Trypanosoma equiperdum in the horse – a neglected threat?

Trypanozoma equiperdum *bij het paard – een onbekende bedreiging?*

[1 2 3]Y. Ahmed, [3]A. Hagos., [3]B. Merga , [2]A.Van Soom , [2]L. Duchateau , [2,4] B.M.Goddeeris , [2]J. Govaere

[1]Wollo University, School of Veterinary Medicine, PO Box 1145, Dessie, Ethiopia
[2]Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820, Merelbeke, Belgium
[3]Addis Ababa University, College of Veterinary Medicine and Agriculture, PO Box 34, Bishoftu, Ethiopia
[4]KU Leuven, Faculty of Bioscience Engineering, Kasteelpark Arenberg 30, 3001 Leuven, Belgium

jan.govaere@ugent.be

ABSTRACT

Dourine is a contagious disease caused by *Trypanosoma equiperdum* that is transmitted directly from animal to animal during coitus. Dourine is known as an important disease in many countries, and it threatens equidae worldwide. It is reported to be widespread in South America, Eastern Europe, Russia, Mongolia, Namibia and Ethiopia. The disease can be carried to various parts of the world through the transportation of infected animals and semen. Since knowledge of the prepatent infectiousness of a recently infected animal is lacking, introduction of the disease is in principle an ever-present threat. Definitive diagnosis depends on the identification of the parasite by means of direct microscopy. This is rarely possible in practice and therefore, diagnosis in the field is based on the observation of typical clinical signs, together with serological tests. This paper is an endeavour to review briefly and compile information on the appearance and importance of Dourine in terms of its epidemiological and clinical features, as well as on its diagnosis, treatment and prognosis.

SAMENVATTING

Dourine is een infectieuze en venerisch overdraagbare ziekte bij paarden die veroorzaakt wordt door *Trypanosoma equiperdum*. Deze aandoening is endemisch in Zuid-Amerika, Mongolië, Namibië, Ethiopië, Rusland en Oost-Europa en heeft een negatieve invloed op de gezondheid van de betreffende paardenpopulaties.

De ziekteverspreiding kan bespoedigd worden door het transport van geïnfecteerde dieren en sperma. De huidige kennis over de infectiviteit van het agens tijdens de prepatentperiode bij besmette dieren is minimaal. Aldus kan Dourine in landen verspreid worden die voorheen vrij waren van de ziekte. De eigenlijke diagnose is gebaseerd op de identificatie van de parasiet. Gezien dit in de praktijk meestal niet haalbaar is, wordt het klinisch beeld samen met niet-specifieke serologische testen gebruikt om de ziekte vast te stellen. In dit artikel wordt een overzicht gegeven over het belang van Dourine bij het paard. Verder worden de epidemiologie, het klinische ziektebeeld alsook de mogelijkheden van diagnostiek en behandeling besproken.

INTRODUCTION

Trypanosomosis is a parasitic disease caused by different species of flagellated protozoa belonging to the genus Trypanosoma, which inhabit the blood and various body tissues and fluids of vertebrate hosts. Non-Tsetse Transmitted Animal Trypanosomoses (NTTAT) result from infection by *Trypanosoma* (*T). vivax, T. evansi* and *T. equiperdum* (Touratier, 2000). However, the extent of tissue invasion varies among the different parasite species (Igbokwe, 1996; Radostitis et al., 1996). The disease is frequently fatal and is a serious constraint to agricultural production in large parts of sub-Saharan Africa, exhibiting direct impact on live-

stock productivity, livestock management and human settlement, and indirect impact on crop agriculture (Swallow, 2000).

Trypanosoma (T.) equiperdum differs substantially from other trypanosome species that are transmitted by invertebrate vectors. *T. equiperdum* is transmitted venereally directly from one infected horse to another. The trypanosomes, which are present in the seminal fluid and mucous membranes of the genitalia of the donor animal, are transferred to the recipient during sexual intercourse. Moreover, *T. equiperdum* further differs from other trypanosomes in that it is primarily a tissue parasite that rarely invades the blood (Barrowman 1976; Vulpiani et al., 2013; OIE, 2013).

Dourine caused by *T. equiperdum* is characterized mainly by fever, edematous swelling of the genitalia, cutaneous plaques and eruptions and neurological signs including incoordination, paralysis, ocular lesions (such as conjunctivitis, keratitis and mild corneal opacity), anemia and progressive emaciation (Barrowman 1976; Luckins 1994, Vulpiani et al., 2013). Clinical signs related to Dourine have been documented to be often obvious, but final diagnosis requires demonstration of the parasite through serological and molecular tests (OIE, 2013).

Among the various species of Trypanosoma, *T. equiperdum* and *T. evansi* infection in equines cause similar signs. In view of this fact, the actual course of the disease caused by these two trypanosome species was uncertain due to the close similarity in their ultrastructure, genetic makeup and antigenic nature, as has been demonstrated by the fact that the two species have shown similar genetic and antigenic expression (Touatier, 2000; Verloo et al., 2001; Claes et al., 2003a,b). However, recent findings of whole genome analysis of *T. evansi* and *T. equiperdum* provided new insights of their distinction and their relation with the different *T. brucei* subspecies (Birhanu et al., 2016; Cuypers et al., 2017). Both are evolved from *T. brucei* but with different geographical origins. The *T. evansi* genomes are related to the *T. brucei* genomes from Western Africa, whereas the *T. equiperdum* genomes are related to the *T. brucei* genomes from Eastern Africa (Carnes et al., 2015; Cuypers et al., 2017).

Currently, there are fragmented reports and findings about the disease in its occurrence, clinical signs, efficacy of treatment, etc. Therefore, the objective of this paper is to review and compile information on the appearance and importance of Dourine in terms of its epidemiological and clinical features, as well as on its diagnosis, treatment and prognosis.

