CHAPTER 2.1.17.

TRYPANOSOMA EVANSI INFECTIONS
(INCLUDING SURRA)

SUMMARY

Definition of the disease: Trypanosoma evansi causes a disease known as Trypanosomosis¹ ('surra'). It affects a number of species of domesticated animals in Asia, Africa and Central and South America. The principal host species affected varies geographically, but buffalo, cattle, camels and horses are particularly affected, although other animals, including wildlife, are also susceptible. It is an arthropod-borne disease. Several species of haematophagous flies, including Tabanus spp. and Musca spp., are implicated in transferring infection from host to host mechanically vectors. In Brazil, vampire bats have been implicated in transmission.

Description of the disease: The disease in susceptible animals is manifested by pyrexia, directly associated with parasitaemia, together with a progressive anaemia, loss of condition and lassitude. Recurrent episodes of fever and parasitaemia occur during the course of the disease. Oedema, particularly of the lower parts of the body, urticarial plaques and petechial haemorrhages of the serous membranes are often observed. Abortions have been reported in buffaloes and camels. There are indications that the disease causes immunodeficiencies.

Identification of the agent: The general clinical signs of T. evansi infection are not sufficiently pathognomonic for diagnosis. Laboratory methods for detecting the parasite are required. Examination of the host blood is problematic as trypanosomes can be detected only when there is a high parasitaemia. Under these circumstances examination of wet blood films, stained blood smears or lymph node material might reveal the trypanosomes. In other, more chronic cases, such as the carrier state, the examination of thick blood smears, as well as methods of parasite concentration and the inoculation of laboratory animals are required.

Serological tests: Infection gives rise to specific antibody responses and a variety of antibody detection tests have been introduced for laboratory and field use. Some have been partially validated, but await large-scale evaluation and standardisation. Among those that are used regularly in the laboratory are immunoenzyme assays, card agglutination tests and latex agglutination tests. For field use both card agglutination tests (CATT) for T. evansi and latex can be applied, yet an individual test format (pen side test) is currently unavailable. Assays for detection of circulating antibodies have high measures of validity. Estimates of predictive values of different serological tests indicate that enzyme linked immunosorbent assays (ELISA) for detecting IgG antibodies are more likely to classify correctly uninfected animals, and CATT are more likely to classify correctly truly infected animals. An IgG ELISA would thus be suitable for verifying that animals are free from infection, prior to movement or during quarantine. In situations where there is overt disease, CATTs can be used to target individual animals for treatment with trypanocidal drugs. For declaring a disease-free status, serial testing – ELISA followed by re-testing of suspect samples by CATT – is recommended. It must be stressed however, that there are considerable antigenic similarities among the different species of pathogenic trypanosomes, hence in areas where tsetse-transmitted trypanosomoses occur cross-reactions may occur with any serological test employed.

Requirements for vaccines and diagnostic biologicals: No vaccines are available for the disease.

¹ Nomenclature of parasitic diseases: see the note in Chapter. 2.4.18 Trypanosomosis (Tsetse-borne).
A. INTRODUCTION

The clinical signs of surra, the disease caused by Trypanosoma evansi, are indicative but are not sufficiently pathognomonic and diagnosis must be confirmed by laboratory methods. The disease in susceptible animals, including cattle, buffalo, camels (dromedary and bactrian), horses, pigs, sheep and goats, is manifested by pyrexia, directly associated with parasitaemia, together with a progressive anaemia, loss of condition and lassitude. Recurrent episodes of fever and parasitaemia occur during the course of the disease. Oedema, particularly of the lower parts of the body, urticarial plaques and petechial haemorrhages of the serous membranes are often observed. Abortions have been reported in buffaloes and camels (8, 17) and there are indications that the disease causes immunodeficiency (5, 21).

There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species to disease. Disease may manifest as an acute or chronic condition, and in the latter case may persist for many months. The disease is often rapidly fatal in camels, buffaloes, horses, cattle, llama and dogs, but mild and subclinical infections can also occur in these species. Wild animals such as deer and capybara can become infected. Animals subjected to stress – malnutrition, pregnancy, work – are more susceptible to disease.

