

TESCHOVIRUS ENCEPHALOMYELITIS

SUMMARY

Teschovirus encephalomyelitis was first described as a particularly virulent, highly fatal encephalomyelitis of pigs and was previously known as Teschen disease (or enterovirus encephalomyelitis). It is caused by strains of porcine teschovirus serotype 1 (PTV-1) of the genus *Teschovirus*, family Picornaviridae. Less severe forms of the disease were first recognised in the United Kingdom, where it was called Talfan disease, and in mainland Europe, where it was called poliomyelitis suum or benign enzootic paresis. In addition to PTV-1 strains, the milder form of the disease can be caused by other PTV serotypes, including PTV-2, -3, -4, -5, -6, -9 and -10.

The disease was first described in Teschen, Czechoslovakia in 1929. During the 1940s and 1950s it caused serious losses in European countries and was spread to other continents. More recently outbreaks have occurred in Haiti and the Dominican Republic. The clinical disease is otherwise rare and has not been reported in Western Europe since 1980. However, there has been serological evidence that virus variants, which are not pathogenic or of low pathogenicity, circulate in pig populations.

Identification of the agent: The virus has affinity for the central nervous system and therefore suspensions of brain and spinal cord from affected pigs are used as inocula for virus isolation. The virus propagates successfully on monolayers derived from swine tissue, in particular from kidney. If PTV is present, it gives rise to specific cytopathic effects characterised by rounded refractile cells. For PTV identification and serotyping, suitable tests are employed using specific antisera or monoclonal antibodies against standard strains of PTV. Virus neutralisation tests and indirect fluorescent antibody tests are preferred. Reverse-transcription polymerase chain reaction amplification of parts of the viral genome is possible, but as yet no specific tests have formally been accepted for diagnosis.

Serological tests: As the seroprevalence of PTV-1 may exceed 60% in healthy pig populations in Central Europe, and identical clinical signs may be caused by other viruses, including other serotypes of PTV, a single serological test for PTV-1 giving positive results does not indicate that the neurological signs observed are actually caused by a PTV-1 infection. A four-fold rise in titre together with typical signs should be considered to be an indication that PTV-1 infection caused clinical disease. For screening for specific antibodies in pig populations, it is recommended to use the virus neutralisation test in microtitre plates or the enzyme-linked immunosorbent assay.

Requirements for vaccines: When clinical disease was common, vaccines were available and used; however, as the disease is now rare, vaccines are no longer available.

A. INTRODUCTION

Teschovirus encephalomyelitis (previously Teschen/Talfan diseases, and later enterovirus encephalomyelitis) is an acute condition of pigs characterised by central nervous system (CNS) disorders. Teschen is the name of the town in the Czech Republic where the disease was first recognised in 1929 (Klobouk, 1931; 1933). In the 1950s, the disease spread throughout Europe and caused huge losses to the pig breeding industry. Less severe forms of the disease were first recognised in the United Kingdom, where it was called Talfan disease, and in Denmark, where it was called poliomyelitis suum; these were benign enzootics of swine. *Teschovirus encephalomyelitis* has not been reported in Western Europe since 1980 (Austria) and the disease is now considered rare. In the last 20 years (since 1996) disease was reported to the OIE by the following countries: Belarus (1996, 1999 and 2005), Japan (2002), Latvia (1997 and 2000–2002), Madagascar (1996–2000, 2002 and 2004–2005), Moldavia (2002–2004), Romania (2002), Russia (2004), Uganda (2001) and Ukraine (1996–2005). In most of these cases it is not

known if diagnosis was made purely on clinical grounds or in conjunction with laboratory tests; the exception being in Japan in 2002 (Yamada *et al.*, 2004).