APPEARANCE AND IMPORTANCE OF DOURINE

Dourine is known in most countries of the world as a notifiable disease (OIE, 2013) and it threatens equidae around the globe. The disease is reported to be widespread in South America (Samper and Tibary, 2006; Sellon and Long, 2007; Perrone et al., 2009; Sanchez et al., 2015), Mongolia (Clausen et al., 2003), Namibia (Kumba et al., 2002), Eastern Europe (Discontools, 2011) and Ethiopia (Alemu et al., 1997; Clausen et al., 1999; Fikru et al., 2010; Hagos et al., 2010a,b). Recently, the disease has also been observed in the south of Europe, in Italy (Scacchia et al., 2011; Pascucci et al., 2013).

The disease can be carried to various parts of the world through the transportation of infected stallions, mares, donkeys and semen. The spread of the causal agent by AI has not been confirmed; however, it could potentially occur since *T. equiperdum* is present in seminal fluid and genital tissues (Lelli et al., 2012). Besides venereal transmission, close contact between mare and foals, e.g. during nursing (Brun et al., 1998; Lelli et al., 2012), contaminated equipment, such as an artificial vagina and breeding phantom, or contaminated personnel can also cause transmission (Metcalf, 2001; Samper and Tibary, 2006).

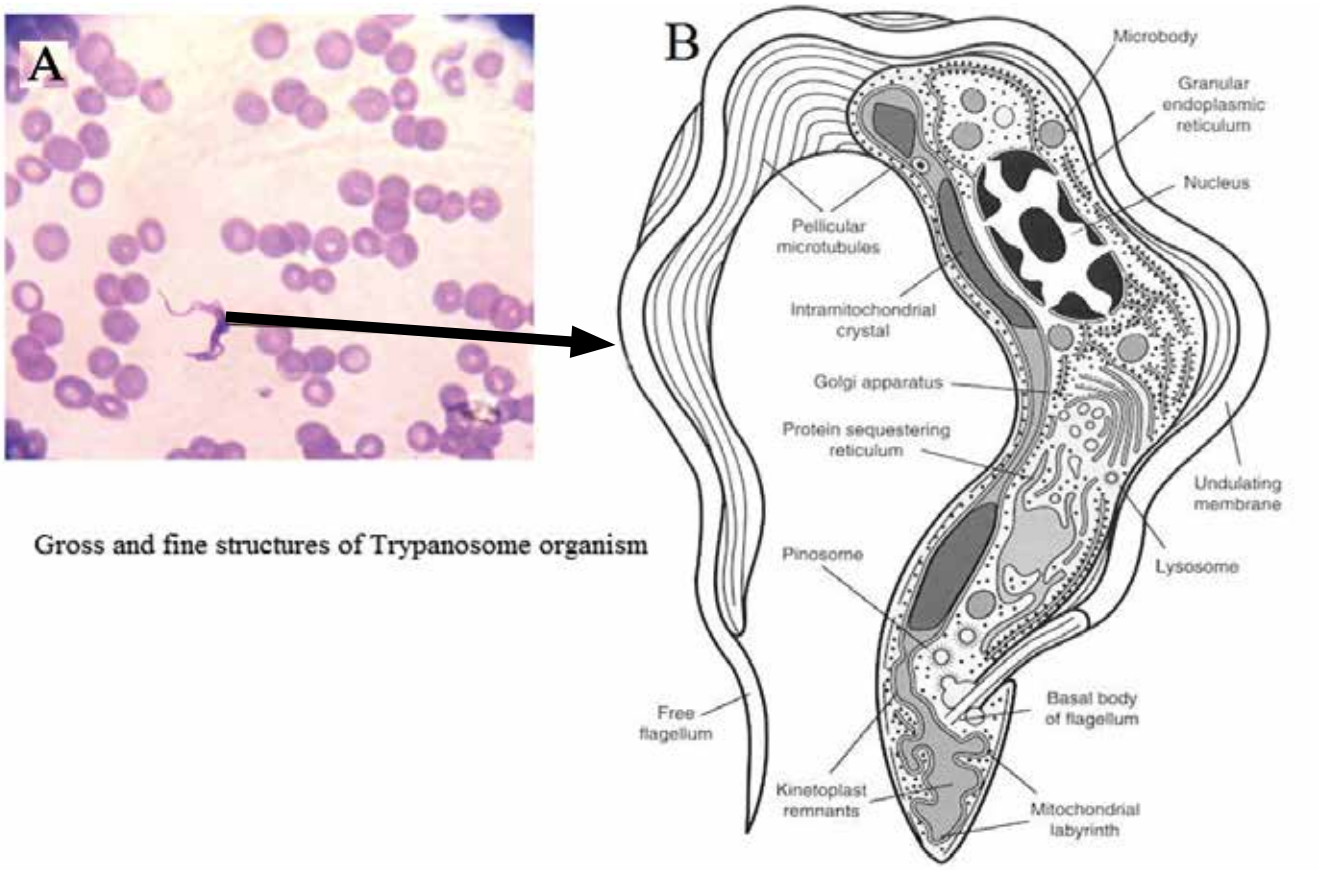
Today, despite the numerous benefits of shipping semen internationally, some serious threats remain unclear when the semen is contaminated with a communicable disease. Import regulations to prevent entry of the disease from endemic countries will require a negative Dourine test for horses and horse gametes. The current regulations vary between countries (IHSES, 2007; Calistri et al., 2013), which makes it difficult to comply with all of them when dealing for instance with cryopreserved semen. The import regulations vary from requiring only a permit for importation to several months of quarantine of the stallion. Due to the lack of knowledge of the prepatent infectiousness of the semen of recently infected animals, the transmission of disease through the transport of either the breeding stock or their gametes is a threat for the equine industry (Metcalf, 2001).

