CHAPTER 3.1.21.

SURRA IN ALL SPECIES (*TRYPANOSOMA EVANSI* INFECTION)

SUMMARY

Description of the disease: Trypanosoma evansi *causes a trypanosomosis known as 'surra'. It affects a large number of wild and domesticated animal species in Africa, Asia, and Central and South America. The principal host species varies geographically, but camels, horses, buffalos and cattle are particularly affected, although other animals, including dogs, cats and wildlife, are also susceptible. It is an arthropod-borne disease; several species of haematophagous flies, including tabanids and Stomoxys, are implicated in transferring infection from host to host, acting as mechanical vectors. In Brazil, vampire bats are also implicated in a unique type of biological transmission. Peroral transmission is the main way of transmission to carnivores.*

The general clinical signs of T. evansi *infections – pyrexia directly associated with parasitaemia together with a progressive anaemia, loss of condition and lassitude – are not sufficiently pathognomonic for diagnosis. 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 sometimes observed in horses. Abortions have been reported in water buffaloes and camels. Nervous signs are common in horses and dogs. The disease causes immunodeficiencies that may be of high impact when interfering with other diseases or vaccination campaigns (foot and mouth disease and haemorrhagic septicaemia for example).*

Detection of the agent: *Laboratory methods for detecting the parasite are required. In early infection or acute cases, when the parasitaemia is high, examination of wet blood films, stained blood smears or lymph node materials might reveal the trypanosomes. In more chronic cases, or more generally when the parasitaemia is low, the examination of thick blood smears, as well as methods of parasite concentration and the inoculation of laboratory rodents are required. In apparently healthy carriers (animals without clinical signs), parasites are rarely observed and mouse inoculation gives the best results. Several primer pairs targeting the subgenus* (Trypanozoon) *or the species-specific* (T. evansi) *parasitic DNA sequences are available for diagnosis by polymerase chain reaction (PCR). PCR is more sensitive than parasitological examination, but it may give false-negative results when the parasitaemia is very low; in these cases, suspicion of potential carriers can only be confirmed by serological examination.*

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. The most relevant are immunofluorescence test (IFAT), enzyme linked immunosorbent assays (ELISA) and card agglutination test (CATT/*T. evansi*). For field use, only CATT/*T. evansi *can be applied. Rapid diagnostic tests (RDT) are currently unavailable. Estimates of predictive values indicate that ELISA for detecting IgG is more likely to classify correctly uninfected animals, while the CATT is more likely to classify correctly truly infected animals. ELISA would thus be suitable for verifying the disease-free status of animals prior to movement or during quarantine. CATT can be used to target individual animals for treatment with trypanocidal drugs. For declaring a disease-free status, serial testing – CATT and ELISA followed by re-testing of suspect samples – is recommended in association with PCR. In areas where* T. cruzi, T. equiperdum *or tsetse-transmitted trypanosomes occur, cross-reactions may occur with any serological test employed.*

Requirements for vaccines: *No vaccines are available for the disease.*

A. INTRODUCTION

Infection with *Trypanosoma evansi* (subgenus *Trypanozoon*) causes a disease named *surra* in India (mainly in horses and bovines), also called, amongst others, *El Debab*, *El Gafar, Tabourit* or *MBori* in North Africa (mainly in camels), or *Mal de Caderas* or *Murrina* in Latin America (in horses). The clinical signs of surra are indicative but are not pathognomonic, thus, diagnosis must be confirmed by laboratory methods. The disease in susceptible animals, including camels (dromedary and bactrian), horses, water buffalo, cattle, sheep, goats, pigs, deer, dogs and cats is manifested by pyrexia, directly associated with parasitaemia, together with a progressive anaemia, loss of condition and lassitude. Such recurrent episodes lead to intermittent fever (as high as 44°C in horses [Gill, 1977]) and parasitaemia during the course of the disease. Oedema, particularly of the lower parts of the body, rough coat in camels, urticarial plaques and petechial haemorrhages of the serous membranes are sometimes observed in male equids, oedema of the genital organs may present a clinical feature that may be confused with dourine. In advanced cases, parasites invade the central nervous system (CNS), which can lead to neurological signs (progressive paralysis of the hind quarters and, exceptionally, paraplegia), especially in horses, but also in other host species, before complete recumbency and death. Abortions have been reported in buffalos and camels (Gutierrez *et al*., 2005) and there are clear indications that the disease causes immunodeficiency (Desquesnes *et al.,* 2013a).

There is considerable variation in the pathogenicity of different strains and the susceptibility of different host species. The disease may manifest as an acute or chronic form, and in the latter case may persist for several months, possibly years. The disease is often rapidly fatal in camels, horses and dogs. It may also be fatal in water buffalo, cattle, sheep, goat, pig and llamas, however, these host species, as well as camels, may develop mild or subclinical infections. Wild animals such as deer, capybara (*Hydrochoerus hydrochaeris*) and coati (*Nasua nasua*) can become infected and ill (including death), but they may also recover and constitute a reservoir. 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, and morphologically resembles the slender forms of the tsetse-transmitted subspecies, *T. brucei brucei, T. b. gambiense* and *T.b. rhodesiense.* The initial molecular characterisation of *T. evansi* strains isolated from Asia, Africa and South America indicated that they were very homogeneous and suggested a single origin (Ventura *et al.,* 2002), other works suggest that *T. evansi* could have emerged from *T. brucei* in several instances (Lai *et al.,* 2008). Analysis by random amplified polymorphic DNA and endonuclease fingerprinting showed that *T. evansi* and *T. equiperdum* isolates form a closely homogeneous group. The difficulties in differentiating *T. equiperdum* from the other species of *Trypanozoon* have been stressed (Zablotskij *et al.,* 2003), and the existence of *T. equiperdum* was even questioned.

