eastern subtype.
**Siberian subtype.
[§]Mean microneutralization titers are reported.
[¶]Calculated values.
[^]One subject had anti-JEV antibodies and was excluded.
[#]No SCR is reported; however, minimum NT titer against Sofjin strain was 21.
[¨]Subjects with IgG titer ≥1:100/subjects with a 4-fold increase in IgG titers.
[¶¶]On enrollment, 117 subjects were TBEV-naïve; however, it is unclear how many serum samples were collected at each time-point. ELISA, enzyme-linked immunosorbent assay; HI, hemagglutination inhibition; IFA, indirect immunofluorescence assay; GMT, geometric mean titer; NR, not reported; NT, neutralization test.

The rapid immunization schedule was found to be highly immunogenic against all subtypes.[17,49,51] On microneutralization assay (μNT), all 41 subjects vaccinated with 3 doses of FSME-Immun were seropositive against 2 TBEV-FE strains (Sofjin and Oshima 5–10) and the Vasilchenko strain belonging to TBEV-Sib. In comparison with the TBEV-FE strains, the mean μNT titer against the Vasilchenko strain was lower (1:319/1:409 and 1:224); the difference, however, was not statistically significant.[51] In one Russian study[49] the rapid schedule of FSME-Immun was investigated by means of ELISA and HI after 2 doses administered 2 weeks apart. On ELISA one month after the 2nd dose, almost all subjects (38 of 39) proved to have seroconverted; on HI, this proportion was slightly lower (33 of 39). The rapid protocol of FSME-Immun Junior was also highly immunogenic in children and adolescents, with up to 100% seroconversion following 2 doses.[52]

A progressive waning of antibody titers against both TBEV-FE and TBEV-Sib was reported in a study[50] in which 15 subjects, who had previously been immunized with 3 doses of Encepur according to the conventional schedule, were followed up for 7 y.

Although SCRs remained 100%, the authors observed about 4.7- and 1.9-fold reductions in GMTs (from 1 month to 7 y after the 3rd dose) of neutralizing antibodies against strains P-73 and Kolarovo-2008 (from 1:182 to 1:39 and from 1:208 to 1:112, respectively). Similar results were documented by means of ELISA IgG, with a nearly 2.5-fold decrease (from 1:2750 to 1:1120) in GMTs. A subsequent booster increased GMTs by about 3.5 times on NT against both heterologous strains (post-booster GMTs against P-73 and Kolarovo-2008 strains of 1:138 and 1:471, respectively). A similar pattern was noted on ELISA, with GMTs rising to the level of one month post-dose 3 (1:2750).

Comparison of immunogenicity against European and non-European subtypes

In 5 studies[40,41,43,44,51] the humoral immune response was tested against strains belonging to both heterologous subtypes and homologous TBEV-Eu subtype. Across all 5 studies, SCRs/SPRs against homologous and heterologous subtypes were similarly high. Specifically, in 2 studies[43,51] after completion of the primary vaccination course of 3 doses (both conventional and rapid schedule) and in one study[44] after 2 doses of FSME-Immun, seropositivity against homologous and heterologous subtypes was documented in all vaccinees. Chiba et al.[43] also reported high SPRs (NT $\geq$ 1:20): 100% (n = 8) against the Hochosterwitz TBEV-Eu strain and the Sofjin TBEV-FE strain, and 87.5% against the Oshima 5–10 TBEV-FE strain after 2 FSME-Immun doses. In one Russian study,[41] which also assessed a 2-dose regimen of FSME-Immun, 86.7% of vaccines proved to have seroconverted on ELISA based on the Neudörfl strain, while 83.3% had seroconverted on HI based on TBEV-FE strain 139. Similarly, 3 doses of Encepur were able to induce an NT titer >1:20 against the Sofjin TBEV-FE strain and against 10 different European isolates.[40]

GMTs or individual titers were reported in 3 studies,[40,43,44] while in one paper[51] mean μNT titers were calculated (**Table 4**). Of these studies, only in 2[43,51] were statistical tests performed to compare antibody titers against different strains. Neither study found a significant difference at the 5% level; however, the results of statistical tests were not reported.

As shown in **Table 4**, in only one study[40] was a significant variation observed in neutralizing antibody levels against single strains, with 4 significant post-hoc multiple comparisons between prototype Sofjin and TBEV-Eu isolates (**Table 4**).

