

# Immunogenicity of a booster vaccination against tick-borne encephalitis

## Imunogenost poživitvenega cepljenja proti klopnemu meningoencefalitisu

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### Izvleček

**Izhodišča:** V Sloveniji je klopni meningoencefalitis endemičen, kljub temu je manj kot 10 % ljudi redno cepljenih. Na delež cepljenih pomembno vpliva obvezno cepljenje med letoma 1993 in 2003, ko je bilo cepljenje proti klopnemu meningoencefalitisu obvezno za vse slovenske vojaške nabornike.

**Metode:** V našo raziskavo smo vključili 73 moških iz celjske regije, ki so bili cepljeni proti klopnemu meningoencefalitisu s cepivom FSME-Immun® (Baxter) s tremi odmerki, nato pa so cepljenje prekinili za obdobje 8–16 let. Sodelujoče smo serološko testirali pred in po prejetju prvega poživitvenega odmerka. Udeleženci so prihajali na drugi odvzem krvi 21–196 dni po prejetju poživitvenega odmerka, povprečna vrednost 55 dni (mediana 43). Uporabljen je bil encimsko imunski test Enzygnost®.

**Rezultati:** Pri analizi smo ugotovili, da je bila raven protiteles pred prejemom revakcinacije pri večini udeležencev zaščitna (67/73; 91,8 %, 95 % i.z. 86–98 %), po prejetju poživitvenega odmerka pa je bila presežena zaščitna vrednost pri vseh udeležencih, ki so oddali drugi vzorec krvi. Geometrična sredina koncentracij protiteles pred poživitvenem odmerkom je bila 56 U/ml, po poživitvenem odmerku pa 316 U/ml.

**Zaključki:** Z našo raziskavo smo z uporabo encimsko imunskega testa potrdili dolgotrajno zaščito po osnovnem cepljenju s tremi odmerki proti klopnemu meningoencefalitisu pri moških, mlajših od 50 let. Rezultati podpirajo skrbno proučitev trenutno priporočenega intervala revakcinacij.

### Abstract

**Background:** Tick-borne encephalitis is endemic in Slovenia, but still less than 10 % of people are regularly vaccinated. The proportion of vaccinated individuals was significantly influenced by obligatory vaccination for all Slovenian military conscripts between 1993 and 2003.

**Methods:** Our study includes 73 men from the Celje region, who were vaccinated with three doses of vaccine against tick-borne meningoencephalitis FSME-Immun® (Baxter), but afterwards they stopped the vaccination for a period of 8 to 16 years. The participants were serologically tested before and after the first booster dose. The second blood sample was taken 21 to 196 days after the application of a booster dose, the mean spacing of 55 days (median 43). We used the enzyme immunoassay Enzygnost®.

**Results:** The result of the analysis showed that in most of the participants the level of antibodies before receiving revaccination was protective (67/73; 91.8 %, 95 % CI 86 %–98 %); after receiving a booster dose, the protective value was exceeded in all participants who had submitted a second blood sample. Geometric mean concentration was 56 U/ml before the booster dose and 316 U/ml after the booster dose.

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**Conclusions:** In our study, long-term protection after primary vaccination with three doses against tick-borne encephalitis in men younger than 50 years was confirmed by using enzyme immunoassay. The results support careful consideration of currently recommended revaccination interval.