When *T. evansi* evolved from *T. brucei*, its kinetoplastic DNA was subjected to alterations or loss, in part or total; as a consequence, it can no longer implement a cycle in tsetse flies (Lai *et al,* 2008), and it is mainly mechanically transmitted by haematophagous flies. In fact, *T. evansi* was considered a malignancy of *T. brucei* (Lun *et al.,* 2015). The main mechanical vectors of *T. evansi* are tabanids (amongst which *Tabanus*, *Chrysops* and *Haematopota* are predominant) and *Stomoxys* flies (*Stomoxys* spp., *Hematobia* sp., etc); they are responsible for immediate transmission at a short distance amongst animals living, pasturing or watering together. Cats and dogs do not appear to play a significant role in ongoing transmission of the disease, but they generally act as sentinel animals. Like other members of the *Trypanozoon* subgenus, *T. evansi* is able to penetrate through mucosal membrane, which allows not only vertical transmission *in utero*, but also peroral transmission, principally observed in domestic and wild carnivores, and vampire bats in Latin America, but may also be responsible for mother-to-foal transmission in peripartum. Due to frequent peaks of parasitaemia, the risk of iatrogenic transmission must also be considered when serial treatments are applied (Desquesnes *et al.,* 2013b).

In Latin America, *T. evansi* has found a vertebrate vector: the vampire bat, such as *Desmodus rotundus*. These haematophagous mammals are infected when feeding on hosts such as infected horses or cattle, they develop the infection and may recover and stay under chronic infection; during this period, trypanosomes invade the salivary glands. They are then able to transmit *T. evansi* when biting their congeners in the bat colony, and to livestock when feeding; thus vampire bats are both hosts, reservoirs and vectors of the parasite. Several wild mammals act as potential reservoirs of *T. evansi* in Latin America, with capybara, the largest rodent in the world, being the most important sentinel to detect the presence of surra, as significant parasitaemia levels are not associated with clinical impact (Desquesnes *et al.*, 2013a; 2013b).

Like all pathogenic trypanosomes, *T. evansi* is covered by a dense protein layer consisting of a single protein called the variant surface glycoprotein (VSG). This acts as a major immunogen and elicits the formation of a series of specific antibodies. The parasites are able to evade the consequences of these immune reactions by switching the VSG, a phenomenon known as antigenic variation.

Surra should be considered for differential diagnosis when cases presenting fever and/or anaemia are observed in the field. Anaemia is a reliable indicator of trypanosome infection, but it is not in itself pathognomonic. On the other hand, animals with a mild subclinical infection can have parasitaemia without evidence of anaemia. Reliable diagnosis requires laboratory confirmation of the infection.

In enzootic areas, routine diagnoses can be made using parasitological techniques, while serological surveys can be carried out, preferably by enzyme linked immunosorbent assays (ELISA). Card agglutination test (CATT)/*T. evansi* can be used to target individual animals for treatment with trypanocidal drugs.

Where a definitive confirmation of the absence of infection is needed (e.g. for importation into a disease-free area), serial examinations are required, including the antibody detection ELISA and a sensitive agent detection method, such as polymerase chain reaction (PCR). Testing by mouse inoculation should be limited and used only if fully justified.

In areas where *T. cruzi, T. equiperdum* or tsetse-transmitted trypanosomes are present, cross-reactions may occur with any serological test employed so that the exact trypanosome status of an animal may not be established in full.

Surra is not known as a zoonotic disease, however, some rare human cases have been described, notably in India and Vietnam (Van Vinh Chau *et al*., 2016). Consequently, suspect animal samples should be handled at an appropriate biosafety and containment level determined by biorisk analysis as described in Chapter 1.1.4 *Biosafety and biosecurity: Standard for managing biological risk in the veterinary laboratory and animal facilities*.

As there is no vaccine against trypanosomoses, the only option is treatment of the animals using trypanocidal drugs; OIE has published an article setting out quality control approaches for trypanocidal drugs: https://www.ncbi.nlm.nih.gov/pubmed/25812206 or http://dx.doi.org/10.20506/rst.33.3.2320

B. DIAGNOSTIC TECHNIQUES

A variety of diagnostic tests is available and researchers are still working to improve existing tests and develop new ones. Current diagnostic tests vary in their sensitivity and specificity, the ease with which they can be applied and their costs. The choice of a particular test will be guided by economic principles, by the availability of expertise and especially by the diagnostic requirements. For example, different degrees of sensitivity and specificity are required to confirm the infection in an individual animal as compared with the detection of infection at the herd level. Similarly, the diagnostic test(s) to establish the parasitological prevalence of trypanosomosis is (are) different from those required to establish the presence or absence of the disease in an area. Reliable diagnosis may be achieved by combining appropriate diagnostic tests. Reliable interpretation of results from diagnostic tests will depend on test validity, as well as on proper sample selection/collection, the sample size, and the way the diagnostic tests are conducted (see Table 1). Detailed diagnostic techniques (including figures) of most of the tests described in Section B can be found in the "Compendium of standard diagnostic protocols for Animal Trypanosomoses of African Origin", available online:
https://www.woah.org/en/document/compendium-of-diagnostic-protocols-of-the-oie-reference-laboratory-for-animal-trypanosomoses-of-african-origin/

Table 1. Test methods available 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
Detection of the agent[(a)]						
Thin GSBS (or lymph or oedema fluid)	–	+	+	+++	++	–
DNA detection/PCR	+++	+++	+++	+++	+++	–
Wet blood film	–	–	–	++	–	–
TGSBF	–	–	–	++	++	–
HCT (Woo)	+++	+++	+++	+++	+++	–
BCT (Murray)	+	+	++	++	++	–
AECT	–	+	++	++	–	–
Detection of immune response						
CATT/ *T. evansi*	++	++	+++	+++	++	–
IFAT *T. evansi*	++	+++	+++	+++	++	–
ELISA *T. evansi*	+++	+++	+++	+++	+++	–
TL RoTat1.2 test	–	–	++	–	+	–

Key: +++ = recommended for this purpose; ++ recommended but has limitations;
+ = suitable in very limited circumstances; – = not appropriate for this purpose.
GSBS = Giemsa-stained blood smear; PCR = polymerase chain reaction; TGSBF = thick Giemsa-stained blood film;
HCT = haematocrit centrifuge technique; BCT = buffy coat technique; AECT = anion exchange chromatography technique;
CATT = card agglutination test; IFAT = indirect-fluorescent antibody test;
ELISA = enzyme-linked immunosorbent assay; TL = trypanolysis test.
[(a)]A combination of agent identification methods applied on the same clinical specimen is recommended (See Section B.3.2).