Owing to the presence of raw data in only 2 studies, and considering between-study differences in methodology, the strains analyzed and their numbers, we decided not to pool titers of neutralizing antibodies.

Head-to-head RCTs on the immunogenicity of Western and Russian TBE vaccines

Four RCTs[17,41,42,49] compared the immunogenicity of Russian and Western vaccines. As shown in **Table 5**, 3 trials were classified as being at unclear risk of bias, while the fourth RCT was judged to be at high risk of bias owing to reporting bias (pre-specified outcome of seroprotection was not reported in the results). Inter-rater agreement was substantial (K: 0.63, 95% CI: 0.35 – 0.92).

All studies used FSME-Immun as a Western vaccine, while the comparator Russian vaccine was IPVE in 2 studies[41,42] and EnceVir in another 2 studies.[17,49] Children's formulations of FSME-Immun and EnceVir were compared in one trial.[17] In all studies, the immunogenicity of vaccines was evaluated after 2 doses, while one of the 4 studies[49] reported SCRs after each of 3 doses. Two studies[41,42] used a commercial HI kit based on strain 139; one study[17] used an ELISA kit based on strain 205, while the fourth study[49] used both HI and ELISA commercial kits. Two RCTs evaluated conventional schedules,[41,42] while the other 2 studies[17,49] evaluated rapid schedules.

Vorobjeva et al.[41] found no significant difference in SCRs on HI after 2 doses of FSME-Immun and IPVE (FSME-Immun: 83.3% [n = 30], IPVE: 91.4% [n = 35]). By contrast, among previously seronegative children and adolescents, SCR after 2 doses was higher in the FSME-Immun group than in the IPVE group (FSME-Immun: 100% [n = 75], IPVE: 91.4% [n = 70]) as reported by Pavlova et al.[42] On ELISA, Shutova et al.[49] recorded higher SCRs among subjects vaccinated with EnceVir than among FSME-Immun vaccinees (FSME-Immun: 71.8% [n = 39], EnceVir: 100% [n = 29]) 2 weeks after the 2nd dose. However, this difference disappeared one month after the 2nd dose (97.4% and 100%). HI performed by the same authors revealed similar SCRs at both time-points (FSME-Immun: 79.5% and 84.6%, EnceVir: 89.7% and 93.1%, respectively). Likewise, on ELISA one month after 2 doses of FSME-Immun Junior and the pediatric formulation of EnceVir, each administered 2 weeks apart, SCRs were very similar (FSME-Immun: 72.7% [n = 44], EnceVir: 78.6% [n = 42]), reaching 100% for both vaccines 6 months after the 2nd dose.[17]

Three studies[41,42,49] reported SCRs on HI on sera collected one month after the 2nd dose, and 2 studies[17,49] reported SCRs on ELISA one month after 2 doses. We therefore performed 2 separate pooled analyses. In the analysis of HI SCRs, a substantial level of heterogeneity was observed, which justified the use of a random effects model. As shown in **Figure 2**, no significant association (p = 0.83) emerged between SCRs on HI after 2 doses of FSME-Immun or the Russian vaccines. It can be seen that a probable source of the heterogeneity observed is the trial by Pavlova et al.,[42] which showed a significantly higher SCR in subjects immunized with FSME-Immun than in those immunized with IPVE vaccine. Notably, the study population in this trial consisted of 7–17-year-olds, while vaccinees in other 2 trials were adults. Removal of this trial from the meta-analysis completely reset heterogeneity (I^2 = 0%, Q = 0 [p = 0.98]) without, however, altering the pattern of the pooled effect (Mantel-Haenszel risk ratio [RR] = 0.91 [95% CI: 0.80 – 1.03], p = 0.14).

The second pooled analysis did not find any such heterogeneity (I^2 = 0%, Q = 0.55 [p = 0.46]); no difference emerged in SCRs determined by ELISA among vaccinees who had received 2 doses of EnceVir or FSME-Immun 2 weeks apart (Mantel-Haenszel RR = 0.95 [95% CI: 0.84 – 1.08], p = 0.44). Adding the results of ELISA after 2 doses of FSME-Immun and EnceVir, as obtained from a field effectiveness study by Prochorova et al.,[46] did not alter the pooled estimates (Mantel-Haenszel [RR] = 1.02 [95% CI: 0.94 – 1.10], p = 0.68).

Table 4. Comparison of neutralizing antibody titers against homologous and heterologous subtypes reported in single studies.