Although Dourine has a high mortality rate of up to 50%, some infected animals have been observed to recover spontaneously (Equimed, 2009; Ricketts et al., 2011; OIE, 2013). So far, there has been no known natural reservoir of the parasite other than infected equids. Donkeys and mules are more resistant than horses and may therefore remain unapparent carriers, in which the disease may often pass unperceived, even though their semen and vaginal secretions contain the infective trypanosomes (OIE, 2013).

ETIOLOGY

Trypanosomes are flagellated and elongated spindle-shaped protozoa with an average length of 20-30 µm and a width of 1.5 to 3.5 µm. The trypanosome cell has a blunted posterior end and a free flagellum at the anterior end (Matthews, 2005) (Figure 1).

The name *T. equiperdum* was postulated by Doflein in 1901 as cited in OIE (2013). *T. equiperdum* is a member of the non-tsetse-transmitted trypanosome group. The trypanosomes, which are strictly parasi-



Gross and fine structures of Trypanosome organism

tic, are flagellar protozoa that belong to the phylum of Sarcomastigophora, the order of Kinetoplastidae, the family of Trypanosomatidae and the genus of Trypanosoma, under the Salivarian group. The subgenus Trypanozoon includes the pathogenic species *T. evansi*, *T. brucei* and *T. equiperdum. T. brucei* is further divided into three subspecies: the animal pathogen *T. b. brucei* (ruminants, equines), and the two human pathogens responsible for human sleeping sickness, *T. b. gambiense* and *T. b. rhodesiense* (Hoare, 1972). *T. equiperdum* has recently come to be considered a subspecies of *T. brucei* based on molecular analysis (Claes et al., 2003b; Li et al., 2005, Lun et al., 2010; Schnaufer, 2010; Carnes et al., 2015; Wen et al., 2016).

Kinetoplastid flagellates contain their eponym kinetoplast DNA (kDNA), consisting of two types of interlocked circular DNA molecules: dozens of maxicircles and thousands of minicircles. The maxicircles have typical mitochondrial genes, most of which are translatable only after RNA editing. The minicircles encode guide RNA's, which are required for decrypting the maxicircle transcripts (Lai et al., 2008). *T. brucei* is a kinetoplastid trypanosome whereas *T. evansi* and *T. equiperdum* are dyskinetoplastic trypanosomes. *T. evansi* lack the maxicircle genes, but *T. equiperdum* does have, although with major deletions of some genes. So far, no akinetoplastic (lacking all kDNA) *T. equiperdum* have been observed (Birhanu et al., 2016). Two biological features differentiate *T. evansi* and *T. equiperdum* from the *T. brucei*. First, they do not use tsetse flies as the vector for transmission, since *T. evansi* is transmitted by biting flies and *T. equiperdum* by sexual contact (Desquesnes et al., 2013; Brun et al., 1998). Secondly, *T. evansi* and *T. equiperdum* are dyskinetoplastic, lacking part of or all (akinetoplastic) of their kDNA (Lai et al., 2008). Partial dyskinetoplastidy or total akinetoplastidy locks the trypanosome in the blood stream form trypamastigotes in the host, and transmission between vertebrates becomes purely mechanical, without further development in a vector (Bringuard et al., 2006).

Trypanosomes of subspecies *T. equiperdum* are rarely observed in the bloodstream of the host because they are normally localized in the capillaries of the mucous membranes of the urogenital tract (Brun et al., 1998). However, a few trypanosomes occasionally appear in the peripheral blood of animals (Brun et al., 1998; Fikru et al., 2010; Hagos et al., 2010c). The fact that foals have been found to be infected with *T. equiperdum* may indicate that the parasite can also be directly transmitted through udder lesions or milk, or during passage through the birth canal upon parturition (Schultz, 1935; Wang, 1988; Brun et al., 1998). Vertical transmission of *T. evansi* has already been reported in camels (Narnaware et al., 2016).

The true *T. equiperdum* strains successfully isolated and available for various studies are OVI, BoTat 1.1, Dodola 940 and 943 and the Mongolian isolates (IVM-t1) (OIE 2013; Birhanu et al., 2016, Suganuma et al., 2016).

CLINICAL SIGNS

Clinical symptoms, although not pathognomonic, can be of great help in diagnosing the disease in endemic areas. The presence of edematous swellings of the external genitalia, the development of plaques of the skin, and the appearance of nervous signs such as ataxia and incoordination are highly indicative for the disease (Vulpiani et al., 2013).

Substantial weight loss leading to severe emaciation, weakness and a generally poor condition has been reported in naturally infected as well as experimentally infected horses (Vulpiani et al., 2013). Hagos et al. (2010a, c) also reported marked muscular atrophy in the gluteal region, emaciation, weakness and poor body condition as important clinical signs in horses. However, the vulvar or preputial lesions of Dourine might be difficult to clinically differentiate from other diseases such as Equine Coital Exanthema (EHV3) in the early stages and in latent cases (Barrowman, 1976). Equine Herpes Virus (EHV3) infection is manifested by the appearance of vesicles, ulcers and depigmented spots on the vaginal mucous membrane (Blanchard et al., 1992; Studdert, 1996; Allen and Umphenour, 2004), which is similar to the genital form of Dourine. By contrast, however, EHV3 is self-limited and is not accompanied by emaciation, ataxia or incoordination.