The causal agent of teschovirus encephalomyelitis is porcine teschovirus serotype 1 (PTV-1), which belongs to the species *Teschovirus A*, genus *Teschovirus*, family *Picornaviridae* (Betts, 1960; Klobouk, 1933; Knowles *et al.*, 2012). Originally the PTVs were classified within the genus *Enterovirus* and the original 11 porcine enterovirus (PEV) serotypes, PEV-1 to PEV-11, were placed in three groups – I, II and III – on the basis of cytopathic effect (CPE) produced, serological assays and replication in different cell cultures (Knowles *et al.*, 1979). PEV-1 to PEV-7 and PEV-11 to PEV-13 were identified as group I. Based on nucleotide sequencing and phylogenetic analysis, the PEV group I viruses have now been placed in the genus *Teschovirus*. PEV-1 to -7 have been renamed PTV-1 to -7, and PEV-11 to -13 were renamed PTV-8 to 10; additional types PTV-11 to PTV-13, have also been described (based on nucleotide sequence differences in VP1) (Boros *et al.*, 2012; Cano-Gomez *et al.*, 2011; Krumbholz *et al.*, 2002). PEV group II contained PEV-8, which has now been reclassified as porcine sapelovirus 1, species *Sapelovirus A*, genus *Sapelovirus*. Group III consisted of PEV-9 and PEV-10, and has now been reclassified as enterovirus G1 and G2, species *Enterovirus G*, genus *Enterovirus*.

Outbreaks of severe teschovirus encephalomyelitis in pigs occurred in Haiti in February 2009, and PTV-1 was isolated from brain samples of sick pigs. Phylogenetic analyses on the polyprotein gene indicated that the Haitian isolate was closely related to other PTV-1 strains, including the strain Konratice that was isolated in Czech Republic from pigs with Teschen disease (Deng *et al.*, 2012). Surveillance studies indicated that infection was prevalent in multiple regions in Haiti and the Dominican Republic.

PTV-2, -3, -4, -5, -6, -9 and -10 have been isolated from pigs with milder forms of the disease (Witte von *et al.*, 1994). PTV infections often do not produce clinical signs. Serotypes may be differentiated using a virus neutralisation (VN) test (Betts, 1960; Knowles *et al.*, 1979), complement fixation test (Knowles & Buckley, 1980) or indirect fluorescent antibody (IFA) test (Auerbach *et al.*, 1994; Romanenko *et al.*, 1982).

PTV infections only occur in swine (including wild boar); other animal species, including humans, are not known to be susceptible.

Differential diagnoses include pseudorabies (Aujeszky's disease) and classical swine fever (acute form). In addition, Japanese encephalitis, *Streptococcus suis* and haemagglutinating encephalomyelitis may occasionally produce similar clinical signs. Non-infectious aetiologies, in particular toxicities, must also be considered.

PTV may be identified serologically using standard antisera that have been prepared by hyperimmunisation of guinea-pigs, rabbits, or colostrum-deprived piglets with standard strains of PTV serotypes 1–11; these reagents are not known to be available for types 12 and 13.

The virus enters the animal via the oral or nasal cavity. The incubation period is about 14 days. The main signs of the prodromal stage are fever up to 41.5°C, lassitude, anorexia and locomotor disturbances. This stage is followed by hypersensitivity, tremors, clonic spasms of the legs, flaccid paralysis, opisthotonos and nystagmus; convulsions may be observed in young pigs. In the final clinical stage, paralysis proceeding from the hind part through the loins to the fore part of the body is observed. Paralysis of the thermoregulatory centre results in hypothermy. When respiratory muscles are paralysed, the animal dies of suffocation.

For histological diagnosis, samples of cerebrum, cerebellum, diencephalon, medulla oblongata and cervical and lumbar spinal cord are collected. The samples are fixed in formaldehyde and sections are stained using conventional histological methods. The virus multiplies in the CNS causing a nonsuppurative polio-encephalomyelitis with lymphocytic perivascular cuffs, especially in the spinal cord (Klobouk, 1931). Pathological changes are observed in the grey matter of the diencephalon, cerebellum, medulla oblongata and in the ventral horns of the spinal cord, consistently including dorsal root ganglia and trigeminal ganglia (ganglioneurites) and to a lesser extent in the cerebral hemispheres. Lesions may involve the dorsal horns of the spinal cord in very young animals. Degeneration of neurons (swelling, chromatolysis, necrosis, neuronophagia, axonal degeneration) and their replacement by microgliosis (astrocytosis, astrogliosis) develops in the late stage of the disease (Cantile & Youssef, 2016).

Detection of teschovirus antigens by immunohistochemistry on fixed, paraffin-embedded CNS sections is very difficult and not consistently possible. If suitable specific antisera or monoclonal antibodies are available, as well as specific detection techniques, correlation of pathological changes with the location of the agent may be possible on fixed, paraffin-embedded sections of the CNS.