## Introduction

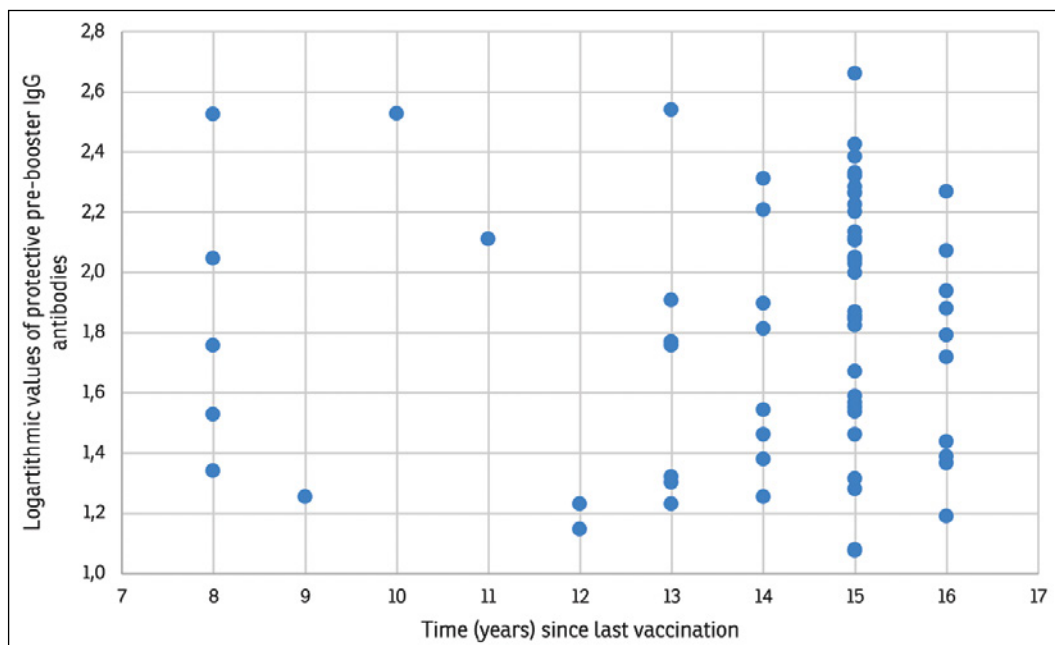
Slovenia is among the countries with the highest incidence of tick-borne encephalitis (TBE). In 2014, the number of reported cases of TBE was the lowest since 1995 and much lower than the average since 1995 (about 250 reported cases per year). There were 101 cases of TBE reported, which means 4.9/100 000 population. That is the lowest number of reported cases after 1992. In 2014 there was also one death due to TBE infection reported (the patient was infected in 2013). TBE cases were reported in all regions in Slovenia, except in Gorica region. Like every year, the highest incidence was again noted in Gorenjska region and Koroška region, where the incidence was 8.3 and 12.6/100 000 population respectively.<sup>1</sup> It is however important to emphasize that the incidence in a given region may vary significantly over time, as confirmed by data from Austria.<sup>2</sup> From the 5<sup>th</sup> of September 2012 onwards, TBE must be reported to the European Union (EU). The European Centre for Disease Prevention and Control (ECDC) published a definition of the disease for epidemiological monitoring, which enables a unified reporting and classification of TBE cases on the basis of an appropriate clinical picture and laboratory findings. The unified definition provides a better comparison of data on the burden of disease in the EU and a more precise definition of the risk of infection.<sup>3</sup>

The most effective way to prevent TBE is vaccination. The vaccine is one of the safest and most effective.<sup>4-10</sup> In Slovenia, two vaccines are registered. Both use chicken embryonic cells, inactivated with formaldehyde and contain-

ing the adjuvant aluminum hydroxide. The first vaccine is FSME-Immun® (Baxter), the second one is Encepur® (Novartis). In Slovenia, compulsory vaccination against TBE was introduced in 1986 for occupationally exposed persons. The payer of vaccination was the person's employer. Since 1990, vaccination is also compulsory for those pupils and students who are exposed to infection with TBE virus during training. This vaccination is paid by the Health Insurance Institute of Slovenia. Since 1991, vaccination against TBE is recommended for all persons over 1 year of age who reside in endemic area or plan activities in the endemic area. In these cases, vaccination is self-funded.<sup>11</sup> Between 1993 and 2003, vaccination against TBE was mandatory for all military conscripts (at the cost of the Slovenian Armed Forces), which means that most men born between 1974 and 1984, had received primary vaccination against TBE. Vaccinations were carried out in nine Slovenian regional institutes of public health, which were responsible for the vaccination of conscripts in their area. The Regional Institute of Public Health Celje was an authorized institution (under Slovenian law) for the vaccination of regional conscripts and for conscripts' personal data management (continuous data management). Since 2003, when the Slovenian Armed Forces became a professional army, TBE vaccination is compulsory for professional soldiers only.<sup>12</sup>

The primary vaccination consists of three doses of the vaccine; vaccination is carried out (depending on the recipient's

**Figure 1:** Logarithmic values of protective antibodies before the booster dose ( $\geq 10$  U/ml), considering the interval (in years) between the last dose (3<sup>rd</sup> dose) and the serologic testing



age) either with children's doses or with a vaccine for adults. The first booster dose (revaccination) is carried out after three years with one dose, while all of the following revaccinations are carried out at five-year intervals. After 60 (FSME-Immun) or 50 years of age (Encepur) revaccinations are advised at three-year intervals.<sup>11</sup>

The aim of our study was to determine the immune response/levels of antibodies against TBE before and after the first booster dose in persons who had been vaccinated as former conscripts against TBE in the years from 1993 to 2003 with three doses of FSME-Immun (primary vaccination). Invitation to boosting vaccination and participation in the survey was sent to all conscripts from the register in Celje region ( $n = 7473$ ). Only 76 persons responded. They were vaccinated at the Institute of Public Health Celje, which covers a region of about 300 000 inhabitants.