Lesions in the genitalia are documented to be the first symptoms after *T. equiperdum* infection. In stallions, moderate edema of the scrotum and preputial sheath (Figures 2 E and F) is seen, accompanied by both preputial and urethral purulent discharge. In mares, edema of the vulva, accompanied by ulcers along the rim of the vulva, vaginal discharge mainly of the mucopurulent type with foul odour, and edema of the mammary gland and cloudy off-white mammary secretions have been documented as the prominent signs in the genital form of the disease. Later in the progression of the disease, edema of all ventral body parts, and cutaneous plaques all over the body can be seen. At this stage, the edematous plaques, especially on the genitalia, frequently ulcerate and become depigmented scars (Hagos et al., 2010a; Vulpiani et al., 2013) (Figure 2 D). This depigmentation has been indicated to be due to severe dermatitis with hydropic degeneration and necrosis of the keratinocytes and necrosis of basal cells including the melanocytes with excess free melanin pigment within the epidermis (Gizaw et al., 2017).

The next stage of Dourine is characterized by progressive anemia, with or without fever, and nervous disorders, mainly manifested as paraplegia and paralysis of the hind limbs, showing severe muscle atrophy and emaciation before death (Hoare, 1972; Stephen, 1986) (Figures 2 A, B and C).

Nervous signs such as stiffness, weakness, lameness in one or more hind legs, staggering, lack of coordination, inability to stand upright after prolonged sternal or lateral recumbence, ataxia and facial paralysis have also been reported by Hagos et al. (2010a) and Vulpiani et al. (2013).

DIAGNOSIS

Diagnosis has to be based on demonstrating the presence of the parasite itself or indirectly by antibody detection. However, there is no defined serological assay for T. *equiperdum* nor molecular markers

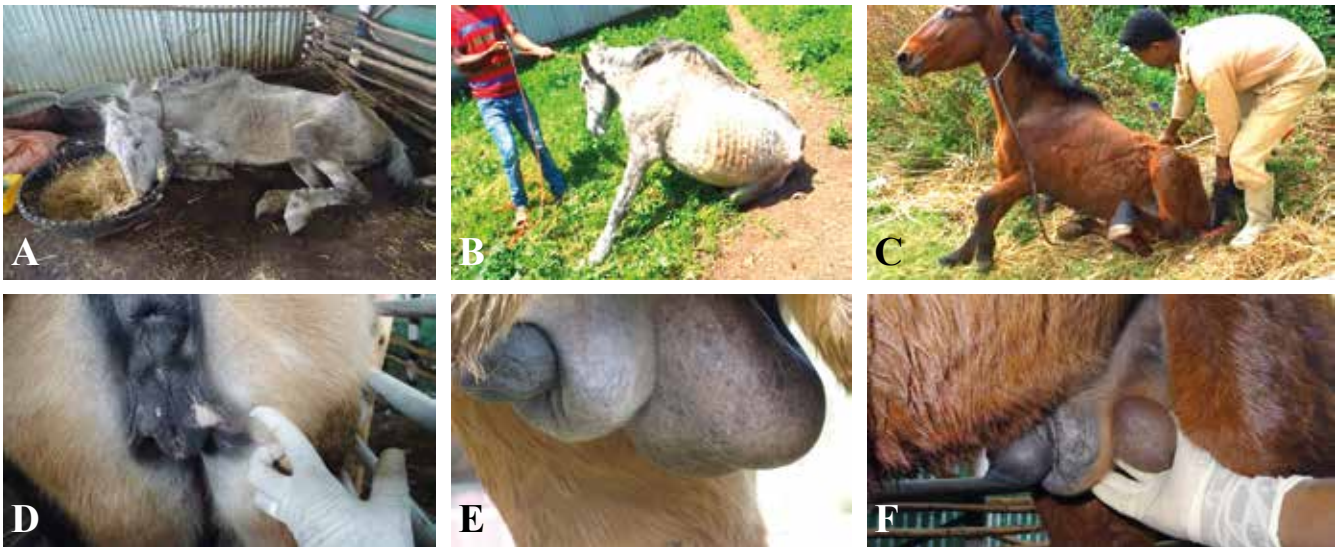


Figure 2. Symptomatology of Dourine infected horses. A. and B. Mare with weight loss and hind quarter paralysis. C. Stallion with hind quarter paralysis. D. Mare with depigmented scar at the vulva. E. and F. Swelling at the prepuce and scrotum from experimentally infected horses with *T. equiperdum* (mares are infected by artificial insemination after spiking the semen by *T. equiperdum* Dodola 943 and stallions are infected by the blood transfusion from these mares via intra venous route. Ethical clearance for this experiment was obtained from the Ethical Review Committee of Addis Ababa University College of Veterinary Medicine and Agriculture to use animals for experimentation (Ref. No: VM/ERC/004/07/015).

that can be used for clinical use so far. The clinical signs and gross lesions in diseased animals are suggestive, but cannot always be identified with certainty, especially in the early stages or in latent cases, and in cases of surra due to *T. evansi,* which exhibits similar clinical signs (OIE, 2013). Other conditions, such as equine coital exanthema or chronic irritation (e.g. urine scalding), might cause some similar clinical features (Blanchard et al., 1992; Studdert, 1996; Allen and Umphenour, 2004).

Differentiation between *T. equiperdum* and *T. evansi* based on parasite morphology is difficult in areas where both organisms are found (Brun et al., 1998; Sanchaz et al., 2015; Suganuma et al., 2016). So far, there are no defined serological assays for the true *T. equiperdum* and also no molecular markers for clinical use. Previously, Claes et al. (2004) and Fikru et al. (2010) used a PCR to amplify a portion of the RoTat 1.2. VSG coding gene in order to detect *T. equiperdum*. More recently, it has been shown that RoTat 1.2 VSG is not present on *T. equiperdum* (Birhanu et al., 2016), thus making previously reported findings equivocal.