Biologically T. evansi is very similar to T. equiperdum, the causative agent of dourine (2), and morphologically resembles the slender forms of the tsetse-transmitted trypanosomes, T. brucei, T. gambiense and T. rhodesiense. Molecular characterisation indicates that various strains of T. evansi isolated from Asia, Africa and South America have a single origin. Molecular characterisation using random amplified polymorphic DNA techniques and endonuclease fingerprinting showed that isolates of T. evansi and T. equiperdum formed a closely homogeneous group. One possibility of this finding is that T. equiperdum is not a genuine species per se and that the clinical outcome of disease is related primarily to the hosts’ immune response. Like all pathogenic trypanosomes, T. evansi is covered by a dense protein layer consisting of a single protein called the variable surface glycoprotein. This acts as a major immunogen and elicits the formation of specific antibodies. The parasites are able to evade the consequences of these immune reactions by switching the variant surface glycoprotein, the phenomenon known as antigenic variation.

B. DIAGNOSTIC TECHNIQUES

1. Identification of the agent

The classical direct parasitological methods for the diagnosis of trypanosomosis, namely examining blood or lymph node material, are not highly sensitive. In regions where other Trypanozoon spp. occur in addition to T. evansi, specific identification by microscopy is not possible. Specific DNA probes (19, 24) may enable identification of trypanosome species by nonradioactive DNA hybridisation. A species-specific polymerase chain reaction (PCR) based on T. evansi specific antigen (RoTat 1.2 VSG) has been developed, but has not been validated in the field (3).

• Direct methods

a) Usual field methods

i) Blood sampling

Trypanosoma evansi is a parasite of the blood and tissues often inhabiting the deep blood vessels in cases of low parasitaemia. For this reason, it is recommended that blood for diagnosis be obtained from both the peripheral and deep blood vessels. However it should be realised that less than 50% of infected animals may be identified by examination of peripheral blood.

Peripheral blood is obtained by puncturing a small vein in the ear or tail. Deeper samples are taken from a larger vein by syringe. Cleanse an area of the ear margin or tip of the tail with alcohol and, when dry, puncture a vein with a suitable instrument. Ensure that instruments are sterilised or disposable instruments are used between individual animals, so that infection cannot be transmitted by residual blood.

ii) Wet blood films

Place a small drop of blood on to a clean glass slide and cover with a cover-slip to spread the blood as a monolayer of cells. Examine by light microscopy (×200) to detect any motile trypanosomes.

iii) Stained thick smears

Place a large drop of blood on the centre of a microscope slide and spread with a toothpick or the corner of another slide so that an area of approximately 1.0–1.25 cm in diameter is covered. Air-dry for
1 hour or longer, while protecting the slide from insects. Stain the unfixed smear with Giemsa’s Stain (one drop of commercial Giemsa + 1 ml of phosphate buffered saline [PBS, 2.4 g Na₂HPO₄·2H₂O, 0.54 g NaH₂PO₄·2H₂O, 0.34 g NaCl], pH 7.2), for 25 minutes. After washing, examine the smears by light microscopy at high magnification (×500–1000). The advantage of the thick smear technique is that it concentrates the drop of blood into a small area, and thus less time is required to detect the parasites. The disadvantage is that the trypanosomes may be damaged in the process, and the method is therefore not suited for species identification in case of mixed infections.

iv) **Stained thin smears**

Place a drop of blood 20 mm from one end of a clean microscope slide and draw out a thin film in the usual way. Air-dry briefly and fix in methyl alcohol for 2 minutes and allow to dry. Stain the smears in Giemsa (one drop Giemsa + 1 ml PBS, pH 7.2) for 25 minutes. Pour off, stain and wash the slide in tap water and dry. Unfixed smears can be stained by covering them with May–Grünewald stain for 2 minutes, then adding an equal volume of PBS, pH 7.2, and leaving the slides for a further 3 minutes. Pour off and add diluted Giemsa for 25 minutes. Pour off, wash the slides with tap water, and dry. Examine at high magnification (×400–1000). This technique permits detailed morphological studies and identification of the trypanosome species. Rapid staining techniques also exist (Field’s stain, Diff Quick®).

v) **Lymph node biopsies**

Samples are usually obtained from the prescapular or precrural (subiliac) lymph nodes. Select a suitable node by palpation and cleanse the site with alcohol. Puncture the node with a suitable gauge needle, and draw lymph node material into a syringe attached to the needle. Expel lymph on to a slide, cover with a cover-slip and examine as for the fresh blood preparations. Fixed thin or thick smears can also be stored for later examination.