1. Detection of the agent

The classical direct parasitological methods for the diagnosis of trypanosomosis, namely microscopic examination of blood or lymph node material, are not highly sensitive, but a number of techniques, including enrichment of the sample, rodent inoculation and DNA methods may increase the sensitivity. In regions where other species of *Trypanozoon* might be present in addition to *T. evansi*, species-specific discrimination requires molecular tools and cannot be accomplished by microscopic examinations.

1.1. Direct microscopic examination

1.1.1. Blood sampling

Trypanosoma evansi is a parasite of the blood and tissues. As for other trypanosomes, it is recommended that blood for diagnosis be obtained from peripheral ear or tail vein, even if the jugular vein is most often preferred for practical reasons. However, it should be realised that less than 50% of infected animals may be identified by examination of 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 (lancet, needle). Ensure that instruments are sterilised or use disposable instruments to avoid iatrogenic transmission of the infection by residual blood.

1.1.2. Wet blood films

Place a small drop of blood (2–3 µl) on to a clean glass slide and place over it a cover-slip to spread the blood as a monolayer of cells. Examine by light microscopy (200×) to detect any motile trypanosomes. Improved visualisation can be obtained with dark-ground or phase-contrast microscopy (200–400×). The sensitivity of this method is low, approximately 10 trypanosomes per µl, which is frequent in early or acute infections only. This examination can be applied to case confirmation, however, due to its very low sensitivity, it is mostly used to follow-up experimentally infected animals.

1.1.3. Thick Giemsa-stained blood film (TGSBF)

Place a large drop of blood (10 µl) 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. Placing the slide in a horizontal position, stain the unfixed smear with Giemsa's Stain (one drop of commercial Giemsa + 1 ml of phosphate-buffered saline, pH 7.2), for 25 minutes. After washing and drying, examine the smears by light microscopy at a magnification of 500× with oil immersion. 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, which are more visible owing to the haemolysis of the unfixed red cells. 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 potentially mixed infections. Consequently, thin blood smears are generally preferred to thick blood films.

1.1.4. Giemsa-stained blood smears (GSBS)

Place a small drop of blood (3–5 µl) at 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 1 minute 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. Nowadays, commercially available fast stains are most often used, which allow fixation and staining within a few seconds. Slides are then washed in tap water and dried. Examine at a magnification of 400–1000× with oil immersion. This technique permits detailed morphological studies and identification of the *Trypanozoon* subgenus (which, sometimes, allows the species to be defined, according to the epizootiological context), but its sensitivity is very low (it can detect parasitaemia >500,000 trypanosomes/ml of blood). Nevertheless, when positive, this examination brings a reliable and subgenus-specific confirmation of the infection.

1.1.5. Lymph node biopsies or oedema fluid

Samples are usually obtained from the prescapular or precrural (subiliac) lymph nodes, preferably when they are enlarged. 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. Similar examination can be done by collection of oedema fluid.

1.2. Concentration methods

In most of its hosts, *T. evansi* can induce mild clinical or subclinical carrier state infections with low parasitaemia in which it is difficult to demonstrate the presence of the parasites. In these circumstances, concentration methods are necessary, as they increase the sensitivity of microscopic examination; being based on the observation of moving parasites, these methods should be completed no later than 2–4 hours after blood collection.

1.2.1. Haematocrit centrifugation technique (also known as Woo's technique, or HCT)

Collect blood (2 × 70 µl) into two heparinised capillary tubes (75 × 1.5 mm). Close the wet end with plasticine and centrifuge at 14,000 ***g*** for 5 minutes (generally 12,000 rpm in a haematocrit centrifuge machine). The capillary tube is examined and the value of the haematocrit is expressed as a percentage of packed blood cells to total blood volume (%packed cell volume [PCV]); this gives an indication on the anaemia of the animal. The capillary tube is then placed in a groove made with pieces of slide glued to a slide, for microscopic observation. Trypanosomes are large cells that concentrate at the junction between the buffy coat and the plasma, which is observed under the microscope (100–200×). Light conditions must be set to induce refringency of the cells to increase the visibility of the moving trypanosomes; this can be obtained by lowering the position of the light condenser or with intermediary positions of the turret light condenser. Specially designed reading chambers for HCT can be obtained at the OIE Reference Laboratories for surra[1]; they enable submerging of the capillary tube in water to avoid light diffraction, however, they require long-focal objectives. The fresher the sample, the better is the sensitivity as strong parasitic movements make trypanosomes more visible. This technique can detect around 50–200 trypanosomes/ml of blood (Desquesnes, 2004). The buffy coat sample can also be extracted from the capillary tube to be prepared for PCR. HCT is the most employed and one of the best concentration techniques for *T. evansi* detection; its specificity is limited to the subgenus level (*Trypanozoon* versus *Duttonella*, *Nannomonas*, *Schizotrypanum*, *Megatrypanum*).

1.2.2. Buffy coat technique (also known as Murray's technique, or BCT)

This technique is very similar to the previous one. Collect blood into heparinised capillary tubes and centrifuge as above. Scratch the tube with a glass-cutting diamond pencil and break it 0.5 mm below the buffy coat layer – the upper part thus contains a small top layer of red blood cells, the buffy coat (white blood cells and platelets) and some plasma.