## Methods

On the basis of serological tests with enzyme linked immunosorbent assay

(ELISA), we evaluated concentrations of IgG antibodies 8–16 years after completed primary vaccination. We used an enzyme immunoassay Enzygnost®. Detection limit was 5.2 U/ml. The protective threshold, considered for specific IgG of antibodies, is  $\geq 10$  U/mL.<sup>13</sup>

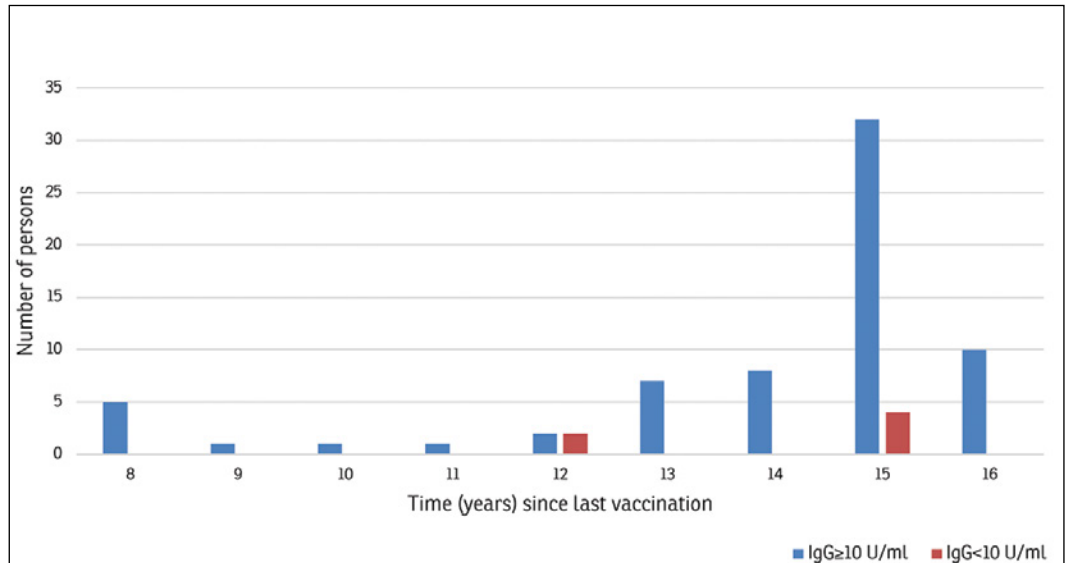
We analyzed the data by the Epi Info program. Concentrations of antibodies were transformed into logarithms to calculate the geometric mean concentrations (GMCs). This research was carried out with the approval of the National Medical Ethics Committee of the Republic of Slovenia (No. 33/02/12).

## Results

Our study included 76 participants. We excluded three persons from the statistical analysis, due to possible cross-reactivity of the flavivirus antibodies (two persons who were vaccinated against yellow fever and one person who convalesced from hepatitis C). Therefore, the statistical analysis is based on a sample of 73 persons.

The median age of participants was 34 (range 28–41) years.

**Figure 2:** The number of persons with protective ( $\geq 10$  U/ml) and unprotective pre-booster specific IgG antibody levels, considering the interval (in years) between the last (3<sup>rd</sup>) dose of basic vaccination and the serologic testing, (n = 73)



The median interval between the last dose of basic vaccination (3<sup>rd</sup> dose) and serologic testing was 15 (8–16) years (Figure 2).

Participants gave the second blood sample from 21 to 196 days after receiving a booster dose, the mean time interval of 55 days (median 43).

In most participants (67/73; 91.8%, 95% CI 86%–98%) the antibody levels before the booster dose exceeded the protective value  $\geq 10$  U/ml (Figure 2). After the booster dose, the protective value was established in all participants who had submitted the second blood sample (69/69; 100%).

