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# Tick-borne Encephalitis Vaccines

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## Abstract

Tick-borne encephalitis (TBE) is a disease that is found from western Europe across Asia and into Japan. In recent years the incidence rate has been increasing as has the endemic range of the virus. Tick-borne encephalitis is caused by three genetically distinct sutypes of viruses within a single TBE virus (TBEV) serocomplex. These three subtypes consist of Far-eastern subtype TBEV (TBEV-FE), Siberian subtype (TBEV-Sib) and European subtype (TBEV-Eu). Each of these subtypes cause clinically distinct diseases with varying degrees of severity. Development of the first vaccines for TBEV began in the late 1930s shortly after the first isolation of TBEV-FE in Russia. In the 1970s Austria began large scale vaccine production and a nationalized vaccine campaign that significantly reduced the incidence rate of TBE. Currently there are four licensed TBE vaccines, two in Europe and two in Russia. These vaccines are all quite similar formalin-inactivated virus vaccines but the each use a different virus strain for production. Published studies have shown that European vaccines are cross-protective in rodent studies and elicit cross-reactive neutralizing antibody responses in human vaccines. European vaccines have been licensed for a rapid vaccine schedule that could be used in response to a significant outbreak and reasonable neutralizing antibody titers can be achieved after a single dose although a second dose provides nearly complete and long-lasting protection. This review focuses on the current status of licensed TBE vaccines and provides a brief summary of technology currently being developed for new vaccines.

**Keywords:** Tick-borne encephalitis; vaccine; arbovirus; biodefe

## Introduction

The tick-borne encephalitis (TBE) serocomplex of flaviviruses (Family Flaviviridae, genus Flavivirus) includes a number of viruses that cause disease in humans. These include the TBE viruses (TBEV) of which there are three subtypes, Kyasanur Forest disease virus (KFDV) and the closely related Alkhurma (ALKV) and Nanjianyin viruses, Omsk hemorrhagic fever virus (OHFV) and Powassan virus (POWV). A subtype of POWV is deer tick virus (DTV). Also within the TBE serocomplex is Langat virus (LGTV), a naturally attenuated virus that is generally apathogenic in humans following natural infection. Members of the TBE serocomplex are genetically distinct, but closely enough related serologically that they are often difficult to distinguish by antibody-based assays.

The diseases caused by the TBE serocomplex viruses range from asymptomatic or mild febrile illness to hemorrhagic fever or severe encephalitis with significant morbidity and mortality. OHFV, KFDV and ALKV are most frequently associated with hemorrhagic disease while POWV and TBEV infections can result in encephalitic disease. The three subtypes of TBEV can be distinguished genetically and often by clinical presentation. European subtype TBEV (TBEV-Eu) is generally a biphasic disease, occasionally resulting in neurologic disease, but with a low case fatality rate. In contrast, Far-eastern subtype TBEV (TBEV-FE) is more frequently associated with severe neurologic disease, relatively high case fatality rate and an increased propensity for neurological sequelae in survivors. The Siberian subtype TBEV (TBEV-Sib) is intermediate in disease severity, but has been associated with chronic infection [1-5].

The incidence of TBE in Europe has increased significantly over the past 40 years despite active vaccination programs in many European countries [6,7]. The spread of TBE cases has been attributed to a number of factors including incomplete vaccine coverage, increased abundance of ticks, an increased range of the *Ixodes ricinus* tick, changes in human life-style, socio-economic conditions and climate change [8]. There are 10,000-12,000 cases of TBE reported annually throughout Europe and Asia (Tick-borne encephalitis International Scientific Working Group (TBE-ISW), http://www.tbe-info.com) [9]. KFD accounts for 100-500 cases per year in India with outbreaks in both humans and non-human primates [10]. The documented incidence of OHFV, ALKV and POWV infection is very low.

Transmission of TBE serocomplex viruses typically occurs through the bite of an infected tick although outbreaks sometimes can also be associated with consumption of un-pasteurized milk products from infected sheep or goats, a disease termed "biphasic milk-fever" [2]. Percutaneous injury in laboratory or medical settings along with direct exposure to blood or bodily fluids from infected humans or animals are also potential routes of exposure. Infection from inhalation of TBE virus aerosols has been documented after laboratory accidents [11,12]. Quoting the CDC Special Pathogens Branch internet website: "Laboratory infections were common before the use of vaccines and availability of biosafety precautions to prevent exposure to infectious aerosols".