Partially expel the contents of this piece on to a slide; avoid expelling more than 5–8 µl of plasma, but make sure the buffy coat has been expelled (the small disk of the buffy coat should be visible to the naked eye), press on a cover-slip to spread the buffy coat and examine by dark-ground, phase-contrast or similar microscopy under the previously described refringency conditions at a magnification of 200–500×. Trypanosomes are mostly present at the periphery of the thick buffy coat material. Expelling the buffy coat from the capillary tube is a delicate step and will affect the reproducibility of the technique. HCT (Section B.1.2.1 above) is more advisable than BCT as the latter is highly dependent on the technical skill of the operator and presents a low level of reproducibility.

Both the Woo and the Murray techniques allow anaemia to be estimated by measuring the PCV and may be used in surveys of herds at risk. The value of the haematocrit (considered as low when <24% for example in cattle) can be used as an indicator to select a subset of samples to be submitted to the more expensive, but also more sensitive, PCR analysis.

1.2.3. Mini-anion exchange chromatography technique (mAECT)

When a blood sample from animals infected with salivarian trypanosomes is passed through an appropriate DEAE-cellulose (diethylamino-ethylcellulose, such as DE52 GE Healthcare) anion-exchange column, the host blood cells, being more negatively charged than trypanosomes, can be adsorbed onto the anion-exchanger (in pH and ionic strength conditions adapted to the host species), while the trypanosomes are eluted, retaining viability and infectivity (Lanham & Godfrey, 1970). This technique is mostly used for the purification of parasites from the blood (for example, for parasite antigen preparation), but miniature systems have been developed, especially for diagnosis in humans. The sensitivity of this technique can be increased by approximately tenfold by the use of buffy coat preparations rather than whole blood.

i) *Preparation of phosphate buffered saline glucose* (*PSG*), *pH 8*

Na_2HPO_4 anhydrous (13.48 g); $NaH_2PO_4.2H_2O$ (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

1 See the list of OIE Reference Laboratories: https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3

glucose to maintain a suitable concentration. For blood of mice, domestic and wild ruminants and dogs, 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 (however, PBS must be autoclaved before adding glucose).

ii) *Equilibration of DEAE-cellulose*

Suspend 500 g of DEAE-cellulose in 2 litres of distilled water. Mix for 20 minutes with a plastic-coated magnetic stirrer at low speed (metallic contact with DEAE-cellulose is proscribed). Adjust the pH to 8 with phosphoric acid. Allow to settle for 30 minutes. Discard the supernatant fluid containing the finest particles that might block the column. Repeat the procedure three times using PSG buffer as described above. Store the equilibrated concentrated suspension of DEAE-cellulose (slurry) at 4°C for a short period, or at –20°C for longer conservation.

iii) *Packing of equilibrated DEAE-cellulose*

Place a 2 ml syringe without the plunger on a test-tube rack, complete with a flexible tube that can be closed with a clamp to act as a tap. 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 packing for 5 minutes before 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.

iv) *Adsorption of blood eluate of the trypanosomes*

Gently place 100–300 µl of heparinised blood (or preferably buffy coat) on the surface of the cellulose column; allow it to penetrate the cellulose, but do not let the cellulose dry before pouring on the eluting buffer. Progressively add 1.5 ml of PSG and start collecting the eluate into a finely tapered Pasteur pipette with a sealed end. The cellulose column should remain wet throughout the procedure. 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.

A similar method used in cattle, pig and goat is also referred to as the miniature anion exchange chromatography method. However, this technique, being time consuming and expensive, is rarely used for animal diagnosis. Conversely, large amounts of blood or buffy coat from experimentally infected rodents can be applied to large columns, for massive parasite isolations, to produce parasites/antigens for serological tests.

1.3. Animal inoculation

Due to increasing bioethical concerns and the tendency to eliminate the use of animals for biological testing, animal inoculation should be limited as much as possible and only used if fully justified. Laboratory animals may be used to reveal subclinical (non-patent) infections in domesticated animals. *Trypanosoma evansi* has a broad spectrum of infectivity for small rodents, and so rats and mice may be used. Rodent inoculation is not 100% sensitive (Monzon *et al.,* 1990) but further improvement in its efficacy can be obtained by the use of buffy coat material. Such a procedure is able to detect as few as 1.25 *T. evansi*/ml blood. This technique is suitable when highly sensitive detection is required.

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 every 48 hours post-infection to detect and/or monitor the parasitaemia. The incubation period before the initial appearance of the parasites and their virulence will depend on the trypanosome strain, on the concentration of the inoculum, and the strain of laboratory animal used; however in most cases it is very short (5 ± 2 days), but can extend to 2 weeks in some cases (Monzon *et al.,* 1990). Sensitivity of this *in vivo* culture system may be increased by the use of immunosuppressed laboratory animals. Drugs such as cyclophosphamide or hydrocortisone acetate may be used for this purpose. Such a procedure is only justified when the detection of a potentially

infected host is of high importance, for example, to confirm a suspected case in high value animals, or to isolate a parasite for further characterisation.

1.4. Detection of trypanosomal DNA

Detection of minute amounts of trypanosomal DNA is a possible means of identifying animals with active infections as the parasitic DNA does not remain for more than 24–48 hours in the blood of the host after the trypanosomes are killed (Desquesnes, 2004).

1.4.1. Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) based on DNA sequences of various taxonomic levels is used. The gold standard, to date, for detection of the *Trypanozoon* subgenus are the TBR primers (Masiga *et al.,* 1992). Other primers (see Table 2) have been reported and are being evaluated; some of them are specific for *Trypanozoon* and others for *T. evansi* ± *T. equiperdum* (Desquesnes & Davila, 2002; Holland *et al*., 2001; Panyim *et al.,* 1993). To date, the most sensitive test is that of satellite DNA using TBR primers (Masiga *et al.,* 1992); the sensitivity of the other primers is being compared under various conditions, including in laboratory rodents, but can only be validated with a sufficient batch of field samples from natural hosts. The use of TBR primers is recommended, at least in the first instance, and, if necessary, confirmed by other sets of *Trypanozoon*-specific primers such as ESAG6/7 (Holland *et al*., 2001) or TEPAN (Panyim *et al*., 1993).