Vaccine	N of doses	Antibody titer against TBEV-FE or TBEV-Sib strains	Antibody titer against TBEV-Eu strains	Difference[†]	Ref
FSME-Immun	2	Oshima 5–10: 40.0[§¶*] Sofjin: 47.6[§¶*]	Hochosterwitz : 87.2[§¶*]	Kruskal-Wallis test: H = 4.55, p = 0.10	[43]
		IR99–2f7: 58.4[§*] KH98–5: 37.6[§*] VL99-m11: 62.2[§*]	Hochosterwitz: 96.7[§*]	Kruskal-Wallis test: H = 5.02, p = 0.17	[44]
	3	Oshima 5–10: 80.0[§^*] Sofjin: 63.5[§^*]	Hochosterwitz: 127.0[§^*]	Kruskal-Wallis test: H = 2.29, p = 0.32	[43]
		Sofjin: 319[**] Oshima 5–10: 409[**] Vasilchenko: 224[**]	Neudörfl: 360[**] K 23: 338[**]	ANOVA: F = 2.05, p = 0.089	[51]
Encepur	3	Sofjin: 56[*]	K 23: 307[*]; IX 10: 134[*]; Hypr: 110[*]; Trpisovsky: 114[*]; Petracova: 129[*]; 274/II: 110[*]; Gbelce: 98[*]; Cg 1: 55[*]; Dobrostan: 31[*]; Absettarov: 46[*]	ANOVA: F = 16.94, p < 0.001. Tukey post test: least significant difference in mean $\log_e$ titers at 5% level is 0.69. Mean $\log_e$ titer against Sofjin strain is significantly lower than those against 4 of 10 TBEV-Eu strains (K23, IX 10, Trpisovsky and Petracova)	[40]

Notes:
[†]Comparison was performed since none of the papers had reported results of statistical tests.
[§]Calculated values.
[¶]Two subjects had anti-Japanese encephalitis virus antibodies and were excluded; the authors reported GMTs of 44, 43 and 65 against Oshima 5–10, Sofjin and Hochosterwitz strains, respectively.[^] One subject had anti-JEV antibodies and was excluded.
[*]Geometric mean titers are reported.
[**]Mean microneutralization titers are reported. TBEV-Eu, European subtype of TBEV; TBEV-FE, Far Eastern subtype of TBEV; TBEV-Sib, Siberian subtype of TBEV.

Owing to the paucity of studies, we did not check for publication bias, nor could sensitivity analysis be performed.

The HI titers one month post-dose 2 reported by Pavlova et al.[42] did not differ significantly between subjects immunized with FSME-Immun and those immunized with IPVE: standardized mean difference (SMD) = 0.15 (95% CI: -0.18 – 0.48), p = 0.37. SMD in antibody titers determined on HI by Shutova et al.[49] was significantly lower among FSME-Immun vaccinees than EnceVir vaccinees only one month after the 2nd vaccine dose (SMD = -0.95 [95% CI: -1.45 – −0.44] $P < 0.001$) but not 2 weeks after the 2nd dose, when the sign change in SMD was noted (SMD = 0.45 [95% CI: -0.04 – 0.94] p = 0.069). We do not report the results of the pooled analysis on HI titers one month post-dose 2 recorded in these 2 trials because of the unacceptably high heterogeneity level ($I^2 = 92.1\%$; Q = 12.65, $P < 0.001$).

Two trials[17,49] reported the GMTs determined on ELISA after 2 doses of FSME-Immun and EnceVir administered 2 weeks apart. One[49] found that significantly lower titers were elicited by FSME-Immun than by EnceVir both 2 and 4 weeks post-dose 2 (SMD = −2.94 [95% CI: −3.64 – −2.24] $P < 0.001$ and SMD = −1.20 [95% CI:−1.73 – −0.68] $P < 0.001$, respectively). By contrast, the trial by Feldblium et al.[17] did not find any significant difference between FSME-Immun Junior and the pediatric formulation of EnceVir either one or 6 months post-dose 2 (SMD = 0.31 [95% CI: −0.11 – 0.74] p = 0.15 and SMD = 0.01 [95% CI: -0.41 – 0.43] p = 0.96). Again, no pooled analysis of ELISA titers was done

Table 5. Judgments on risk of bias in each trial included.