Direct diagnosis: parasitological techniques

Wet, thick blood films can be examined microscopically (x400) under a coverslip. Detection of Trypanosomes moving in between the erythrocytes is a simple, although non-species specific, parasitological test. However, with a detection limit as high as 10,000 trypanosomes/ml, the technique has a very low sensitivity. Giemsa or stained thin blood films have a similarly low sensitivity. The sensitivity can be increased by applying concentration techniques such as used in the Woo test (Woo, 1970). This test includes a microcentrifugation of 50µl whole blood at 3000 g for 5 minutes. Subsequently, the capillary tubes are mounted in a special holder and the buffy coat is examined microscopically at magnification of ×100 to look for live parasites (Woo, 1970; Reid et al. 2001; Fikru et al., 2010). On microscopic evaluation, *T. equiperdum* and *T. evansi* cannot be differentiated from one another, a fact which makes the test less reliable in areas where both organisms are prevalent (OIE, 2012).

Immunohistochemistry and immunofluorescence

A more recent study managed to identify and localize trypanosome antigen directly in the tissue by immunochemistry, using Mayer's hematoxylin, in addition to an immunofluorescence staining, using diamidin phenylindole (DAPI) (Pasucci et al., 2013).

Animal inoculation

Mice, rats, rabbits and dogs are susceptible to laboratory adapted *T. equiperdum* strains (Brun et al., 1998; Claes et al., 2005; Akhmetova, et al. 2016). However, beside the animal welfare issues, it is often difficult to obtain a first passage from samples obtained from the host, and animal inoculation is of little use as a routine method of diagnosis.

Blood from suspected animals can be used as inoculants for laboratory rodents. Under laboratory conditions, dogs can develop Dourine. Different routes of infection, such as subcutaneous, intraperitoneal, intravenous, intraurethral and intravaginal transmission, were tested and all gave rise to clinical signs of Dourine. In dogs, inoculation of *T. equiperdum* produces the typical picture of Dourine with trypanosomes present in the lesions, but not in the blood, and the infection may last from one to several months (Stephen, 1986; Claes, 2003).

Strains of *T. equiperdum* were successfully isolated after intratesticular injection in the rabbit with blood or material from infected horses (Claes, 2003; Claes et al., 2005). Similarly, after the injection of udder secretions from clinically diseased mares into the scrotum of rabbits, *T. equiperdum* could be isolated successfully from the scrotal tissue homogenate of the rabbit. The new isolate from that rabbit could also be re-isolated from scrotal edema of another rabbit after successive inoculation, even after freezing of the inoculum in liquid nitrogen (Pascucci et al., 2013). However, blood and genital washes from serologically positive horses did not lead to infection when inoculated into mice and puppies (Alemu et al., 1997; Hagos et al., 2010a; Pascucci et al., 2013). Attempts to transmit the parasite to animals other than horses (rats by intra peritoneal way and dogs subcutaneously) were also unsuccessful with inocula of blood and Cerebro Spinal Fluid (CSF) known to contain living trypanosomes (Barrowman, 1976).

DNA-based technique

A highly sensitive method for detecting even a single parasite is the polymerase chain reaction (PCR) based on an amplification of trypanosomal DNA with a sensitivity of 0.5 pg of parasite DNA or one single parasite in 10µl blood (Wuyts et al., 1994). Using PCR of a 135 bp portion of a highly repeated region within the Trypanozoon subgenus and the OVI strain of *T. equiperdum* as positive control was used by Pascucci et al. (2013) to detect the organism from blood and other tissue fluids such as uterine and vaginal washings, cerebrospinal fluid, joint fluids, mammary secretion and urine. Moreover, solid tissues from udder, mammary and iliac lymph nodes, vulva, clitoris and uterus showed positive results for the parasite in Dourine affected animals.

Serology: Indirect diagnosis

From earlier studies, it was known that the diagnosis of *T. equiperdum* by standard parasitological techniques is difficult owing to the low numbers of parasites present in blood or tissue fluids and the frequent

absence of clinical signs of disease in the prepatent and the chronic phases. Consequently, the demonstration of trypanosomal antibodies in the serum has become the most important diagnostic technique in determining whether an animal is currently infected or has been previously in contact with the parasite (OIE, 2013). Serological testing by the complement fixation test (CFT) has been widely used in health certification of horses for export (Wassal et al., 1991).

Humoral antibodies are present in infected animals, whether they exhibit clinical signs or not. The CFT has been used to confirm clinical evidence and to detect latent infections (OIE, 2013). The reliability of CFT and IFAT for known *T. equiperdum* has been reported by Cauchard et al. (2014). However, uninfected equids, particularly donkeys and mules, often give inconsistent or nonspecific reactions because of the anti-complement effects of their sera. In this case, the indirect fluorescent antibody test (IFAT) is more adequate. Enzyme-linked immunosorbent assays (ELISAs) are also used (OIE, 2013).

However, the diagnostic antigens and antibodies currently available for use in sero-diagnostic tests are not specific for *T. equiperdum,* but react due to the cross-reactivity with the other Trypanosome spp. Significant improvements in Dourine sero-diagnosis will require the development of more specific *T. equiperdum*-subunit antigens. A recent publication reported the successful in vitro cultivation of *T. equiperdum* OVI parasites that can be used in complement fixation tests (Bassarak et al., 2016). This might be of great help in obtaining a specific diagnosis for *T. equiperdum*, since the OVI strain is one of the few genuine *T. equiperdum* strains generally available in reference laboratories (Claes et al., 2003b). Until all of these requirements have been achieved, however, the diagnosis of Dourine will of necessity involve the detailed history and the clinical and pathological findings, on top of the serology, to establish confirmation of the disease (Calistri et al., 2013).