In areas and host species potentially infected with other *Trypanozoon* such as *T. brucei brucei*, species confirmation can be obtained with more specific primers such as RoTat 1.2 (Claes *et al*., 2004; Verloo *et al.,* 2001) or EVAB, for non-RoTat 1.2 strains (Ngaira *et al.,* 2005). RoTat1.2 primers are good candidates for a *T. evansi*-specific diagnosis, however (i) they exhibit a lower sensitivity than TBR primers (Elhaig & Sallam, 2018), (ii) they fail to detect *T. evansi* type B (Njiru *et al*, 2006), and, (iii) they amplify a similar 205 bp product as expected for *T. evansi* with some *T. brucei* and *T. equiperdum* strains (Abou El-Naga *et al*., 2012). If *T. equiperdum* is suspected, other epizootioological information must be considered (amongst which clinical signs and mode of transmission are determinant) as there is not, so far, a simple and reliable molecular test that can distinguish *T. evansi, T. equiperdum* and *T. brucei* sspp. (Gizaw *et al*., 2017).

When trypanosome subspecies that affect human are suspected, more specific primers can be used to detect *T.b. gambiense* (Radwanska *et al*., 2002b) or *T. b. rhodesiense* (Radwanska *et al*., 2002a); however, targeting single copy genes, they exhibit a much lower sensitivity than TBR, potentially leading to inconclusive results when the PCR is negative.

If *Trypanosoma* spp. of other subgenera may be suspected, primers amplifying the internal transcribed spacer 1 (ITS1) of the ribosomal DNA may be used since they can detect several subgenera and species in a single reaction (Njiru *et al*., 2005), however with limited sensitivity. Other techniques such as the loop-mediated isothermal amplification (LAMP) (Thekisoe *et al.,* 2005) and real-time PCR (Sharma *et al*., 2012), have been developed; they need to be further evaluated and validated, in comparison with the standard PCR using TBR primers; be that as it may, these techniques are more expensive than the classical PCR.

DNA preparation is an important step that determines the success and the sensitivity of the PCR. It can be done on plain blood (generally collected with anticoagulant), or, preferably, on the buffy coat to increase the sensitivity of the test (Desquesnes & Davila, 2002). Several classical techniques are available (Penchenier *et al*., 1996), including commercial kits and the classical phenol–chloroform preparation. Blood conserved 1/1 in 70% alcohol, or on dry filter paper can also be used (Hopkins *et al*., 1998).

Being dependent on the amount of DNA available, the sensitivity of the PCR is proportional to the parasitaemia. PCR is thus more sensitive in highly susceptible hosts (camels, horses, dogs, etc.) than in hosts of mild or low susceptibility (cattle, buffalo, pigs, etc.). Using a suitable DNA preparation and the most sensitive primers available (TBR), PCR can detect as few as 1–5 trypanosomes/ml of blood (Panyim *et al.,* 1993; Penchenier *et al.,* 1996), or only 10 per ml in buffaloes with a quantitative real-time PCR.

Table 2. Primers for* Trypanozoon,T. evansi *and* Trypanosoma *spp. characterisation

Specificity	Primer sequences (5' → 3')	References
Trypanozoon: *T. b. brucei, T. b. gambiense, T. b. rhodesiense, T. evansi & T. equiperdum*)	TBR1: CGA-ATG-AAT-ATT-AAA-CAA-TGC-GCA-G	Masiga *et al.,* 1992
	TBR2: AGA-ACC-ATT-TAT-TAG-CTT-TGT-TGC	
T. evansi (type A) and some *T. brucei & T. equiperdum*	RoTat1.2F: GCG-GGG-TGT-TTA-AAG-CAA-TA	Claes *et al*., 2004
	RoTat1.2R: ATT-AGT-GCT-GCG-TGT-GTT-CG	
T. evansi (type B)	EVAB1: CAC-AGT-CCG-AGA-GAT-AGA-G	Njiru *et al.*, 2006
	EVAB2: CTG-TAC-TCT-ACA-TCT-ACC-TC	
T. evansi and some *T. brucei & T. equiperdum*	TEPAN1: AGT-CAC-ATG-CAT-TGG-TGG-CA	Panyim *et al*., 1993
	TEPAN2: GAG-AAG-GCG-TTA-CCC-AAC-A	
T. evansi and some *T. brucei & T. equiperdum*	ESAG6/7F: ACA-TTC-CAG-CAG-GAG-TTG-GAG	Holland *et al*., 2001
	ESAG6/7R: CAC-GTG-AAT-CCT-CAA-TTT-TGT	
T. brucei gambiense	Tgs-GP F: GCT-GCT-GTG-TTC-GGA-GAG-C	Radwanska *et al.*, 2002b
	TgsGP R: GCC-ATC-GTG-CTT-GCC-GCT-C	
T. brucei rhodesiense	Tbr F: ATA-GTG-ACA-AGA-TGC-GTA-CTC-AAC-GC	Radwanska *et al.*, 2002a
	Tbr R: AAT-GTG-TTC-GAG-TAC-TTC-GGT-CAC-GCT	
Pan-tryp.: *T.vivax, Trypanozoon, T.congolense* savannah forest, Kilifi, *T. lewisi,* etc.	TRYP1S: CGT-CCC-TGC-CAT-TTG-TAC-ACA-C	Desquesnes *et al*., 2002
	TRYP1R: GGA-AGC-CAA-GTC-ATC-CAT-CG	
Pan-tryp.: *T. vivax, Trypanozoon, T. congolense* savannah forest, Kilifi, etc.	ITS1 CF: CCG-GAA-GTT-CAC-CGA-TAT-TG	Njiru *et al.*, 2005
	ITS1 BR: TTG-CTG-CGT-TCT-TCA-ACG-AA	

PCR offers high sensitivity and specificity required for detection of trypanosome infection (Masiga *et al.,* 1992), but it may give false-negative results. Experimental studies in sheep have shown that PCR can remain negative for long intervals during aparasitaemic periods (Desquesnes, 2004), while in buffalo the diagnostic sensitivity of a PCR was only 78%, which is similar to mouse inoculation (Holland *et al*., 2001). Nevertheless, PCR is the most sensitive technique for detection of active infection.