Laboratory diagnosis of the disease is based on identification of the virus in the CNS of affected pigs, and on the detection of specific antibodies in the blood of convalescent animals.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of *Teschovirus encephalomyelitis* and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification¹						
Virus isolation	–	+++	–	+++	+	–
Antigen detection	–	–	–	++	++	–
Real-time RT-PCR	–	+	–	++	++	–
Detection of immune response						
AGID	–	–	–	+	–	–
CFT	–	–	–	++	–	–
ELISA	–	–	–	++	+	–
VN	–	–	–	+++	+++	+++

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose; n/a = purpose not applicable.

RT-PCR = reverse-transcription polymerase chain reaction; AGID = agar gel immunodiffusion; CFT = complement fixation test; ELISA = enzyme-linked immunosorbent assay; VN = virus neutralisation.

1. Identification of the agent

1.1. Isolation of the virus

Progress in diagnosis of teschovirus encephalomyelitis and vaccine production has been made possible by the propagation of virus in cell culture (Madr, 1959; Mayr & Schwoebel, 1957).

Samples of brain and spinal cord are collected from pigs slaughtered at an early clinical stage of the disease. When not processed immediately, the samples should be placed in a solution prepared from equal parts of phosphate buffered isotonic saline solution (PBS), pH 7.4, and glycerol. Pieces of tissue are minced to prepare a 10% (w/v) suspension in PBS. The suspension is centrifuged at 800 **g** for 10 minutes and the supernatant fluid is used for inoculation of cell cultures. Monolayer cultures of primary porcine kidney or established cell lines derived from porcine tissue are suitable for isolation of PTV.

1.1.1. Test procedure

- i) Test tubes or tissue culture vessels with monolayer cell cultures are used. Growth medium is discarded and tubes or vessels are inoculated with 0.1 ml of suspect tissue homogenate.
- ii) Inoculated test tubes are placed on a roller drum or tissue culture vessels are placed on a tray and incubated for 1 hour at 37°C.
- iii) The inoculum is discarded; the tubes or tissue culture vessels are washed with PBS and replenished with 1–20 ml (depending on the type of tissue culture vessel used) of maintenance medium without calf serum.

¹ A combination of agent identification methods applied on the same clinical sample is recommended.

- iv) The tubes are examined microscopically each day. If the sample contains PTV, characteristic CPE will be seen after 3–4 days. The CPE is characterised by small foci of rounded refractile cells. After several passages the virus grows better and produces complete CPE. The identity of PTV can be confirmed by the use of specific antiserum or monoclonal antibodies. The VN or the IFA test is best suited to this purpose. Once an isolate has been identified serologically as PTV, piglet inoculation is the only certain means of determining that the given isolate is pathogenic.

1.2. Virus neutralisation test for porcine teschovirus identification

The virus harvested from cell cultures is diluted in cell culture maintenance medium over the range 10^{-1} to 10^{-6} in tenfold steps. For teschovirus serotyping, 12 rows of each dilution are prepared; 50 μ l of standard antisera to PTV-1–11 diluted 1/10 is added to rows 1–11 and 50 μ l of negative serum is added to the last row; if antisera to types 12 and 13 are available, a second plate would be required. Mixtures are incubated overnight at 4°C or for 1 hour at 37°C and thereafter inoculated into roller tube cultures or into wells of microtitration plates with confluent monolayer cell cultures. The inoculated cell cultures are incubated at 37°C. Assessment is carried out 72 hours later and every following day up to day 10, depending on when the CPE is seen. The identification of a PTV serotype is confirmed if the titre of the isolated virus in the presence of that antiserum is at least 10^3 lower than that virus incubated with negative serum.

1.3. Indirect fluorescent antibody test for the confirmation of porcine teschovirus antigen in cells

The IFA test is based on the reaction of the antigens in infected cells with specific antibodies in positive serum (Romanenko *et al.*, 1982). The reaction is visualised by a fluorescein isothiocyanate (FITC)-conjugated antiglobulin, using a microscope with a UV or a blue light source. The antigen is detectable in cells 12 hours after the infection with PTV, i.e. before the development of CPE. Polyclonal antisera often show cross-reactivity with different PTV types, which can confuse the interpretation of results.