b) **Concentration methods**

In most hosts *T. evansi* can induce mild clinical or subclinical carrier state infections with low parasitaemia in which it is difficult to demonstrate the parasites. In these circumstances, concentration methods become necessary.

i) **Haematocrit centrifugation**

Collect blood (70 µl) into at least two heparinised capillary tubes (75 × 1.5 mm). Seal at the dry end by heat and centrifuge, sealed end down, at 3000 g for 10 minutes. Place the capillary tube between two pieces of glass (25 × 10 × 1.2 mm) glued to a slide. Place a cover-slip on top at the level of the buffy coat junction where the trypanosomes will be concentrated. Flood the space around this part of the tube with water or immersion oil, and examine the buffy coat area under the microscope (×100–200). This technique can detect around 400 trypanosomes/ml. A simpler alternative is to examine the centrifuged capillary tube by placing a drop of immersion oil on the tube and ensuring that there is contact between the objective lens and the immersion oil.

ii) **Dark-ground/phase-contrast buffy coat technique**

Collect blood into heparinised capillary tubes and centrifuge as above. Scratch the tube with a glass-cutting diamond and break it 1 mm below the buffy coat layer – the upper part thus contains the top layer of red blood cells (RBCs), the buffy coat (white blood cells) and some plasma. Partially expel the contents of this piece on to a slide, cover with a cover-slip and examine under dark-ground, phase-contrast or ordinary illumination.

As an alternative to the electrically powered haematocrit centrifuge, hand-powered micro-centrifuges have been used successfully for detection of trypanosomosis in cattle and camels (9, 14).

iii) **Haemolysis techniques**

Sodium dodecyl sulphate (SDS) can be used as a reagent to haemolyse RBCs to facilitate detection of motile trypanosomes in parasitised blood samples. As SDS is toxic, contact with skin, inhalation and ingestion should be avoided. SDS solution can be stored for several months at ambient temperature. Both the SDS solution and the blood samples should be used at a temperature above 15°C. At lower temperatures the trypanosomes may be destroyed.

Two general procedures, namely wet blood film clarification and haemolysis centrifugation, can be used².

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² All necessary materials and instructions can be obtained from the Institute of Tropical Medicine, Laboratory of Serology, Nationalestraat 155, B-2000 Antwerp, Belgium.
- **Wet blood film clarification method**

  This method uses the partial lysis of RBCs to facilitate the detection of motile trypanosomes. The method requires an SDS solution: 1% SDS dissolved in Tris/glucose/saline, pH 7.5, inoculating loops (10 µl), slides and cover-slips (24 × 24 mm), and a drop of fresh meat or heparinised blood. Dissolve 100 mg of SDS in 100 ml of isotonic Tris/NaCl/glucose buffer, pH 7.5 (Trizma base 14.0 g, NaCl 3.8 g, glucose 10.8 g; dissolve chemicals in 750 ml distilled H2O, add 90–100 ml 1 N HCl and adjust pH to 7.5 then make up to final volume of 1000 ml with distilled H2O). This buffer can be stored in small vials at ambient temperature for several months. The test does not give a significantly higher sensitivity than wet film technique. In addition, the SDS can cause problems when focusing the microscope and the movements of trypanosomes can be severely curtailed owing to the high viscosity of the SDS.

  Put approximately 10 µl of blood on a slide. Add 10 µl of SDS solution using a dip inoculation loop and mix gently. Apply a cover-slip and examine the preparation without delay at low magnification (×100 or ×200).

- **Haemolysis centrifugation technique**

  Nearly complete lysis of RBCs is required for this procedure. The materials needed include: SDS solution (0.1% SDS dissolved in Tris/glucose/saline, pH 7.5), conical centrifuge tubes, ordinary test tubes, large and fine plastic tapering pipettes with attached bulb, slides, cover-slips (24 × 24 mm or 24 × 32 mm), and heparinised blood.

  Using a pipette or syringe, transfer nine volumes (maximum 6.3 ml) of SDS solution into an ordinary test tube. Aspirate one volume (maximum 0.7 ml) of heparinised blood into a bulb pipette and expel it just above the surface of the SDS solution; mix quickly and thoroughly. Avoid foam formation, which may result in destruction of the trypanosomes. Wait for 10 minutes so as to achieve complete haemolysis. Pour the haemolysed suspension into a conical centrifuge tube and spin at approximately 500 g for 10 minutes. With a clean bulb pipette, remove as much supernatant as possible without disturbing the sediment. Using a fine tapering bulb pipette, remove more supernatant, leaving 10–20 µl of undisturbed sediment at the bottom.