1.5. Antigen detection

Circulating antigen detection in blood or serum is also a way to detect active infection. Several attempts to develop such tests have not yet reached a satisfactory level to be recommended for routine diagnosis (Desquesnes, 2004).

2. Serological tests

Many historic serological methods have been used but are no longer recommended. The IFAT (Desquesnes 2004) is still useful for small-scale surveys. The trypanolysis test (Van Meirvenne *et al*., 1995) is used for individual confirmation of positivity because of its high specificity. The other tests are no longer used because they have been replaced by the more easily standardised techniques of ELISA (Desquesnes, 2004; Reid & Copeman, 2003) and CATT (Bajyana Songa & Hamers, 1988; Njiru *et al*., 2004) are the methods of choice in most circumstances.

Evaluations of ELISA and CATT have been carried out in camels, horses, cattle, buffaloes and pigs (Desquesnes *et al.,* 2009; Holland *et al*., 2005; Reid & Copeman, 2003, amongst others). Tests should preferably be carried out on plasma or serum, but 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 (Hopkins *et al*., 1998). It is vitally important that serological tests be validated and standardised if they are to be suitable for correctly identifying infected animals; cross evaluation in different laboratories is thus required. Standard criteria for interpreting the tests might have to be developed for each animal species and standardised at least at a regional level (Desquesnes, 2004). As

T. evansi is considered to be polyphyletic, having evolved from *T. brucei* (Lai *et al.,* 2008), it is necessary to take into account the various strains that may be present in a given area (RoTat *versus* non-RoTat for example).

2.1. Indirect immunofluorescent antibody test (IFAT)

Although the technique is not adapted to large-scale surveys, it is still useful to screen a small number of samples in laboratories that are carrying out the test for other purposes and/or that are not carrying out the ELISA. Cost of reagents is medium, around 0.5€/test, but the technique is time consuming.

2.1.1. Test procedure

The antigen consists of dried blood smears containing from five to ten *T. evansi* per field at magnification 500×, collected from a highly parasitaemic mouse or rat (3–4 days post-infection). Smears are dried at room temperature for 1 hour and fixed with acetone (± ethanol) for 5 minutes. When kept dry, the fixed smears may be stored at –20°C for several months. Better results are obtained using purified trypanosomes separated from the rat's buffy coat on a DEAE-cellulose column (Lanham & Godfrey, 1970) using a mixture of 80% cold acetone and 0.25% formalin in a normal saline solution.

On testing, the slides are first subdivided into several circles of 5 mm diameter with nail varnish using mounting media (Teflon-coated multispot slides may also be used), then washed in PBS, pH 7.2, at room temperature for 10 minutes.

After washing, a positive and a negative control serum and the field sera to be tested (diluted 1/50 in PBS), are added and allowed to react at 37°C for 30 minutes in a humid chamber. The slides are washed three successive times in PBS for 5 minutes each. A rabbit or goat anti-bovine IgG (for tests on bovine sera) conjugated to fluorescein isothiocyanate or other fluorescein-conjugated antiserum specific to the animal species tested is then added at a suitable dilution and left at 37°C for 30 minutes in a humid chamber. The slides are rewashed in PBS, mounted with 50% glycerol in PBS with immunofluroescence mounting media, and examined by fluorescence microscopy. The glycerol solution should be stored at 4°C and renewed every 2 weeks.

The fluorescein conjugate should be stored at –20°C in small aliquots to avoid repeated freezing and thawing. The tube should be shielded from light in some way, for example by wrapping in aluminium foil. The conjugate is diluted in PBS, pH 7.2, or in PBS containing Evans blue 1/1000 (w/v) as a counterstain to facilitate discrimination between positive (green) and negative (red) fluorescence. In general, monospecific anti-IgG (gamma-chain) conjugates give the most specific results.

The IFAT-*T. evansi* seroconversion can take 60–90 days. Compared with the CATT, IFAT is more sensitive, probably because it can detect more aparasitaemic animals, but its specificity is lower. In borderline cases, the interpretation is subjective and reproducibility has sometimes been questioned. For these reasons, ELISA is a more advisable technique.

2.2. Enzyme-linked immunosorbent assay (ELISA)

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 concentrated in the buffy coat by centrifugation and 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 5%, together with a protease inhibitor cocktail[2] subjected to five freeze–thawing cycles, and ultrasonicated three times for 2 minutes on ice to ensure complete disintegration of the organisms. This preparation is centrifuged at 4°C and 10,000 ***g*** for 10 minutes. The

2 For example: complete solution for protease inhibitor; Roche Molecular Biochemicals®

supernatant is collected and the protein concentration estimated by UV readings at 260 and 280 nm or by colorimetry. The soluble antigen obtained can be stored in small aliquots at –80°C for several months or at –20°C for shorter period. It can also be freeze-dried and stored at –20°C.