1.3.1. Test procedure

- i) Monolayers of porcine kidney cells on cover-slips are inoculated with the suspected material. Positive and negative controls should be processed in parallel with the test specimens.
- ii) After incubation for 12–16 hours, the cover-slips are removed, washed twice in PBS, air-dried and fixed in cold acetone for 5–15 minutes.
- iii) The cover-slips are placed into a wet box and flooded with rabbit or pig hyperimmune anti-PTV serum optimally diluted 1/10 with PBS or with PTV-specific monoclonal antibody at working dilution.
- iv) The wet box is closed and incubated at 37°C for 60 minutes.
- v) The cover-slips are removed and washed three times in PBS, then flooded with FITC-conjugated anti-rabbit or anti-pig goat serum, at a previously assessed working dilution, and incubated at 37°C for 30 minutes.
- vi) The cover-slips are then washed three times with PBS, air-dried and mounted in 0.1 M Tris-buffered glycerol, pH 8.6.

After processing, the cover-slips are examined microscopically. The control slides are examined first to confirm that the fluorescence observed is specific. The fluorescence is apple green in colour and occurs in the cell cytoplasm and at the periphery of the nucleus. Instead of cover-slips, multispot slides or multiwell plates can also be used.

1.4. Reverse-transcription polymerase chain reaction

The reverse-transcription polymerase chain reaction (RT-PCR) provides a method for detection and differentiation of specific gene regions of porcine teschoviruses (Palmquist *et al.*, 2002; Zell *et al.*, 2000). The nested RT-PCR with specific primer sets has been used to differentiate between PTVs and PEVs (Zell *et al.*, 2000). PCR is more rapid and less laborious than virus isolation by tissue culture technique and serotyping. However, the PCR technique is currently restricted to specialised laboratories.

2. Serological tests

As the seroprevalence of PTV-1 may exceed 60% in healthy pig populations in some countries of Central Europe, and identical clinical signs may be caused by other viruses – including other serotypes of PTV – a single serological test for PTV-1 giving positive results does not indicate that neurological signs observed are actually caused by PTV-1. A four-fold rise in titre together with typical signs should be considered to be an indication that PTV-1 infection caused clinical disease. Another reason that paired serum samples are needed for confirmation of the significance of titres is that cross-reactions have been reported with orphan teschoviruses.

Pigs that have recovered from disease, or those with inapparent disease, produce specific antibodies. Several serological methods are available for their detection, of which the microtitre VN test using pig kidney cell cultures is the most useful (Mayr & Bibrack, 1971). An ELISA has been developed that is more sensitive and rapid (Hubschle *et al.*, 1983).

For serological diagnosis it is necessary to have standard strains of PTV serotypes propagated in cell cultures and hyperimmune serum monospecific for PTV types.

2.1. Standard strains of porcine teschoviruses

2.1.1. Characteristics

Following long experience, the strain 'Zabreh', isolated in Czechoslovakia during the period of peak incidence of the disease, was selected as the standard strain to generate the severe form of teschovirus encephalomyelitis. The pathogenicity of the strain is maintained by intracerebral passages in healthy, colostrum-deprived piglets. The virus produces typical signs of teschovirus encephalomyelitis after an incubation period of 5–7 days. For serological diagnosis, the following strains of PTV serotypes should be used as standard strains: type 1: Talfan, type 2: T80, type 3: O2b, type 4: PS36, type 5: F26, type 6: PS37, type 7: F43, type 8: UKG/173/74, type 9: Ger-2899/84, type 10: Ger-460/88, type 11: Dresden, type 12: CC25/SPA/2006, type 13: wild boar/WB2C-TV/2011/HUN.

2.1.2. Stock virus

Standard strains are propagated on monolayers of cell culture either from primary porcine kidney or testes or on an established cell line, for example PK-15. A 10% suspension in PBS, pH 7.4, is prepared from the brain and spinal cord of piglets infected experimentally with PTV. Some types are isolated from faeces. The suspension is centrifuged and the supernatant is used for the inoculation of cell cultures. The procedure for the cultivation of PTV in cell cultures is as follows:

The growth medium is removed from the cell culture and after rinsing with buffered saline, cells are inoculated with the virus suspension at 37°C. The size of the inoculum should be equal to 10% of the growth medium. After 1 hour of incubation at 37°C, the inoculum is decanted, the culture vessel is rinsed with buffered saline, and the cells are overlaid with the appropriate volume of serum-free medium supplemented with antibiotics. CPE is apparent within 48 hours, and the monolayer disintegrates more or less completely during the next 48–72 hours. In the subsequent three to five passages in cell culture, the development of the CPE accelerates and the concentration of virions increases. Titration of the virus is performed in tube cultures or on microtitre plates. A cell-adapted strain usually reaches TCID₅₀ (50% tissue culture infective dose) titres of 10⁶–10⁷ per ml.