	Risk of bias			
Domain	**Vorobjeva et al. 1996 [41]**	**Pavlova et al. 1999 [42]**	**Shutova et al. 2009 [49]**	**Feldblium et al. 2013 [17]**
Random sequence generation	Unclear	Unclear	Unclear	Unclear
Allocation concealment	Unclear	Unclear	Unclear	Unclear
Blinding of participants	Unclear	Unclear	Unclear	Unclear
Blinding of personnel	Unclear	Unclear	Unclear	Unclear
Blinding of outcome assessment	Unclear	Unclear	Low	Unclear
Incomplete outcome data	Unclear	Unclear	Low	Low
Selective reporting	Low	Low	Low	High
Other bias	Unclear	Unclear	Unclear	Unclear

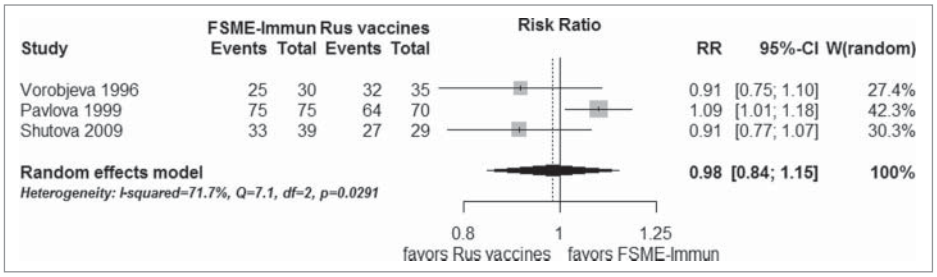

Figure 2. Forest plot of seroconversion rates on hemagglutination inhibition assay by means of commercial kit one month after 2 doses of FSME-Immun and Russian vaccines: random effects model.

owing to the high heterogeneity ($I^2 = 94.8\%$; $Q = 19.40$, $P < 0.001$).

Discussion

This paper provides a comprehensive review of the cross-subtype protection elicited by both currently available Western vaccines. These findings could be used for future research in the field. The main strength of our investigation is that we systematically searched the Russian language literature. Indeed, most of the papers and all the head-to-head RCTs included in the review were published in Russian; this is not surprising since both IPVE and EnceVir are marketed only in the Russian Federation and some post-Soviet countries. The inclusion of non-English literature may make a review more comprehensive, increase the precision of pooled estimates and reduce systematic errors[53,54]; moreover, it has also been shown that the need to include non-English papers may depend on the topic of the review.[55] In the case of TBE, the inclusion of Russian studies is particularly appropriate for several reasons: all vaccines are commercialized in Russia,[1,2] the TBEV population is very heterogeneous,[36] the study of TBE has a long history, dating back to its description by Silber et al.,[56] and the first "brain-made vaccine" was prepared in the former Soviet Union.[38]

Cross-subtype protection provided by the currently available TBE vaccines is biologically plausible, since the 3 main subtypes are closely related both genetically and antigenically.[1] The grading of scientific evidence in support of the hypothesis of vaccine-induced cross-protection has been estimated in a WHO position paper;[57] this included 5 studies,[44,47,48,51,58] and attributed a final score of 2 (out of 4), concluding that the currently available vaccines protect against all 3 subtypes, though suggesting that the true effect may be substantially different from the estimated effect.

To answer our research questions, we were able to identify a higher number of studies. With regard to the SCRs/SPRs against TBEV-FE, the protection given by the Western vaccines against almost all the strains tested may be judged adequate. In 2 Japanese papers,[43,44] all subjects had NT titers of 1:20 or higher against 5 heterologous isolates; an NT titer of 20 was later shown to be the lower threshold of protective IgG activity.[59] The only exception concerns TBEV-FE strain P-69, for which only one third of vaccinees were seropositive after 3 doses of Encepur. It is noteworthy that this strain, which was isolated from the blood of a tick-exposed healthy subject, forms a separate subclade within TBEV-FE.[47] Similarly high immune responses have been found against Siberian isolates, with seropositivity rates as high as 100%.[44,50,51] However, in human studies, only 3 TBEV-Sib strains were tested. Indeed, the small number of strains tested is cited in the WHO position paper as a serious limitation in the designs of the studies examined.[57]