Flaviviruses are typically transmitted by either mosquitoes or ticks although there is a small subset of viruses that are not believed to require an arthropod vector for transmission [13]. The flaviviruses account for majority significant number of the arthropod-borne viral diseases worldwide. Tick species associated with transmission of TBE serocomplex viruses include the hard ticks *Ixodes ricinus*, *Ix.*

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*persulcatus*, Dermacentor spp. and Hyalomma spp. Soft ticks from the genus Ornithodoros have also been associated with transmission of some tick-borne flaviviruses including ALKV [14,15]. There has been some suggestion that tick-borne flaviviruses are transmitted by mosquitoes, but this has not been conclusively proven.

The flaviviruses are a family of small, single-stranded RNA viruses with a positive-sense genome and a host-derived lipid envelope. The viral genome encodes a single polyprotein that is co- and post-translationally cleaved into ten individual proteins, three structural and seven non-structural. The three structural proteins (C-capsid, prM/M-premembrane/membrane and E-envelope) organize to produce the viral particle. The non-structural proteins are principally associated with viral replication, polyprotein cleavage and may have additional roles in regulating the host immune response [16,17]. The viral E protein is the major surface antigen for the flaviviruses and contains the receptor-binding domain and fusion peptide. The viral prM/M protein functions as a chaperone for the E protein and also blocks the fusion peptide during virus assembly to prevent fusion with exocytic vesicles. The prM protein is cleaved by furin following particle assembly to produce the mature fusogenic virus. Co-expression of the prM and E genes in cell culture systems have been used to produce subviral particles which were used in early characterization of the membrane fusion process of flavivirus entry and have also have been tested as potential vaccine candidates. Vaccine candidates for several flaviviruses, including West Nile and dengue viruses utilize a purified recombinant E protein that has been produced in insect cell cultures [18-22].

Tick-borne encephalitis was first described in 1936 as a neurologic disease in far eastern Russia (then the Soviet Union) that had been recognized in 1932. In 1937 a large expedition supported by the Soviet government identified *Ix. persulcatus* ticks as the likely vector for the contagion. In the same year separate groups isolated the virus and were able to demonstrate that this virus was the causative agent for the encephalitic disease seen in far-eastern Russia. The virus was subsequently named "Far Eastern encephalitis virus" [23]. The isolation of the virus also provided the first opportunity to develop a vaccine to prevent infection. Smorodintsev developed a formalin-inactivated vaccine that was used to vaccinate forest worker [24]. Subsequent studies characterizing clinical disease described two similar yet distinct diseases, one found in the far-east which was more severe and a less severe disease in western Russia and parts of Eastern Europe. The latter disease was termed Western encephalitis to distinguish the two diseases [24]. Western encephalitis was also known as biphasic milk fever given an apparent relationship with the consumption of unpasteurized milk from infected animals. Studies during outbreaks in Czechoslovakia isolated the causative agent of Western encephalitis and identified the virus as related to Far eastern encephalitis virus [25-27]. Far-eastern encephalitis was found to have a higher case fatality rate and increased incidence of long-term sequelae than Western encephalitis. Additional studies identified a third intermediate subtype of what was now known as tick-borne encephalitis. This third subtype was called the Siberian subtype and has subsequently been shown to be associated with chronic infection [1-5,28]. Following the advent of viral genome sequencing, genetic analysis supported clinical descriptions in clearly defining three distinct subtypes of TBEV. These are now termed TBEV-FE (Far-eastern), TBEV-Eu (European) and TBEV-Sib (Siberian) [29].

In 1957 a large outbreak of hemorrhagic disease in India was described in bonnet macaques and humans. This disease was termed Kyasanur Forest disease (KFD), given its locale, and subsequent studies isolated the causative agent and characterized the virus as related to TBEV [30,31]. KFDV was clearly distinct from TBEV as this virus caused a disease that had both hemorrhagic and neurologic components while TBEV was only associated with encephalitis. For four decades KFDV was thought to only exist in India. However, in 1996 Alkhurma virus was isolated from cases of hemorrhagic disease near Jeddah, Saudi Arabia. This virus was characterized and found to be closely related to KFDV both serologically and genetically. In 2009, Nanjianyin virus was identified in south central China and found to virtually identical to KFDV [32].