The fluid harvest is checked for specificity using known specific hyperimmune antiserum. Treatment with 5% chloroform and cultivation in human and bovine cell cultures and chicken embryos is used to exclude contamination with other viruses. PTV is chloroform resistant and multiplies only in cultures of swine origin. Immunofluorescent antibody staining is useful to detect possible contaminants that are also chloroform resistant and propagate on cells of swine origin (e.g. parvovirus), or that are non-cytopathic. The stock virus should be dispensed into small aliquots and preserved at –60°C. Frozen virus retains its properties for several years. For stock virus that is to be used in the neutralisation test, a constant dose of 100 TCID₅₀ is recommended.

2.2. Specific hyperimmune serum

Specific hyperimmune serum is obtained by repeated immunisation of guinea-pigs, rabbits or colostrum-deprived piglets with PTV. Although the animals are selected from specific pathogen free breeds, they are nonetheless tested before immunisation for absence of antibodies against PTV. The

standard strains should be used. Rabbits are immunised either intravenously, using virus suspension alone, or subcutaneously or intraperitoneally, using the virus suspension with 10% oil adjuvant. Good results may be obtained by administering three doses of 2 ml of virus suspension plus 0.2 ml oil adjuvant, at intervals of 2 weeks. The rabbits are bled 10 days after the last immunisation. Piglets are immunised in the same way. The harvested sera are clarified by centrifugation and stored in small aliquots at -20°C . The sera are titrated using a neutralisation test and constant antigen. Only sera with an antibody titre of at least 1/256 can be used for the identification of the virus.

2.3. Virus neutralisation test in microtitre plates

The test is performed in flat-bottomed cell culture microtitre plates, using low passage porcine kidney or testes cells or cell lines derived from porcine cells. Stock virus is grown in cell monolayers. The virus harvested from cell cultures is clarified by centrifugation and stored in aliquots at -70°C . Culture medium, such as Eagle's complete medium or LYH (Hanks balanced salt solution with yeast extract, lactalbumin and antibiotics), is used as diluent. The virus harvested from cell cultures is clarified by centrifugation and stored in aliquots at -70°C or as 50/50 mixture with glycerol and can be stored at -20°C .

2.3.1. Test procedure

- i) Inactivate swine sera for 30 minutes at 56°C .
- ii) The sera to be tested are diluted in cell culture medium in twofold steps from 1/2 to 1/64, four wells per dilution and 50 μl volumes per well.
- iii) Controls include positive and negative sera, cells and medium control.
- iv) Add to each well 50 μl of virus stock previously diluted in culture medium to provide 100 TCID₅₀.
- v) Incubate for 1 hour at 37°C with the plates covered. The residual virus stock is also incubated.
- vi) Make back titrations of the residual virus stock in four tenfold dilution steps using 50 μl per well and four wells per dilution.
- vii) Add 50 μl of porcine kidney cell suspension at 5×10^5 cells per ml.
- viii) After further shaking, lids are put on and the plates are incubated at 37°C in a 5% CO₂ atmosphere for 2–3 days or longer, to a maximum of 8 days.
- ix) Examine the plates microscopically for CPE. The test should be validated by checking the back titration of virus and titration of positive control serum. Virus should give a value of 100 TCID₅₀ with a permissible range of 30–300. The standard positive serum should give a titre within 0.3 log₁₀ units from its predetermined mean. A negative serum should give no neutralisation at the lowest dilution tested, i.e. 1/2.
- x) The VN results are determined by the Spearman–Kärber method as the dilution of serum that neutralised the virus in 50% of the wells.
- xi) Virus neutralisation titres are regarded as positive if the corresponding serum neutralises the virus at an initial serum dilution of 1/8 or higher.

2.4. Enzyme-linked immunosorbent assay

An alternative method for the detection and titration of specific antibodies against PTV is the ELISA technique (Hubschle *et al.*, 1983). The test is performed in microtitre plates using PTV grown on cell cultures as antigen. The technique can be carried out using the following steps.