  Very carefully collect the entire sediment and put on to a microscope slide. Apply a cover-slip and examine the preparation without delay at low magnification (×100 or ×200).

- **Mini-anion exchange centrifugation technique**

  When a blood sample from animals infected with salivarian trypanosomes is passed through an appropriate anion-exchange column, the host blood cells, being more negatively charged than trypanosomes, are adsorbed onto the anion-exchanger, while the trypanosomes are eluted, retaining viability and infectivity (15). A simplified field method for detection of low parasitaemia has been developed (26). The sensitivity of this technique can be increased by approximately tenfold by the use of buffy coat preparations rather than whole blood (25).

- **Preparation of phosphate buffered saline glucose, pH 8**

  Na2HPO4 (anhydrous) (13.48 g); NaH2PO4.2H2O (0.78 g); NaCl (4.25 g); distilled water (1 litre). Solutions of different ionic strength are made by diluting the stock PBS, pH 8, and adding glucose to maintain a suitable concentration. For blood of mice, domestic and wild ruminants and dog, add four parts PBS to six parts distilled water and adjust the final glucose concentration to 1%. For blood of pigs and rabbits, add three parts PBS to seven parts distilled water and adjust the final glucose concentration to 1.5%. The PBS/glucose solution (PSG) must be sterile.

- **Equilibration of DEAE-cellulose**

  Suspend 500 g of DEAE-cellulose (diethylamino-ethylcellulose) in 2 litres of distilled water. Adjust the pH to 8 with phosphoric acid. Allow to settle for 30 minutes. Discard the supernatant fluid containing the fine granules. Repeat the procedure three times. Store the equilibrated concentrated suspension of DEAE-cellulose (slurry) at 4°C or in small aliquots at −20°C.

- **Packing of equilibrated DEAE-cellulose**

  Place a 2 ml syringe without the plunger on a test-tube rack complete with needle (20 G × 1.5 inch). Put a disc of Whatman No. 41 filter paper at the bottom of the syringe and moisten by adding a few drops of PSG. Pour 2–2.5 ml of the slurry of equilibrated cellulose into the syringe and allow to pack by elution of the buffer. The height of the sediment should be approximately 3 cm. Wash and equilibrate the column with 2 ml of PSG without disturbing the surface.
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- **Adsorption of blood eluate of the trypanosomes**
  Gently place 100–300 µl of heparinised blood above the surface of the cellulose column. Add ten drops of PSG and discard the first ten drops of the eluate. Progressively add 1.5 ml of PSG and start collecting the eluate into a finely tapered Pasteur pipette with a sealed end. Put the filled pipette, protected by a conical plastic pipette tip, in a tube and centrifuge at 525 g (or up to 1000 g) for 10 minutes. Examine the bottom of the pipette under the microscope (×100 or ×200) using a special mounting device. Alternatively, the eluate could be collected into 50 ml plastic tubes, with conical bottoms, centrifuged at 1000 g and the sediment examined by dark-ground microscopy.

  The cellulose column should remain wet throughout the procedure.

c) **Animal inoculation**

  Laboratory animals may be used to reveal subclinical (nonpatent) infections in domesticated animals. *Trypanosoma evansi* has a broad spectrum of infectivity for small rodents, and so rats and mice are often used. Rodent inoculation is not 100% sensitive (18) but further improvement in its efficacy can be obtained by the use of buffy coat material. Such a procedure was able to detect as few as 1.25 T. evansi/ml blood (25).

  Inoculate heparinised blood intraperitoneally into rats (1–2 ml) or mice (0.25–0.5 ml). Inoculate a minimum of two animals. Bleed animals from the tail three times a week to detect parasitaemia. The incubation period before appearance of the parasites and their virulence depends on the strain of trypanosomes, their concentration in the inoculum, and the strain of laboratory animal used. Sensitivity of this *in-vivo* culture system may perhaps be increased by use of immunosuppressed laboratory animals. Drugs such as cyclophosphamide or hydrocortisone acetate, or X-ray irradiation or splenectomy are used for this purpose.

d) **Recombinant DNA probes**

  Specific DNA probes have been used to detect trypanosomes in infected blood or tissue but are not routinely applied (28, 31). Although molecular methods have a potentially high analytical sensitivity there have been few convincing studies to critically evaluate the diagnostic sensitivity of these tests as compared with other techniques, such as serology.

e) **Detection of trypanosomal DNA**

  Detection of minute amounts of trypanosomal DNA using a PCR procedure is a possible means of identifying animals with active infections, and could have the sensitivity and specificity required (3, 20, 32). Experimental studies in buffalo (10) showed the diagnostic sensitivity of a PCR was only 78%, which is similar to mouse inoculation.