In most studies examining titers of neutralizing antibodies against homologous and heterologous strains, no statistically significant difference among tested strains was established, even though NT titers against heterologous subtypes tended to be lower. It should, however, be noted that all these studies involved few samples, and probably had low statistical power, since the minimum number of vaccinees required in order to observe a clinically significant difference was not calculated. The only exception concerns the relatively old study by Klockmann et al.,[40] who observed a significant variation in GMTs against 11 TBEV isolates; this was attributed by the authors to the presence of minor differences in neutralizing epitopes of single isolates. This explanation is reasonable since, for example, TBE vaccination-induced μNT titers against Omsk hemorrhagic fever virus, which is more distantly related to TBEV, have proved to be significantly lower than those against TBEV strains.[51] Moreover, in the study by Hayasaka et al.,[44] the lowest NT titers were those against TBEV-FE strain KH98-5, while the highest GMTs among heterologous strains were documented against TBEV-Sib strain IR99-2f7. Notably, the amino acid sequence of the E protein of the strain KH98-5 differs from the Neudörfl vaccine strain by 23 amino acids (n = 496), while the difference between IR99-2f7 and Neudörfl strains concerns 16 amino acids (n = 496) (determined by means of the BLAST program in GenBank,[60] data not shown). In later papers[5,51] it was suggested that similar inconsistencies in the immune the response to different strains may have been due to a poorly standardized methodology. To overcome that problem, a novel test system was proposed which enabled unbiased head-to-head comparison of the humoral response against the 3 subtypes by constructing hybrid viruses. These hybrids were created by using the West Nile virus backbone and encoding prM and E structural proteins of single TBEV isolates. According to the authors, this method enabled discrepancies in viral growth and infectivity to be mitigated, while preserving the antigenic characteristics of single wild isolates. Indeed, the 2 studies[5,51] (one was a murine model[5] not included in this paper) that used this approach found similar patterns of vaccine-induced cross-immunogenicity between homologous and heterologous strains.

Another important issue concerning the TBEV-FE and TBEV-Sib isolates tested is their correspondence to the currently

circulating TBEV population. Indeed, of 12 heterologous strains reported in the present paper, 5 were identified more than 40 y ago, while only one (Kolarovo-2008) was isolated less than 10 y ago. This has been emphasized,[39] since Zausaev-like Siberian strains have prevailed in Russia in recent years, but no human studies on the immunogenicity provided by the inactivated vaccines against these strains have been conducted. In their murine model, Morozova et al.[39] documented only partial protection of all inactivated vaccines against the Siberian strain 2086 isolated in 2010. Further research on the immunogenicity of vaccines against modern TBEV isolates is therefore needed.

The fact that very high cross-subtype immunogenicity was provided by the rapid schedule of the Western vaccines (as demonstrated in 3 studies)[17,49,51] is of particular importance in a globalized world, as this modality may be suitable for short-term travelers to TBEV-endemic zones where non-European subtypes circulate (such as the Baltic states, Russia, Mongolia, Northern China or Hokkaido). Indeed, international tourist arrivals to Central/Eastern Europe and North East Asia are steadily rising, reaching more than 230 million in 2012.[61]

Our third research question regarded the direct comparison of Russian and Western vaccines in terms of immunogenicity against Far Eastern isolates. This question was posed with a double aim. First, it could demonstrate the efficacy of the Western vaccines against TBEV-FE; second, the question is of a certain local significance (in Russia) as both FSME-Immun and Encepur are on the market in regions where TBEV-FE circulates. Evidence of moderate quality suggests that TBEV-Eu-based vaccines are as effective as TBEV-FE-based ones against Far Eastern isolates. The use of standardized commercial ELISA and HI kits in selected RCTs enables the above-mentioned methodological issues to be avoided. On the other hand, the results of our pooled analysis should be interpreted cautiously on account of the small number of trials included, the limited number of participants in single RCTs and the moderate methodological quality of these trials. Indeed, the results of meta-analyses of small trials may not be confirmed by subsequent large RCTs for at least 2 reasons: publication bias and the limited methodological quality of pooled studies. Thus, small trials tend to be accepted for publication if they find a statistically significant intervention effect.[62] Moreover, Vickers et al.[63] found a high proportion of positive results among acupuncture trials conducted in Russia/the former Soviet Union and some Asian countries. Although we were not able to check formally for publication bias, its presence seems to be unlikely, since all 4 RCTs concluded that Russian and Western vaccines displayed almost equal immunological performance. Furthermore, it has been shown that the methodological quality of non-English clinical trials may be lower than that of those published in English.[55] We believe that sub-optimal methodological quality observed stems from the later adoption of standards of reporting trials in Russia.