2.4.1. Antigen preparation

- i) Virus is propagated on monolayers of cell culture either from primary porcine kidney or testes or on an established cell line, for example PK-15. The growth medium is removed from the cell culture and after rinsing with buffered saline, cells are inoculated with virus suspension at a low multiplicity of infection. After 30 min of incubation at 37°C , cells are overlaid with the appropriate volume of serum-free medium supplemented with antibiotics. Incubation at 37°C is continued with daily microscopic observations. CPE should be apparent within 48 hours, and the monolayer disintegrates more or less completely during the next 48–72 hours. A cell-adapted strain usually reaches TCID₅₀ (50% tissue culture infective dose) titres of 10^6 – 10^7 per ml.

- ii) The harvested virus is clarified by centrifugation at 200 **g** for 15 minutes, and then precipitated with a final 50% saturated $(\text{NH}_4)_2\text{SO}_4$ for 120 minutes at 4°C.
- iii) After centrifugation at 2000 **g**, the resulting precipitate is suspended in TEN buffer (Tris-hydroxymethyl-methylamine [0.01 M], ethylene diamine tetra-acetic acid [1 mM] and NaCl [0.15 M]), pH 7.4, to 1/100 of the initial volume.
- iv) The concentrated viral suspension is extracted by shaking with freon 3/1 for 10 minutes at 4°C.
- v) Following further centrifugation, the supernatant is divided into two separate phases. The upper aqueous phase, containing the viral antigen, is desalinated by passage through a 2.5 x 40 cm cylinder packed with sephadex G 25.
- vi) The viral solution is finally concentrated by ultracentrifugation at 160,000 **g** for 3 hours.
- vii) The pellet is suspended in TEN buffer, pH 7.4, in approximately 1000th the initial volume of virus.
- viii) Insoluble proteins are separated by light centrifugation, and the supernatant is used as the positive antigen in the ELISA.

2.4.2. Test procedure

- i) Plates are sensitised with pre-diluted antigen in phosphate buffered saline (PBS), pH 7.2, by adding 100 µl to each well. The adsorption of antigen to the surface of the plate takes place overnight at 4°C. Parallel rows of the plate should be treated with negative antigen.
- ii) The plate is washed five times in PBS to remove excess antigen.
- iii) Test sera are diluted 1/20 with PBST (PBS solution containing 0.05% Tween 20). 50 µl of the diluted sera is placed into each of two wells with positive antigen and into two wells with negative antigen. (Negative antigen is prepared as described above except that the tissue culture is not inoculated with virus and cells are disrupted by freezing.) The plate is incubated for 1 hour at 37°C.
- iv) The plates are washed five times with PBST.
- v) A predetermined dilution of horseradish peroxidase conjugated with anti-swine immunoglobulin prepared in rabbits is added in 50 µl quantities to each well. The plates are further incubated for a further 1 hour at room temperature.
- vi) The plates are washed five times in PBS.
- vii) Substrate solution (0.1% ortho-phenyldiamine with 0.03% hydrogen peroxide in PBS, pH 6.0) is added in 100 µl quantities to each well.
- viii) After the addition of substrate, positive samples change colour to dark brown. When a sufficient degree of colour reaction is seen in the wells of known positive sera, the reaction is stopped by addition of 50 µl of 2 M sulphuric acid to each well. The absorbance of the wells is measured at a wavelength of 492 nm, preferably using an automatic multi-channel spectrophotometer with print-out mechanism. Positive and negative sera and non-infected cells should be processed as controls in parallel with the test specimens.
- ix) The absorbance of a serum is the mean reading of two wells with positive antigen minus the mean reading of two wells with negative antigen. Absorbance readings of test sera that exceed by more than twofold the mean reading of standard negative sera are regarded as positive.

C. REQUIREMENTS FOR VACCINES

1. Vaccines against teschovirus encephalomyelitis

During the period of highest incidence of the disease in central Europe and Madagascar, active immunoprophylaxis was an important means for the control of this infection (Traub, 1942). As severe clinical disease has largely disappeared, vaccination has been discontinued and the vaccine is no longer being produced or used anywhere in the world.

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NB: FIRST ADOPTED IN 1991 AS TESCHEN DISEASE. MOST RECENT UPDATES ADOPTED IN 2017.