We calculated the GMCs before and after the booster dose. Before the booster dose the value was 56 U/ml (n = 73),

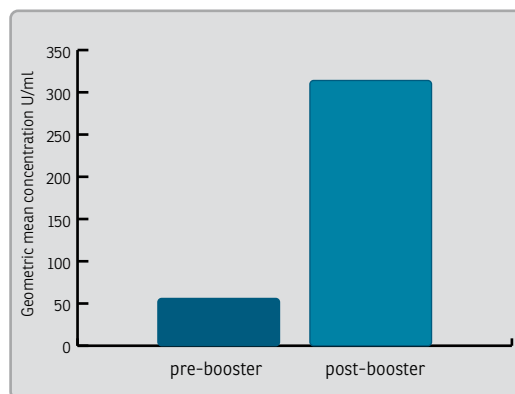
while after the booster dose it was 316 U/ml (n = 69). Therefore, we have witnessed a six-fold increase in the GMCs (Figure 3).

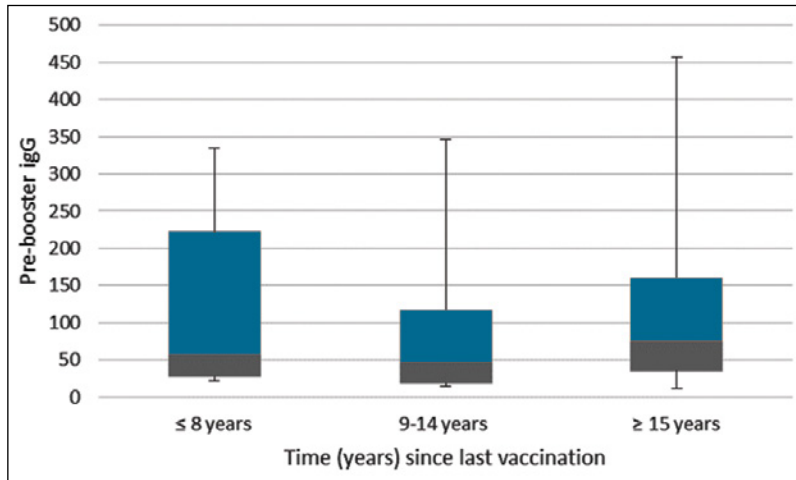
We studied the protective pre and post-booster antibody values considering the interval between the last vaccination dose (3<sup>rd</sup> dose) and the serologic testing. We divided participants into three groups and presented the results in boxplots (Figures 4 and 5).

## Discussion

In this study we investigated the humoral immune response to TBE vaccine in the former conscripts. For the detection of specific IgG antibodies against TBE virus we used the enzyme immunoassay (ELISA). The literature describes two microbiological tests to measure the concentration of antibodies against TBE: ELISA and the neutralization test (NT). Both tests are comparable, but the fact is that they are not always completely the same. In the case of ELISA method, compared to NT, the cross-reactivity of antibodies to other flaviviruses is possible. In our study, we used ELISA method since we excluded all persons who could jeopardize the investigation due to

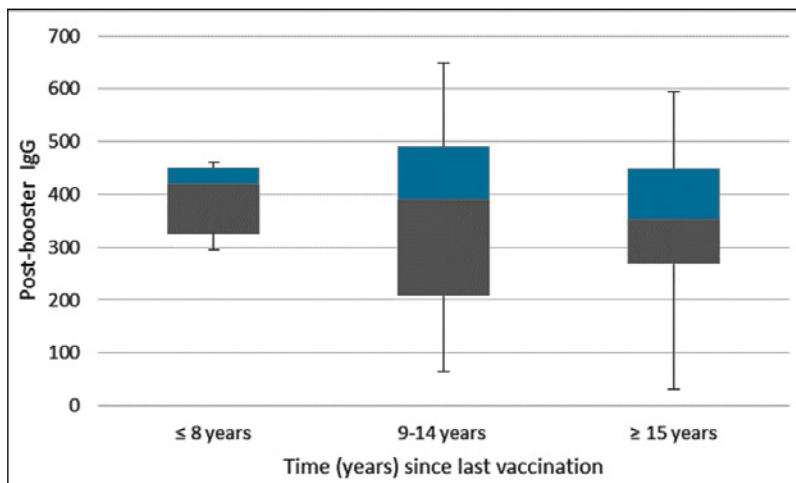
**Figure 3:** Display of geometric mean concentrations, before and after the booster (4<sup>th</sup>) dose





**Figure 4:** Boxplot of protective pre-booster antibody values (protective value of  $\geq 10$  U/ml) considering the interval (in years) between the last vaccination dose (3rd dose) and the serologic testing, Slovenia, 2012/13

a possible cross-reactivity of antibodies to flaviviruses (3 persons).<sup>14</sup> The other reason for using ELISA is that NT is not applicable in the National Microbiology Laboratory routine diagnostics, as the method is difficult and time consuming, particularly when testing a large number of samples. The threshold of protective concentration with ELISA method has not yet been formally defined. In our study we considered the concentration of  $\geq 10$  U/ml as the protective concentration of antibodies, consistently with the