Because of the lack of a specific antigenic marker to differentiate *T. equiperdum* from *T. evansi,* careful attention must be paid when choosing a strain to prepare such an antigen. According to recent data, true *T. equiperdum* needs to be differentiated from *T. evansi* (Claes et al., 2003a, b; 2004). The problem, however, is that many *T. equiperdum* strains have been found to be closely related to certain classes of *T. evansi* in cluster analysis by Random Amplified Polymorphic DNA (RAPD) and Multiplex-endonuclease genotyping (Claes et al., 2003b). With this in mind, the *T. equiperdum* Onderstepoort Veterinary Institute (OVI) and BoTat 1.1 strains have been found to be the most suitable for use as antigen sources. Single Nucleotide Polymorphism (SNP) within the F1-ATP synthase γ subunit gene provided an identifying characteristic of *T. evansi* as distinct from *T. equiperdum* without relying on VSG genes or kinetoplast DNA (Birhanu et al., 2016). A recent findings of whole genome SNP analysis of *T. evansi* and *T. equiperdum* also provided new insights in the origin of both species and their relation with the different *T. brucei* subspecies (Cuypers et al., 2017). This method may be the future key for differentiation of the two species and then developing specific markers for diagnosis.

Agglutination Test for Trypanosomosis

Card and Latex agglutination tests developed for *T. evansi* from RoTat 1.2 antigen have been implemented for Dourine diagnosis because of the cross-reactive nature of the antibodies of some strains of *T. equiperdum (*Claes 2002). Fikru et al. (2010) and Hagos et al. (2010 a,b) have used the test to diagnose Dourine in Ethiopia at the field level. However, there is no RoTat1.2 gene on true *T. equiperdum* strains (Birhanu, et al. 2016; OIE, 2013). Therefore, unless genuine antigens are identified from true strains of *T. equiperdum,* these agglutination tests will be no more functional to diagnose Dourine (OIE, 2013).

Indirect Fluorescent Antibody Test (IFAT)

The Indirect Fluorescent Antibody Test (IFAT) is frequently used in the diagnosis of Dourine as a confirmation test for a positive CFT result, since immunofluorescence is a more reliable and sensitive technique, though its interpretation is both subjective and labour intensive (Wiliamson et al., 1988). This test can be used in surveillance (prevalence of infection) and for the purpose of declaring a population free of the disease (OIE, 2013). This test has been used with success to diagnose Dourine in Italy (Pascucci et al., 2013).

Enzyme-Linked Immunosorbent Assay (ELISA)

Although the CFT has been used for many years to diagnose Dourine, it is considered to be less sensitive than ELISA and it has been suggested that ELISA could replace the CFT for animal health certification. The Enzyme-Linked Immunosorbent Assay (ELISA) is a very sensitive technique and its use for routine diagnostic serology of Dourine would provide a significant advantage over current serological tests if a defined antigen were to be used, since it would permit test standardization and more readily allow comparison of the test results among the different laboratories. In addition, ELISA testing lends itself to a far greater degree of automation, which makes it suitable for large numbers of samples (Wassal et al., 1991; Bishop et al., 1995).

Different authors have stated that the ELISA has a satisfactory concordance ratio with CFT and can be used to supplement CFT (Williamson et al., 1988; Alemu et al., 1997; Clausen et al., 2003). Similarly, Wasal et al. (1991) concluded that ELISA is a very sensitive test for Dourine compared to the CFT and IFA tests.

Recently, Davaasuren et al. (2017) have shown the use of ELISA to diagnose Dourine based on recombinant GM6 antigen (rTeGM6) derived from *T. equiperdum* isolated from the urethral mucosa of a clinically Dourine diseased stallion in Mongolia (Suganuma et al., 2016). The result showed a good diagnostic value in testing the sera of *T. equiperdum*-infected horses. However, it has been already shown to diagnose *T. evansi*-infected water buffalo, cattle, goats and sheep (Nguyen et al., 2015; Nguyen et al., 2014) by this method. This might be helpful in the diagnosis of non-tsetse transmitted horse trypanosomiases in the field, but the technique couldn't differentiate between the two species.

Genotype analysis is able to differentiate *T. evansi* from T. *equiperdum* (Carnes et al., 2015; Birhanu et al., 2016; Cuypers et al., 2017). This may lead to the development of other antigen-specific markers for *T equiperdum* in future ELISA.

TREATMENT

The World Organization for Animal Health (OIE, 2013) currently imposes the slaughtering of CFT-positive horses as an effective control strategy. In general, treatment may result in asymptomatic carrier animals and is as such not recommended in a Dourine-free territory because of fear for the continuing dissemination of the disease by the treated animals (Barrowman, 1976; Losos, 1986; OIE, 2000).

Evidence from in vitro drug sensitivity determination of *T. equiperdum* (Zhang et al., 1992; Brun and Lukins, 1994) indicates that Suramin, Diminazene, Quinapyramine and Cymelarsan are effective against trypanosome species. Hagos et al. (2010c) carried out *in vivo* efficacy testing of Diminazene diaceturate (Diminasan®) and bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®) on mice, which demonstrated that Cymelarsan® is effective, but that Diminasan®, on the other hand, fails even at high doses of up to 28 mg/kg body weight (four times the recommended dose in cattle) to cure any of the mice infected with the Dodola strain from Ethiopia.

Horses treated with Cymelarsan® at doses of 0.25 mg/kg and 0.5 mg/kg body weight showed no detectable parasitaemia 24 h after treatment. The mean PCV levels also improved after treatment, and seroreversion on card agglutination test for trypanosoma was observed starting from 150 and 170 days post treatment (Hagos et al., 2010c). This might have been due to the fact that the absence of the antigen source from the host system stopped the triggering of antibody production and consequently the antibodies were diluted in the serum. The study by Hagos et al. (2010c) showed improvement in body condition following the treatment of chronic infection and there was no relapse. Moreover, the clinical signs of incoordination of the hind legs, weakness and ventral oedema disappeared within 10 days, together with a progressive increase of the PCV. However, in recent reports, both Cymelarsan® and Diminasan® have been found to lead to relapse in treated mice (Habte et al., 2014), and parasites can still be found in the CSF of horses treated with Cymelarsan® (Cauchard et al., 2016).