- **Indirect methods**

  These methods involve tests that demonstrate the effects of the parasite on its host rather than directly detecting the parasite itself.

  a) **Haematology**

  Anaemia is usually a reliable indicator of trypanosome infection, although it is not in itself pathognomonic. However, animals with a mild subclinical infection can have parasitaemia without evidence of anaemia (4).

  Anaemia can be estimated by measuring the packed cell volume and may be used in surveys of herds at risk. The technique is identical to that of haematocrit centrifugation. The capillary tube is examined and the results are expressed as a percentage of packed RBCs to total blood volume.

  2. **Serological tests**

  Historically, many different methods have been used to detect specific humoral antibodies to trypanosomal antigens but, more recently, there has been a tendency to concentrate on more easily standardised techniques such as ELISA (6, 7, 12, 22, 23, 24, 27) card agglutination tests (CATT) (1, 20, 24) and latex agglutination tests (LAT) (11, 16). Extensive evaluation of ELISA and CATT has been carried out in buffaloes in Indonesia and Vietnam (6, 11, 29). The collection of samples can be simplified by using filter paper blood spots for later use in the ELISA, while for the CATT whole blood can be substituted for serum (11). Other innovative modifications that might be developed in the future are the use of a colloidal-dye dipstick test (13) that could enable the tests to be carried out under field conditions. It is vitally important that serological tests are validated and standardised if they are to be suitable for correctly identifying infected animals. This means that standard criteria for interpreting the tests might have to be developed for each animal species as well as each laboratory operating the procedure.
a) **Enzyme-linked immunosorbent assay**

The principle of this technique is that specific antibodies to trypanosomes can be detected by enzyme-linked anti-immunoglobulins using solid-phase polystyrene plates coated with soluble antigen. The enzyme may be peroxidase, alkaline phosphatase or any other suitable enzyme. The enzyme conjugate binds to the antigen/antibody complex and then reacts with a suitable substrate to yield a characteristic colour change either of the substrate itself or of an added indicator (the chromogen).

The antigen for coating the plates is derived from the blood of a heavily parasitaemic rat. The trypanosomes are separated on a DEAE-cellulose column and washed three times by centrifugation in cold PSG, pH 8 (PBS with 1% glucose). The final pellet is suspended in cold PSG to a concentration of 3–5%, and briefly ultrasonicated on ice for 30–120 seconds until disintegration of the organisms is complete. This preparation is centrifuged at 4°C and 40,000 g for 60 minutes. The supernatant is diluted in water so as to obtain a protein concentration of 1 mg/ml. The reagent thus obtained can be stored in small aliquots at –70°C for several months. It can also be freeze-dried and stored at –20°C. Various treatments of the antigen preparations have been applied to improve the accuracy of antibody detection (32).

**Test procedure**

i) Dilute or reconstitute the frozen or freeze-dried antigen with freshly prepared 0.01 M carbonate/bicarbonate buffer, pH 9.6. Add 100 µl to each well of a 96-well microtitre plate and incubate overnight at 4°C or for 1 hour at 37°C.

ii) Remove excess antigen by washing plate with 0.01 M PBS containing 0.05% Tween 20 (PBST). Add test serum dilutions in PBST (100 µl). Include control negative and positive sera. Dilutions must be determined empirically, but are usually between 1/100 and 1/1000.

iii) Incubate plates at 37°C for 30 minutes. Eject contents and wash three times with PBST.

iv) Add a specific peroxidase conjugated species-specific anti-globulin (100 µl) appropriately diluted in PBST (usually between 1/1000 and 1/50,000).

v) Incubate the plates at 37°C for 30 minutes, eject contents and wash three times with PBST.