Omsk hemorrhagic fever was first identified in 1947 in the Novosibirsk and Omsk Oblast regions of Russia. Few cases of OHF have been described, but clinical disease frequently has a hemorrhagic component with evidence of neurologic disease less common [33]. Following isolation of OHFV, serological and genetic characterization clearly identified this virus as a member of the TBEV serocomplex.

Langat virus was first isolated from rodents in Malaysia in 1956 and was initially thought to be TBEV-FE based on serological results [34]. Further analysis found that LGTV was a virus within the TBEV serocomplex that was less virulent that related viruses. Shortly after isolation and characterization of LGTV, the Elantcev 15-20/3 strain of the virus was tested in human trials as a potential vaccine for prevention of more severe disease caused by the more virulent members of the TBE complex. Unfortunately, LGTV vaccination resulted in unexpectedly high incidence (~1:10,000) of neurologic disease among vaccinees [35-38].

Powassan virus (POWV) and the closely related DTV are the only members of the TBEV serocomplex viruses known to be endemic in North America. POWV was isolated in northeast Canada in 1958 [39] and has been found in the northern United States, southern Canada and in far-eastern Asia [2,40]. POWV appears to generally be associated with subclinical disease, but occasional cases of severe encephalitis do occur, most recently in June 2011 in Minnesota (ProMED-Mail, June 29, 2011).

## Disease

Members of the TBEV serocomplex cause a range of diseases from subclinical or mild febrile illnesses to severe and lethal encephalitis or hemorrhagic fever. Although a majority of infections with TBEV-Eu appear to be subclinical [2], clinical descriptions of European TBE indicate a primarily biphasic disease with the first phase a relatively mild flu-like illness that is followed by a symptom-free phase of about one week. Approximately 65% of TBE-Eu patients recover after the first phase of disease. Patients who progress to the second phase of disease generally present with high fevers and evidence of neurologic involvement. Typical presentations include meningitis, meningoencephalitis, poliomyelitic or polyradiculoneuritic symptoms [12]. Neurological symptoms mostly resolve as TBEV-Eu infection has a case fatality rate of 1-2% with little evidence of long-term sequelae although the incidence of long-term effects is higher in older (> 60 years) patients [41].

Illness caused by infection with TBEV-FE is generally more severe than that associated with TBEV-Eu infections. Following a 2-18 day incubation period, disease onset can be very sudden with symptoms including headache, high fever, vomiting, myalgia, photophobia and other indications of neurologic disease including evidence of focal encephalitis and meningitis. TBE in these cases is often described as

mono-phasic with a majority of patients progressing directly to the severe phase of disease [41]. The disease can be complicated by flaccid lower motor neuron paralysis and ascending paralysis or hemiparesis [42]. Neurological sequelae are more common in TBEV-FE cases than is seen following TBEV-Eu infections. Sequelae can include atrophy and paresis of the brachial plexus and neck muscles, paresis of the lower extremities and poliomyelitis-like sequelae. The case fatality rate following severe TBEV-FE infection can be 20-30%.

Disease caused by infection with TBEV-Sib is generally described as intermediate between TBEV-FE and TBEV-Eu. However, TBEV-Sib has been associated with chronic or persistent infections in both humans, and primates, [1-5], a phenomenon that has not been described for either the TBEV-FE or TBEV-Eu subtypes.

Disease seen following infection with KFDV and its subtypes generally consists of a hemorrhagic fever type of illness and may be associated with encephalitis whereas infection with OHFV is biphasic in about 50% of cases with limited neurologic manifestations [33]. Infection with KFDV or ALKV may present as epitaxis, hematemesis, cutaneous bleeding, melena or bleeding from venipuncture sites [43,44].

## Therapeutics

There are currently no licensed therapeutics for the treatment of infections by TBEV or related viruses and recommended treatment is largely supportive. A hyperimmune serum therapy regimen available previously has been discontinued due to an unfavorable outcome in disease observed in at least five treated children [45].