Recently, ex vivo trypanocidal activity of 1-(2-hydroxybenzylidene) thiosemicarbazide against Venezuelan *T. equiperdum* strain has been reported (Parra et al., 2017). The compound exhibits a greater inhibitory activity of the parasite in the culture medium. In another recent report, in vitro laboratory tests of equine antimicrobial peptide (eCATH1) showed promising results relating to its trypanocidal activity on Trypanozoon spp through plasma membrane permeabilization and mitochondrial alteration. The administration of eCATH1 at a dose of 10 mg/kg to *T. equiperdum*-infected mice diminished the mortality rate. This finding suggests that eCATH1 can be considered as a candidate for the development of new therapeutic agents for the treatment of trypanosomosis (Cauchard et al., 2016).

PROGNOSIS

Horses treated with Cymelarsan showed elimination of the parasite from circulation within short periods of time (Hagos et al., 2010; Cauchard et al., 2016). However, since the parasite is a tissue parasite, it may hide in areas that cannot easily be reached by the drugs, thus resulting in relapse (Cauchard et al., 2016).

When horses are left untreated, the majority of cases will perish (Barrowman, 1976), or else they will develop a chronic form of Dourine with clinical signs as described above (Barrowman, 1976; Hagos et al., 2010a, c; Vulpiani et al., 2013). Parasitaemia may disappear after 80 days of infection even though the general body condition continues to progressively deteriorate (Barrowman, 1976; Hagos et al., 2010c). Infected animals became aparasitaemic after 80 days post infection, though they expressed parasitaemia again when challenged with immunosuppressive drugs (Hagos et al., 2010c). Therefore, it is assumed that the parasites can hide themselves from the immune system.

CONCLUSIONS

Dourine is a contagious disease that is caused by *T. equiperdum* and is transmitted directly from animal to animal during coitus. It is a notifiable disease in most countries and it threatens equidae worldwide. Since knowledge about the prepatent infectiousness of semen is lacking, introduction of the disease is in principle an ever-present threat. Clinical signs of the

disease are very similar to Surra caused by *T evansi*. Differentiation of *T. equiperdum* and *T. evansi* in areas where the two organisms coexist remains a challenge due to the absence of a specific antigen and molecular marker for clinical use. Characterization of the true *T. equiperdum* strains and the search for specific genes should be the focus of future research. The World Organization for Animal Health (OIE) imposes euthanasia of diseased animals due to the absence of an effective treatment and to prevent the dissemination of the disease. However, in vitro anti-trypanosomal activity of thiosemicarbazide compounds and equine antimicrobial peptide (eCATH1) seems to be a promising approach in the context of future treatment strategies.

REFERENCES

Akhmetova G.D., Sabirovna S.G., Eldesbaevna T.G., Suttybaevna U.S., Omirtaevna N.B., Mikhdatovich K.D., Bilalovich A.K., Yergalievna M.G., Ualievich A.D. (2016). Isolation virulent strain of the *Trypanosoma equiperdum* for preparation of trypanosoma antigen. *Journal of Biotechnology 231*, 4-109.

Alemu T., Luckins A.G., Philips L.P. Reid S.W.J., Holmes P.H. (1997). The use of ELISA to investigate the prevalence of *Trypanosoma equiperdum* in Ethiopian horses. *Veterinary Parasitology 71,* 239 - 250.

Allen G., Umphenour N. (2004). Equine coital exanthema. In: Coetzer J., Tustin, R. (editors). *Infectious Diseases of Livestock*. Oxford Press, Cape Town, South Africa, pp. 860–867.

Barrowman P.R. (1976). Observations on the transmission, immunology, clinical signs and chemotherapy of Dourine (*Trypanosoma equiperdum*) in horses, with special reference to cerebro-spinal fluid. *Onderstepoort Journal of Veterinary Research 43,* 55-66.

Bassarak B., Moser I., Menge C. (2016). *In vitro* production of *Trypanosoma equiperdum* antigen and its evaluation for use in serodiagnosis of Dourine. *Veterinary Parasitology* 223, 133–140.

Birhanu H., Gebrehiwot T., Goddeeris B.M., Büscher P., Van Reet N. (2016). New *Trypanosoma evansi* Type B Isolates from Ethiopian Dromedary Camels. *PLoS Neglected Tropical Diseases 10*, e0004556. Pmid: 27035661.

Bishop P., Rae P.F., Philips L.P., Boid R., Luckins A.G. (1995). *Trypanosoma equiperdum*: Detection of trypanosomal antibodies and antigen by enzyme-linked immunosorbent assay. *British Veterinary Journal 151,* 715-720.

Blanchard T., Kenney R., Timoney P. (1992). Venereal diseases. *Veterinary Clinics of North America: Equine Practice 8,* 191–203.

Bringaud F., Riviere L., Coustou V. (2006). Energy metabolism of trypanosomatids: adaptation to available carbon sources. *Molecular and Biochemical Parasitology 149*, 1-9.

Brun R., Lun Z.R. (1994). Drug sensitivity of Chinese *T. evansi* and *Trypanosoma equiperdum* isolates. *Veterinary Parasiology 52*, 37-46.

Brun R., Hecker H., Lun Z.R. (1998). *Trypanosoma evansi* and *Trypanosoma equiperdum*: Distribution, biology, treatment and phylogenetic relation (a review). *Veterinary Parasiology 79*, 95-107.

Calistri P., Valeria N., Marcello A., Fabrizio D.M., Manuela T., Mercante M.T. Enzo R., Scacchia M. (2013). Dourine reemergence in Italy. *Journal of Equine Veterinary Science 33,* 83-89.