vi) For peroxidase conjugates a number of substrate/chromogen solutions can be used, consisting of hydrogen peroxide with a chromogen, such as tetramethylbenzidine (TMB), 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and ortho-diphenylenediamine (OPD). A suitable substrate/chromogen solution for peroxidase conjugates is 30% hydrogen peroxide (0.167 ml and 35 mg) in citrate buffer (100 ml), pH 6.0. The citrate buffer is made up as follows: Solution A (36.85 ml): (0.1 M citric acid [21.01 g/litre]); Solution B (65.15 ml): (0.2 M, Na₂HPO₄ [35.59 g/litre]); and distilled water (100 ml). Dissolve 10 mg TMB in 1 ml dimethyl sulphoxide and add to 99 ml of the citrate buffer. A number of these combinations are available commercially in ready-to-use formulations that remain stable at 4°C for up to 1 year.

vii) Add the substrate chromogen (100 µl) to the plates and incubate at room temperature for 15–20 minutes.

viii) Stop the reaction by adding 50 µl 1 M sulphuric acid. Read the absorbance of each well at 450 nm for TMB chromogen. Other chromogens may require the use of a different wavelength. All tests should include known high and medium positive control sera, a negative control serum, and a buffer control.

A large variety of other test procedures exists. For closely related animal species, cross-reacting reagents may often be used (e.g. anti-bovine immunoglobulin for buffaloes) and the use of monospecific anti-IgG conjugates is generally recommended. There are a number of methods that can be used to determine a cut-off point to discriminate between positive and negative results. The simplest method is to base the cut-off on visual inspection of the test results from known positive and negative populations. These results are likely to show some overlap. The operator can choose the most appropriate point to modify the false negative or false positive results depending on the required application of the assay. An alternative is to base the cut-off on the mean ±2 standard deviations (SD) or ±3 SD values from a large sample of negative animals. Finally, if no suitable negative/positive samples are available a cut-off can be based on the analysis of the data from either of the substrate itself or of an added indicator (the chromogen).

b) **Card agglutination tests**

It is well known that certain predominant variable antigen types (VATs) are expressed in common in different strains of salivarian trypanosomes from different areas. This finding was used as a basis for a test for the
diagnosis of *T. evansi*, the card agglutination test – CATT/ *T. evansi* – was developed at the Laboratory of Serology, Institute of Tropical Medicine, Antwerp. The test makes use of fixed and stained trypanosomes of a defined VAT known as RoTat 1.2. Both variable and invariable surface antigens take part in the agglutination reaction. The CATT is available in kit form from ITM, Antwerp. It consists of lyophilised antigen, PBS, pH 7.4, plastic-coated cards, spatulas, positive and negative control sera and a rotator. The lyophilised antigen can be stored at 2–8°C for up to 1 year. Reconstituted antigen can be stored at 2–8°C for 2 days, but preferably should be used within 8 hours.

For screening, dilute test sera 1/4 or 1/8 in PBS on to circles inscribed on the plastic cards. Add 45 µl of the prepared antigen suspension onto circles inscribed on the plastic cards. Add 25 µl of each test serum. Mix and spread the reagents with a spatula and rotate the card for 5 minutes using the rotator provided in the kit. Compare the pattern of agglutination with the illustrations of different reactions provided in the kit. Blue granular deposits reveal a positive reaction visible to the naked eye.

c) **Latex agglutination tests**

A kit is available from ITM, Antwerp. It comprises a lyophilised latex suspension coated with *T. evansi* RoTat 1.2 variable antigens, PBS, positive and negative controls, test cards, plastic spatulas and a rotator.

Reconstitute the antigen-coated latex particles using distilled, deionised water. Mix gently. Add 20 µl of latex suspension onto each black spot on the test cards.

Dilute test sera with PBS (1/2 to 1/4) and add 20 µl to the latex suspension. Include appropriate controls. Mix the reagents carefully with a plastic spatula.

Rotate test cards at 70 rotations/minutes for 5 minutes and view cards under a good light source at the end of the incubation. Positive sera will cause agglutination of the latex particles.

**C. REQUIREMENTS FOR VACCINES AND DIAGNOSTIC BIOLOGICALS**

No vaccines are available for this disease.

**REFERENCES**


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3 CATT/ *T. evansi* kits are available at the Laboratory of Parasite Diagnostic, Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium. (pbuscher@itg.be ; fclaes@itg.be)


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**NB:** There are OIE Reference Laboratories for *Trypanosoma evansi* infections (*including surra*) (see Table in Part 3 of this Terrestrial Manual or consult the OIE Web site for the most up-to-date list: www.oie.int).