In addition to the above-mentioned limitations regarding the small numbers of participants in single studies, the moderate quality of trials and a certain risk of country-related publication bias, the present paper may suffer from other shortcomings. Specifically, most studies that compared immunogenicity against European and non-European subtypes (research question 2) were observational and thus prone to the selection bias. Moreover, to answer our research question 3, we identified only a few RCTs and thus were not able to perform sensitivity analysis nor meta-regression. Finally, although the results reported in the selected RCTs were almost consistent, their moderate quality could reduce the value of the pooled estimates.

In conclusion, to date there is no universally accepted standardized serological correlate of protection against TBE, and all studies on the efficacy of vaccination are based on immunogenicity rather than clinical protection.[64] Indeed, it has been underlined that the *in vitro* presence of neutralizing antibodies against multiple viruses does not guarantee cross-protection against all these viruses *in vivo*.[65] Improvements in TBE surveillance, field studies on the effectiveness of TBE vaccines and the investigation of vaccination failures, especially in countries where different subtypes co-circulate, will further provide useful insights into TBEV vaccination-induced cross-subtype protection.

Methods

Search strategy

To identify eligible studies, the following international databases were systematically searched: PubMed, Web of Science and Scopus. Russian language literature was systematically searched by using the scientific electronic database eLIBRARY.RU, which is the largest information portal in Russia.

In order to ensure maximal retrieval, free text searching was undertaken. In PubMed, the following search syntax was composed: (TBE OR TBEV OR tick-borne encephalitis OR Central European encephalitis OR CEE OR Russian spring summer encephalitis OR RSSE) AND (vaccin* OR immunis* OR immuniz* OR FSME-Immun OR TicoVac OR Encepur) AND (cross-protect* OR ((heterologous OR heterotypic OR cross-subtype) AND protection) OR cross-neutraliz* OR cross-neutralis* OR cross-immun* OR cross-react* OR neutraliz* OR neutralis*). In Scopus and Web of Science, the same entry terms were searched for in "title, abstract, keyword – TITLE-ABS-KEY" and "topic – TS," respectively. Owing the smaller number of Russian language papers, research on the eLIBRARY.RU was performed by using the simpler free text searching "*клещевой энцефалйт & вакцйн**" ("tick-borne encephalitis and vaccin*"). Additionally, a manual search was performed by scanning reference lists of identified studies; Google Scholar was further used in order to identify papers that cited the selected items.

As the development of FSME-Immun – the oldest vaccine currently marketed – began in the early 1970s,[66] the search was restricted to studies published from January 1970 onwards. The last search was performed on 3rd April 2014.

Study eligibility

Studies of any design which assessed the immunogenicity elicited by the Western TBE vaccines against at least one TBEV-FE or TBEV-Sib strain were eligible. Both historic and current formulations of FSME-Immun and Encepur were considered, since

the vaccine antigens have not changed.[1,2,7] Study populations were restricted to healthy flavivirus-naïve subjects of any age vaccinated with at least 2 doses of FSME-Immun or Encepur. The outcome of interest was the humoral immune response to heterologous subtypes, as measured by any serological assay. If an unclear TBEV strain was used in serological assays, the corresponding author of that study was contacted by email. Full-text articles published in English, French, Italian or Russian were eligible.

In the present paper, the unit of analysis was a single serological test; therefore, when the immunologic response was investigated at different time-points and/or by means of different serological assays, but the results were presented in more than one publication, these papers were not considered redundant. By contrast, papers presenting the results of serological assays performed at the same time-points and using sera from the same subjects were deemed redundant. Anyway, if the same research group had produced several similar publications, the senior author was contacted personally for further explanation.

Exclusion criteria were formulated as follows: (1) reviews, commentaries, opinion publications without original data, papers without numeric data; (2) articles published in languages other than English, French, Italian or Russian; (3) redundant publications; (4) animal studies; (5) investigation of only Russian, candidate or obsolete TBE/Langat-based vaccines; (6) explicit statement on documented evidence of TBE or any flavivirus infection or studies in which some participants had anti-TBEV antibodies on enrollment but there were no separate data for seropositive and seronegative subjects; (7) explicit statement on history of yellow fever and/or Japanese encephalitis vaccination; (8) the immune response elicited by Western vaccines was measured only against strains belonging to TBEV-Eu or other members of the mammalian tick-borne flavivirus group or against mosquito-borne flaviviruses.