2.2.1. Test procedure

i) Dilute the soluble antigen at 5 µg/ml in 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 on a shaker-incubator (300 rpm). For this step immunoplates ensuring that the specific activities of the epitopes are preserved during binding to the plate surface[3], are preferred to other plates that may allow epitopes to be obscured or impaired due to more specific binding characteristics.

ii) Remove antigen and add 150 µl of blocking buffer (BB: 0.01 M PBS containing 0.1% Tween 20 and 5% skim milk powder for 1 hour at 37°C. The quality of the skim milk is very critical[4]; optimal skim milk concentration may vary from 0.5 to 7% depending on the skim milk origin. Bovine serum albumin may also be used as blocking agent.

iii) Add test serum dilutions in BB (100 µl), in duplicate or triplicate. Include control negative and positive sera. Final dilution is made at 1/100. Incubate plates at 37°C for 30 minutes. Discard contents and wash five times with washing buffer (PBS-0.1% Tween 20).

iv) Add a specific peroxidase conjugated species-specific anti-globulin (100 µl) appropriately diluted in BB (usually between 1/5000 and 1/20,000). If species-specific conjugates are not available, protein A or protein G conjugates can be used. Incubate the plates at 37°C for 30 minutes, discard contents and wash three times with washing buffer.

v) 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) or ortho-diphenylene-diamine (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, Na_2HPO_4 [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. Add the substrate chromogen (100 µl) to the plates and incubate in the dark, at room temperature for 20–30 minutes.

vi) Read the plates or 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 three known high, medium and low positive and negative control sera, and a buffer control. Results are expressed in relative percentage of positivity (RPP) based on the optical densities of the control samples (Desquesnes, 2004; Desquesnes et al., 2009).

A large variety of other test procedures exists, for example, using purified native antigen or, more recently, using recombinant antigens (Tran *et al.,* 2009). For closely related animal species, cross-reacting reagents may often be used (e.g. anti-bovine immunoglobulin for buffalos) and the use of monospecific anti-IgG conjugates is generally recommended. However, when specific conjugates are not available, nonspecific proteins able to fix on the Fc fragment of the immunoglobulins can be used, such as protein A or protein G. Protein A conjugate has been validated for use in camels (Desquesnes *et al*., 2009).

There is 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 (Desquesnes, 2004). These results are likely to show some overlap. The operator can choose the most appropriate point to adjust the rates of false-positive and false-negative 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

3 For example: Polysorp Nunc® immunoplates

4 For example: ref: 190-12865, Wako Pure Chemical Industries Ltd, Osaka, Japan.

negative animals. Finally, if no suitable negative/positive samples are available, a cut-off can be based on the analysis of the data from animals in an endemic situation. If a bimodal distribution separates infected from uninfected animals, then an appropriate value can be selected. The ELISA is likely to correctly identify uninfected animals (while the CATT would correctly qualify infected ones). A new ELISA/RoTat 1.2 based on the VSG from a *T. evansi* RoTat 1.2 clone – a predominant antigen in *T. evansi* (Verloo *et al.,* 2001) – was successfully used in the field in Vietnam; protocols and reagents are available from the OIE Reference Laboratory in Belgium for use in equines, camelidae and water buffaloes. Another test based on invariant surface glycoprotein has recently been developed at the ITM (Tran *et al.,* 2009) and should proceed to inter-laboratory evaluation.

The VSGs may be too specific to be used as antigen in a universal ELISA *T. evansi* (see below RoTat *versus* non-RoTat parasites), while the ELISA using soluble antigens is not strain specific and this qualifies it as a universal test. Soluble antigens from whole lysate of *T. evansi* are able to detect immunoglobulins directed against *T. evansi* strains present in various host species and geographical areas; they can also detect infections in heterologous systems owing to strong cross reactions with *T. vivax*, *T. congolense* and even *T. cruzi. Trypanosoma evansi* soluble antigen must then be considered as a universal reagent for detection of *T. evansi*, but consideration must be given to species specificity in multispecies areas. The cost of reagents is low, around 0.1€/test, and the technique is fast, allowing 500 samples to be tested a day by experienced technicians.

2.3. 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* (Bajyana Songa & Hamers, 1988). The test makes use of fixed and stained trypanosomes of a defined VAT known as RoTat 1.2. Both variable and invariable surface antigens can be aggregated by specific antibodies (so-called agglutinins), responsible for the agglutination reaction. The CATT/*T. evansi* is commercially available in kit form from the OIE Reference Laboratory in Belgium. It consists of lyophilised stained parasites ('antigen'), PBS, pH 7.4, plastic-coated cards, spatulas, lyophilised 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 1 week, but preferably should be used within 8 hours when kept at 37°C.

For screening, dilute test sera 1/4 or 1/8 in PBS. Add 45 µl of the prepared antigen suspension (previously well shaken to homogenise the parasite suspension) onto circles inscribed on the plastic cards. Add 25 µl of each diluted test serum. Mix and spread the reagents with a spatula and rotate the card for 5 minutes using the rotator provided in the kit (or at 70 rpm on a classical rotor agitator). 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. The cost of reagents is medium, around 200 tests can be carried out a day by one technician.

The CATT/*T. evansi* is suitable for the detection of acute and chronic infections with a high positive predictive value. CATT/*T. evansi* is more likely to classify correctly truly infected animals; it can be used to target individual animals for treatment with trypanocidal drugs. The sensitivity of CATT *T. evansi* is variable from a host species to another; it is generally high in horses, medium in camels and buffaloes, and lower in pigs and cattle (Desquesnes *et al.,* 2011; Hagos *et al*., 2009; Holland *et al*., 2005), and it may be affected by the presence of non-RoTat 1.2 strains in some areas (Hagos *et al*., 2009; Njiru *et al.,* 2004). Animals infected by *T. evansi T. equiperdum, T. bucei* and even *T. vivax* can give a positive response to the CATT/*T. evansi* (Birhanu *et al*., 2015; Desquesnes, 2004; Gizaw *et al.,* 2017). CATT/*T. evansi* is consequently not considered as species specific. Besides this, nonspecific agglutination can be observed in populations of hosts that are not infected by trypanosomes, with variable rates depending on the host species. Nevertheless, CATT/*T. evansi* being (i) the only kit available for surra diagnosis, (ii) possibly used in all mammal species, and (iii) presenting a high positive predictive value, it is recommended in the serodiagnosis of surra.