## Potential as a biothreat agent

Members of the TBEV serocomplex are considered potential biothreat agents due to their pathogenicity, ability to be aerosolized [46], limited vaccine coverage and availability in many areas of the world, a lack of therapeutics and stability in the environment. Reports in the popular press have also suggested that Russian agents worked to develop RSSEV as a bioweapon although the extent and purpose of this effort are not clear. TBEV has been transmitted repeatedly through consumption of contaminated (unpasteurized milk or milk products) as was documented in early reports from outbreaks in Russia and Eastern Europe [1,47-49]. Despite improved safety in production of milk products, transmission of TBEV in milk continues to be a problem in rural communities [50]. However, despite these high risk considerations, the inability of the TBE viruses to be transmitted human-to-human and the relative inefficiency of tick-to-human transmission limits the potential impact if used as a weapon. In addition, the relatively rapid vaccination schedules for licensed European vaccines with high rates of seroconversion following the second dose would provide some protection during an outbreak scenario. In non-endemic areas such as North America, a more significant risk is the introduction of the virus into the tick populations that could potentially allow the virus to become established in a new environment. The ability of the virus to become established requires both competent vectors and susceptible amplifying hosts. It is not clear if either exists in North America and if they share the same ecosystem, even though the presence of POWV suggests that at least certain regions may provide suitable conditions for permanent establishment.

## TBE vaccines

The first effort to derive a TBE vaccine occurred shortly after the discovery of the virus when Chumakov utilized a formalin-inactivated virus preparation to vaccinate forest workers [24]. In the 1960s TBE vaccines were developed using cell culture systems [51-55] and clinical trials demonstrated vaccine efficacy [56]. In 1971 the Institute of Virology at the University of Vienna and the Microbiological Research Establishment at Porton Down, UK began a collaboration to develop a vaccine for use in Europe. This vaccine was based on the Austrian TBEV-Eu strain Neudörfl. Seed stocks were generated in mouse brain and the virus was then cultivated in specific pathogen free (SPF) chicken embryo cells, clarified by centrifugation, inactivated with formalin and then purified to produce the vaccine virus stock [57]. The purified inactivated virus was stabilized with human albumin and combined with aluminum hydroxide that functioned as the adjuvant. In 1976 the Austrian company Immuno took over vaccine production and began marketing the vaccine as FSME-IMMUN®. This vaccine was administered to over 400,000 people and studies found a seroconversion rate of greater than 90% (by hemagglutination inhibition test) following two doses of the vaccine [58]. An additional booster was indicated approximately 9-12 months after the second dose after it was found that antibody titer quickly waned [59]. In 1979 improvements were made to the vaccine preparations to reduce local and systemic side effects to the vaccination and to increase antigen purity [60]. The modified vaccine showed immunogenicity comparable to the original vaccine but with reduced side effects [61]. Later on, mouse-brain derived seed stocks, thiomersal and human albumin were eliminated to further improve the purity of the vaccine and this new formulation was called TicoVac®. However, the removal of the albumin stabilizer without concurrently adjusting the antigen content resulted in an increased likelihood of vaccine-associated fever in infants and young children [62]. In 2001 FSME-IMMUN® containing human serum albumin was reintroduced in doses for both adult and pediatric applications.

In the late 1980s the German firm Behring-Werke developed a second product called Encepur that is based on the K-23 strain of TBEV-Eu isolated in Germany. Encepur was licensed in 1992 and a pediatric formulation of Encepur was released in 1995 [63]. The production processes of Encepur and FSME-IMMUN® are similar with the only significant differences being in the final formulation. Both vaccines have been shown to induce significant protective antibody titers and are considered essentially equivalent. Encepur is now produced by Novartis while FSME-IMMUN® is produced by Baxter. While FSME-IMMUN® is now also available in Canada, neither product is licensed for use in the United States. An additional vaccine is available in China but little is known about its production or efficacy [9].

In the 1960s and 1970s the LGTV strain Elantcev 15-20/3 was tested as a potential live-attenuated vaccine for TBEV. Initial studies were positive with rapid generation of protective responses and safety in non-human primates and humans [1], but in a large scale clinical study, a high rate of viral neuroinvasion and evidence of vaccine-related neurological illness significantly limited enthusiasm for the use of LGTV as a vaccine for its more pathogenic cousins. More recently the LGTV backbone has be used for development of chimeric dengue vaccines [64,65].