**Figure 5:** Boxplot of protective post-booster antibodies values (protective value of  $\geq 10$  U/ml) considering the interval (in years) between the last vaccination dose (3rd dose) and serologic testing, Slovenia, 2012/13

recommendation of the World Health Organization (WHO).<sup>15</sup> Recently the multiplex enzyme-linked immunosorbent assay-based protein array (ELISA-array) has been developed for specific antibody detection against five Flaviviridae viruses (TBE, Japanese B, West Nile, dengue, and yellow fever viruses).<sup>16</sup>

We presented the results of the protective pre- and post-booster antibody values considering the interval between the last vaccination dose (3rd dose) and the serologic testing in boxplots. Due to a small number of participants in the group 1–8 years and the differences in the number of participants between groups, this finding is not relevant. This is the weakest point of this research, but we could not directly influence the number of participants in the study.

In our study, we wanted to investigate the immunogenicity after the primary vaccination, when a person does not come for a booster dose within the scheduled time interval (after 3 years), but comes after a longer period of time. In such cases, during the next visit, we continue with the missing doses of vaccine and the vaccination is never started from the beginning. Longer intervals between doses generally do not reduce the levels of antibodies after the completed missing vaccination, but the protection in the meantime is questionable. All further doses of the vaccine are then received within the normal vaccination scheme.<sup>17-19</sup>

Several articles have been published that prove a long-term immune protection after vaccination against TBE.<sup>20-22</sup> Longitudinal studies have shown that during the first year after primary vaccination GMCs of neutralizing antibodies against TBE are falling, however, their value stabilizes later on.<sup>15</sup> Studies that examined the immune response after receiving  $\geq 1$  booster vaccination



had shown that immunity lasts more than 5 years after receiving the last booster dose.<sup>23,24</sup> Surveys were carried out that examined the immune memory of individuals who had not been vaccinated within the manufacturer's recommended time frame. The results confirmed good immunogenicity even with booster doses that were received later than recommended.<sup>25,26</sup> Another study showed that with the majority of persons the response of antibodies against TBE is present even up to 20 years after the last dose of vaccine. Antibody response after revaccination was also elicited in case when the participants received only one dose of vaccine, or if they were seronegative before the booster dose.<sup>15</sup>

The most effective way to prevent TBE is vaccination. WHO recommends vaccination of the entire population, including children, in highly endemic areas ( $\geq 5$  cases/100 000 per year). In the areas where the incidence of TBE is low or medium (5-year incidence  $< 5$  cases/100 000 per year), vaccination is advised to most exposed groups. WHO also recommends vaccination for persons traveling from non-endemic to endemic areas if extensive outdoor activities are expected in regions up to 1400 meters above sea level.<sup>15</sup> The Central European Vaccination Awareness Group (CEVAG) strongly recommends vaccination against TBE to people older than one year in all countries where there is a high risk of TBE infection. Recommendations for countries with a very low risk for TBE include vaccination for persons traveling to endemic areas.<sup>27</sup> In Slovenia, the vaccination is recommended for all persons older than 1 year who live in the endemic area or provide activities in the endemic area.<sup>11</sup> Booster doses should be administered according to the

manufacturer's instructions: summary of product characteristics (SmPC). The only exception is Switzerland, where the professional body decided differently and carries full responsibility. The recommended booster dose intervals in Switzerland are  $\leq 10$  years (between completed primary vaccination and the first booster dose and between individual booster doses).<sup>15</sup>

Older persons represent a special group.<sup>28</sup> When studying 50–60 year-old persons, the researchers have discovered that they produce lower antibody levels and consequently seronegativity occurs faster than with younger persons.<sup>29</sup> In our study we did not investigate the impact of age on the level of antibody titer as the differences in age among participants were negligible.

## Conclusions

In our study, using an enzyme immunoassay, we confirmed the long-term protection after vaccination against TBE for participants younger than 50 years of age. The studied sample of participants is relatively small ( $n = 73$ ), so the representativeness is questionable, but no more persons responded. The study shows that the protective antibody titers can be detected far beyond the period of 3 years, which is the interval recommended by the manufacturer for the first revaccination, and supports careful consideration of currently recommended revaccination interval.

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