Data extraction

Data from eligible studies were extracted by 2 independent reviewers (AD and EKA) and inserted into an *ad hoc* table, which included the following information: study design, sample size, age of vaccinees, study location, vaccine, number of doses and vaccination schedule, time of serum collection post-immunization, serological assay, TBEV subtypes (both homologous and heterologous) and strains tested, immunogenicity against single strains.

Studies reporting either individual or group data were eligible. If immunogenicity was measured after each successive immunization, data recorded after the 2nd, 3rd or booster doses were extracted separately. If more than one immunologic assay was performed, the results of each were recorded. We were compelled to use a composite outcome for serological response[67] since some studies reported SCRs, SPRs and seroprotection rates after vaccination. The definition of each sero-outcome, if provided by the authors, was extracted. Raw or summarized antibody titers (GMTs, log-transformed GMTs or mean titers), if available, were recorded from each study.

Quality assessment of RCTs

To assess the methodological quality of the RCTs selected, the Cochrane Collaboration's tool for assessing risk of bias[68] was used; this assesses risk of bias (defined as low, unclear and high) in domains of sequence generation and allocation concealment, blinding, incomplete outcome data, selective outcome reporting and other sources of bias. As suggested by the Cochrane Collaboration,[68] the summary assessment outcome of single studies was classified as low (low risk of bias in all domains), unclear (unclear risk of bias in $\geq$1 domain but no domains at high risk of bias), or high (at least one domain with high risk of bias) risk of bias. Assessment was performed independently by 2 authors (AD and EKA); any disagreement was solved by consensus.

Statistical analysis

We compared the immune response to different subtypes in single studies which investigated immunogenicity against both homologous and heterologous strains but did not report the results of statistical tests. When individual titers were available, the Kruskal-Wallis test was used to evaluate differences among 3 or more strains. Since we used a non-parametric test, a simplistic approach for treating censored observations was adopted: left-censored at dilution titer values of antibody titers (for example, $<$20) were treated as substitute values expressed as half of the detection limit; right-censored titers, such as $\geq$640, were set to the maximum dilution specified in the article.[69] When only mean titers and their standard deviations (SDs) or standard errors were available, one-way analysis of variance (ANOVA) was performed. A Tukey's multiple-comparisons test was used to detect differences in log-transformed NT titers against single strains.

Skewed continuous outcomes (GMTs) were treated in accordance with the recommendations of the United States Advisory Committee on Immunization Practices[70] by converting GMTs and their SDs to the natural logarithm ($\log_e$) scale. In studies in which GMTs $\pm$ SD were expressed on $\log_{10}$ scale, we first took their anti-$\log_{10}$ and then reconverted them to the $\log_e$ scale. For studies presenting a range (sample minimum and maximum) of titers, SDs were imputed by dividing the range by 4.[71] The SMD with 95% CIs was used to quantify the differences in $\log_e$-transformed means of antibody titers elicited by Russian and Western vaccines. We planned to pool SMDs of single studies by using Hedges' adjusted g.

The meta-analysis of binary outcomes was performed in order to pool SCRs reported in RCTs on head-to-head comparison of Russian and Western vaccines. Pooled results were expressed as RRs with 95% CIs. In all pooled analyses, random-effects models weighted by the DerSimonian-Laird method were first performed; however, when observed heterogeneity was low ($I^2 <$ 40%), fixed-effects models using the Mantel-Haenszel method for weighting were re-applied. Meta-analysis was not done when heterogeneity was too high ($I^2 >$ 85%).[72] We planned to assess potential publication bias by means of funnel plot and Harbord's test. We also planned *a priori* to perform a leave-one-out sensitivity analysis, in order to ascertain that the estimates were not driven by single trials, and meta-regression to identify study

characteristics that were associated with between-study heterogeneity (such as study quality).

Inter-rater agreement in assessing the methodological quality of RCTs was quantified by means of Cohen's K, which was interpreted as follows: ≤0 – poor, 0–0.20 – slight, 0.21–0.40 – fair, 0.41-0.60 – moderate, 0.61–0.80 – substantial, 0.81–1.0 – almost perfect.[73]

All data were analyzed by means of the R stats package, version 3.0.1.[74]

Disclosure of Potential Conflicts of Interest

A.D., D.P., E.K.A., A.S., R.G., and D.A. declare that they have no competing interests. U.A. is an employee of Baxter S.p. A.

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