Development of a vaccine for KFDV began in the early 1960s with the use of a mouse-brain derived formalin inactivated vaccine based on a TBEV-FE virus that was developed at the Walter Reed Army Institute of Research (WRAIR) laboratory in Washington, DC. Vaccinees receiving the vaccine had few vaccine related side effects, but the vaccine elicited a poor protective immune response as was subsequently abandoned [66-68]. Subsequent development of both mouse brain and

| Product | FSME-IMMUN® | Encepur ™ | EnceVir | TBE-Moscow |
|---|---|---|---|---|
| **Manufacturer** | Baxter Vaccines, Vienna, Austria | Novartis Vaccines and Diagnostics, Germany | Virion Corporation, Tomsk, Russia | Chumakov Institute for Poliomyelitis and Viral Encephalitides, Moscow, Russia |
| **Virus strain (subtype)** | Neudörfl (European subtype) | K-23 (European subtype) | 205 (Far-Eastern subtype) | Sofjin (Far-Eastern subtype) |
| **Production method** | Cultured in primary chicken embryo cells, purified after formaldehyde inactivation by continuous-flow zonal centrifugation | Cultured in primary chicken embryo cells, purified after formaldehyde inactivation by continuous-flow zonal centrifugation. | Cultured in primary chicken embryo cells, purified and concentrated after formaldehyde inactivation; treated with protamine sulfate. | Cultured in primary chicken embryo cells, purified and concentrated after formaldehyde inactivation; treated with protamine sulfate. Lyophilized in excipient. |
| **Excipients** | Aluminium hydroxide, human serum albumin. | Aluminium hydroxide, sucrose. | Aluminium hydroxide, human serum albumin (250µg/dose). | Aluminium hydroxide (final formulation), human albumin (500µg/ dose), gelatin and sucrose. |
| **Distributed product** | Stored as liquid formulation in pre-filled syringe. | Stored as liquid formulation in pre-filled syringe. | Stored as liquid formulation. | Lyophilized in excipient, mixed with saline containing aluminium hydroxide just prior to administration. |
| **Shelf life (2-8°C)** | | | 2 years | 3 years |
| **Countries licensed** | Austria, Germany, Switzerland, Hungary, Czech Republic, Baltic States, UK, Canada | Germany, Austria, Switzerland, Czech Republic, Baltic States, Russia | Russia | Russia |
| **Pediatric vaccine** | 1 to < 16 years old | 1 to < 12 years old | Formulation is licensed for all persons >3 years old. | Formulation is licensed for all persons >3 years old. |
| **Vaccination schedule Conventional:** | 0, 1-3 months, 6-15 months<br>Booster doses for adults as per 2005 Austrian Immunization Plan:<br>< 60 years: first booster after 3 years, subsequently 5-year intervals<br>≥ 60 years: 3-year intervals<br>Booster doses for children per manufacturer: 3-year intervals | 0, 1-3months, 10-15 months<br><br>Booster doses for adults as per 2005 Austrian Immunization Plan:<br>< 60 years: first booster after 3 years, subsequently 5-year intervals<br>≥ 60 years: 3-year intervals<br>Booster doses for children per manufacturer:<br>1st boost at 3-year interval then at 5 year intervals | 0, 5-7 months<br>First booster 12 months after second dose, then boosters every 3 years. | 0, 1-7 months<br>First booster 12 months after second dose, then boosters every 3 years. |
| **Vaccination schedule Accelerated:** | Day 0, day 14, 6-15 months<br>booster doses: as above | Two schedules available:<br>A. Day 0, day 14, 10-15 months. Booster doses as above<br>B. Day 0, 7, 21<br>First booster at 12-18 months, subsequent booster doses as above | 0, 1-2 months<br>First booster 12 months after second dose, then boosters every 3 years. | 0, 1 month<br>First booster 12 months after second dose, then boosters every 3 years. |
| **Immunogenicity (seroconversion) Conventional schedule:** | **Adults:**<br>92.9%-97% after second dose, 100% after third dose<br>**Children:**<br>98.5%-100% after second dose, 100% after third dose | **Adults:**<br>100% after second dose<br>**Children:**<br>95-99% after second dose, 100% after 3rd dose | **Adults:**<br>82-89% after second dose<br>**Children:**<br>84-97% after second dose | **Adults:**<br>84-93% after second dose<br>**Children:**<br>89-96% after second dose |
| **Immunogenicity (seroconversion) Accelerated schedule:** | **Adults:**<br>clinical trials not available<br>**Children:**<br>95% after second dose | **Adults:**<br>100% after primary series (3 doses)<br>**Children:**<br>100% after primary series (3 doses) | **NA** | **NA** |
| **Safety** | Mild-moderate systemic and local reactions common<br>Fever in very young children common<br>Fever in older children occasional<br>Fever in adults infrequent<br>Severe neurologic reactions very rare | | Large scale randomized trials have not been published<br>Moderate reactigenicity identified in small scale trials<br>In 2010-2011, some lots of EnceVir associated with frequent high fever and allergic reactions in children. EnceVir subsequently removed from pediatric use. | |