2.4. Immune trypanolysis test RoTat1.2 (TL RoTat1.2)

Immune trypanolysis test detects specific 'trypanolytic' antibodies directed against a given parasitic strain able to induce trypanolysis in the presence of complement. It is performed with T. evansi variable antigen type RoTat 1.2 and may therefore be positive only with hosts that produce trypanolytic

immunoglobulins directed against RoTat 1.2 VAT (Van Meirvenne *et al.,* 1995). Sera are tested at a 1/4 dilution. Live trypanosomes are incubated for 60 minutes with test serum in the presence of guinea-pig serum as the source of complement. When variant specific antibodies are present in the serum, lysis of the RoTat 1.2 trypanosomes occurs. The sample is considered positive for the presence of anti-RoTat 1.2 antibodies when 50% or more of the trypanosomes are lysed. This test requires the production of complement and the growth of trypanosomes in rodents and is thus costly and ethically concerning. At present, it is mostly used to confirm samples suspected to be positive using other tests. It can be carried out at the ITM, Antwerp, on request. The cost of the test is high.

2.5. Formol-gel test

The formol-gel test is the test of choice in camels but has not been validated in other species. It is carried out by adding two drops of concentrated formalin solution (40% formaldehyde [w/v]) to 1 ml of serum. The test is positive if the serum coagulates immediately and turns white. In negative reactions, the serum remains unchanged or coagulation may take up to 30 minutes to appear.

3. Test applications

Like the majority of biological tests, the methods described above are limited both in terms of sensitivity and specificity. Moreover, test performances and parameters are highly variable, depending on the host species and breed, the species diversity of trypanosomes in the geographical area in which the host occurs, and the epidemiological situation (epizootic/enzootic/sporadic). To date, there is no common test (parasitological, serological or even molecular) that is capable of distinguishing *T. evansi* from the other *Trypanozoon* species or sub-species (Gizaw *et al.,* 2017). The final diagnosis of surra will depend on epizootiological information, clinical signs and laboratory results. For these reasons, a number of test combinations adapted to the different circumstances relevant to a particular host species and geographical area are currently recommended. These are guidelines that should be helpful in achieving the correct diagnoses. Combining at least two of the four methods below is recommended to optimise reliable and specific diagnosis.

3.1. Characteristics and performances of recommended tests for the diagnosis of surra

i) *Microscopic examination:* Microscopic observation (×400–1000 in oil immersion) of a Giemsa-stained thin blood smear (GSBS) from the host, or from a mouse inoculation test, allows identification of the subgenus *Trypanozoon* based on morphology and morphometry of parasites. When fresh samples are available, it is recommended to combine with HCT (or BCT) to increase the sensitivity of microscopic examinations. Observation of a parasite provides a certain diagnosis, however, species identification requires complementary tests if other pathogenic *Trypanosoma* spp. may potentially be present in the host investigated.

ii) PCR-TBR: DNA must be prepared from blood with a commercial kit, a resin, or phenol chloroform method, using a buffy coat obtained by 8000 ***g*** centrifugation of 0.5 ml of blood. PCR is carried out as described above, with TBR primers (Masiga *et al.,* 1992). Result is positive for *Trypanozoon* when a 177 bp product is visible on the agarose gel. Complementary primers can be used (i) to confirm the subgenus (TEPAN or ESAG), (ii) to characterise type A/B: (RoTat1.2 and EVAB), (iii) to characterise human strains (Tgs-GP and Tbr), or (iv) as evidence of other *Trypanosoma* subgenera or species (ITS1 primers); however, the sensitivity of all these primers being lower than that of TBR primers, when negative, they may lead to inconclusive results.

iii) ELISA *T. evansi:* Serum or plasma samples are tested in ELISA-*T. evansi* (soluble antigens from whole *T. evansi* lysate) as described above. A sample is positive when its RPP is above the cut off value established for the host species (appropriate conjugates are defined for each species; see below). Its negative predictive value is very high, unless the host was very recently infected.

iv) CATT/*T. evansi:* Serum or plasma are diluted 1:4 and tested as described by the manufacturer. Positive samples are samples presenting results = or > to one + (doubtful samples are considered as negative samples). Positive predictive value is high, however, nonspecific agglutination may occur. It is generally recommended to combine ELISA *T. evansi* and CATT/*T.evansi* to increase sensitivity and diagnosis reliability; however, in case of discrepancy, it is recommended to repeat sampling and testing.

Conjugates to be used in ELISA *T. evansi* for each host species are: Cattle and buffalo: anti-bovine IgG whole molecule; Pig and elephant: Protein G conjugate; Camels: Protein A conjugate; Dog: anti-dog conjugate; Goat and sheep: anti-goat and sheep conjugates; Rat: anti-rat IgG whole molecule. Conjugates remain to be defined for other host species.

3.2. Association of recommended tests for the diagnosis of surra in animals

3.2.1. Recommended method for sensitive agent detection

A combination of the three techniques GSBS, HCT and a sensitive PCR (based on satellite DNA detection) is recommended for agent detection; when positive, they should be completed with more specific PCR tests. In case of potential mixed trypanosome infections, other primers should be deployed, as indicated in Chapter 3.4.14 *Nagana: infections with salivarian trypanosomoses* (*excluding* Trypanosoma evansi *and* T. equiperdum).

3.2.2. Recommended methods for antibody detection

A combination of ELISA *T. evansi* using soluble antigens from whole trypanosome lysate, and CATT *T. evansi* (RoTat1.2 based test) is recommended in order to potentially detect antibodies directed against all types of *T. evansi*. In case of potential mixed trypanosome infections, because of bilateral cross-reactions amongst trypanosomes, inferences on species-specificity must preferably be based on PCR results.

C. REQUIREMENTS FOR VACCINES

No vaccines are available for this disease.

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NB: There are OIE Reference Laboratories for surra (*Trypanosoma evansi* infection)
(please consult the OIE Web site:
https://www.woah.org/en/what-we-offer/expertise-network/reference-laboratories/#ui-id-3).
Please contact OIE Reference Laboratories for any further information on
diagnostic tests and reagents for surra

NB: FIRST ADOPTED IN 1991 AS SURRA (*TRYPANOSOMA EVANSI*). MOST RECENT UPDATES ADOPTED IN 2021.