**Table 1:**

cell culture derived vaccines utilizing inactivated KFDV met with better success. These vaccines elicited a protective immune response in some vaccinees, but the response was not consistent and did not appear to provide complete protection [69,70]. Currently a formalin inactivated KFD vaccine cultivated in chick embyo fibroblasts has been licensed for use in India. This vaccine has reasonable efficacy, but is only used on outbreak situations and requires an annual booster to retain sufficient protective antibody titers [71].

Two vaccines based on TBEV-FE strains are currently licensed for use in Russia. The TBE-Moscow vaccine was licensed for adult use in 1982 and for children ≥3 years old in 1989. TBE-Moscow is based on the Sofjin strain cultivated in primary chick embryo cells. The virus is formalin inactivated, purified and stabilized with human albumin. The EnceVir vaccine was licensed for both adult and pediatric (≥3 years) use in 2001. EnceVir uses the TBEV-FE strain 205 and is similar in manufacture and preparation to the TBE-Moscow vaccine [9]. Both

Russian vaccines have shown safety and efficacy profiles similar to those seen for the European vaccines. In adults, vaccine associated reactogenicity was limited to primarily local responses following vaccination with either Russian vaccine [9,72]. However, an increased incidence of fever and allergic reactions following vaccination with particular lots of EnceVir led to it being withdrawn from pediatric use pending reformulation [9]. The EnceVir vaccine is currently not recommended for use in children (≤ 17 years old). Protective antibody responses were measured in 90-100% of vaccinees, depending upon the study design and dosing schedule, and surveillance trials following a large-scale vaccination program indicated antibody persistence for ≥3 years following a 3 dose vaccination schedule [9].

Given the significant genetic similarity and serological cross-reactivity between the TBEV serocomplex viruses, vaccines developed for protection against one subtype should be cross protective against all TBEV subtypes. Cross-protection against multiple subtypes has been shown in studies in mice [73] and in human cross-neutralization studies utilizing sera from vaccines [74]. These studies found equivalent neutralization titers against both TBEV-Eu and TBEV-FE subtypes and somewhat lower neutralization titers against OHFV [9, 73,74]. Preclinical studies in mice also found vaccine dose-dependent protection against infection with several TBEV-Eu and TBEV-FE strains in addition to protection against the related Louping Ill virus [75] and POWV (M. Holbrook-personal observations).

## Novel vaccine technologies

The production of inactivated vaccines carries the inherent risk of utilizing large quantities of potentially highly pathogenic viruses and the possibility of incomplete inactivation of viruses. In addition, vaccines based on inactivated viruses as antigens have shown a certain level of adverse reactions, especially in children, that has to be carefully balanced with efficacy and durability [76]. These risks, while minimized by quality control efforts by manufacturers, are real. Subsequently a number of researchers have evaluated alternative strategies for development of vaccines that includes development of live-attenuated viruses [77-79], DNA vaccine technology [80] and the use of subunit vaccines (Coller et al, in preparation). None of these novel vaccine strategies have reached clinical trials.

## Summary

The members of the tick-borne encephalitis virus serocomplex present significant health risks in a large proportion of the world, particularly in Europe and across Asia. With an estimated 10-12,000 annual cases of TBE reported (this figure is believed to significantly underestimate the actual total), these viruses present a real and specific risk in their endemic regions. Fortunately, several established vaccines are very effective for prevention of TBE. Two inactivated virus vaccines manufactured in Central Europe have been used with very good success throughout Europe. These vaccines are also available for travelers from the United Kingdom and Canada. Two vaccines manufactured in Russia appear to be essentially equivalent to those produced in Europe. Perhaps the biggest limitation to the effective use of the TBE vaccines is the ability to maintain complete vaccine coverage within endemic regions. The vaccination schedule requires three doses to stimulate the development of a significant and relatively long-lasting protective antibody response. However, booster vaccinations are required every 3-5 years to maintain protective immunity, especially in an elderly population.

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