Carnes J., Anupama A., Balmer O., Jackson A., Lewis M., Brown R., Cestari I., Desquesnes M., Gendrin C., Hertz-Fowler C., Imamura H., Ivens A., Koreny L., Lai D.-H., MacLeod A., McDermott S.M., Merritt C., Monnerat S., Moon W., Myler P., Phan I., Ramasamy G., Sivam D., Lun Z.-R., Luke J., Stuart K., Schnaufer A., (2015). Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and multiple independent origins for dyskinetoplasty. *PLoS Neglected Tropical Diseases 9 (1),* e3404.

Cauchard J., Carnicer D., Madeline A., Guitton E., Giraudet A., Büscher P., Hebert L., Laugier C. (2016). Evaluation of Melarsamine hydrochloride (Cymelarsan®) efficacy for the treatment of dourine nervous form on experimentally infected ponies. *Journal of Equine Veterinary Science 39*, S45eS55.

Cauchard J., Soldan A., Madeline A., Johnson P., Buscher P., Petry S. (2014). Inter-laboratory ring trials to evaluate serological methods for dourine diagnosis. *Veterinary Parasitology 205,* 70-76.

Cauchard S., Reet N.V., Büscher P., Goux D., Grötzinger J., Leippe M., Cattoir V., Laugier C. Cauchard J. (2016). Killing of trypanozoon parasites by the Equine cathelicidin eCATH1. *Antimicrobial Agents and Chemotherapy 60,* 2610-2619.

Claes F., Verloo D., De Waal D.T., Urakawa T. Matiwa P.,Goddeeris B.M., Buscher P. (2002). Expression of rotat 1.2 cross-reactive VAT. *Annals New York Academy of Science 969*, 174-179.

Claes F. (2003). *Characterization of Pathogenic Trypanosoma spp. how does Trypanosoma equiperdum fit into the Trypanozoon Group*. Catholic University of Leuven, Belgium, PhD. Thesis.

Claes F., Verloo D., De Waal D.T., Majiwa P.A.O., Baltz T., Goddeeris B.M., Büscher P. (2003a). The expression of RoTat 1.2 variable surface glycoprotein (VSG) in *Trypanosoma evansi* and *T. equiperdum*. *Veterinary Parasitology 116,* 209–216.

Claes F., Agbo E.C., Radwanska M., Te Pas M.F., Baltz T., De Waal D.T., Goddeeris B.M., Claassen E. Büscher P. (2003b). How does *T. equperdum* fit into the Trypanozooan genus? A cluster analysis and multiplex genotyping approach. *Parasitology 126,* 425-431.

Claes F., Büscher P., Touratier L., Goddeeris, B. (2005). *Trypanosoma equiperdum:* master of disguise or historical mistake? *Trends in Parasitology 21 (7)*, 316-321.

Claes F., Radwanska M., Urakawa T., Majiwa P.A., Goddeeris B., Büscher P. (2004). Variable surface glycoprotein RoTat 1.2 PCR as a specific diagnostic tool for the detection of *Trypanosoma evansi* infections. *Kinetoplastid Biology and Disease 3,* 3.

Clausen P.H., Chulvun S., Sodnomdarjaa R., Greiner M., Noeckler K., Staak C. Zessin K.H. Schein E. (2003). A field study to estimate the prevalence of *Trypansoma equiperdum* in Mongolian horses. *Veterinary Parasitology 115,* 9-18.

Clausen P.H., Gebreselassie G., Abditcho S., Mehlitz D., Staak C. (1999). Detection of DNA in serological positive but aparasitemic horses suspected of dourine in Ethiopia. *Tokai Journal of Experimental Clinical Medicine 23(6)*, 303-308.

Cuypers B., Van den Broeck F., Van Reet N., Meehan C.J., Cauchard J., Wilkes J.M., Claes F., Goddeeris B., Birhanu H., Dujardin J.C., Laukens K., Büscher P., Deborggraeve S. (2017). Genome-wide SNP analysis reveals distinct origins of *Trypanosoma evansi* and *Trypanosoma equiperdum*. *Genome Biology and Evolution 9 (8)*, 1990–1997.

Davaasurena B., Amgalanbaatar T., Musinguzi SP., Suganuma, K., Otgonsuren D., Mossaada E., Narantsatsral S., Battur B., Battsetseg B., Xuan X., Inouee N. (2017). The evaluation of GM6-based ELISA and ICT as diagnostic methods on a Mongolian farm with an outbreak of non-tsetse transmitted horse Trypanosomosis. *Veterinary Parasitology 244,* 123–128.

Desquesnes M., Holzmuller P., Lai D.H., Dargantes A., Lun Z.R., Jittaplapong S. (2013). *Trypanosoma evansi* and Surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed Research International 2013,* 1-20. doi:10.1155/2013/194176.

Discontools (2011). Trypanosomiasis (African) scores for none tsetse transmitted. http://www.discontools.eu/Diseases/GenerateDiseaseCategories/61?type=pdf accessed on 1 November, 2015.

Equimed (2009). *Dourine, Protozoal-based Venereal Disease*. Accessed on 24/06/2017 from http://equimed.com/diseases-and-conditions/reference/dourine

Fikru R.G., Hagos A., Alemu T., Goddeeris B.M., Claes F. (2010). Comparative diagnosis of parasitological, serological, and molecular tests in dourine-suspected horses. *Tropical Animal Health and Production 42*, 1649–1654.

Gizaw Y., Megersa M., Fayera T. (2017). Dourine: a neglected disease of equids. *Tropical Animal Health Production, 49,* 887–897.

Habte B., Bsrat A., Ashenafi H., Regassa, F. (2014). Efficacy of some trypanocidal drug against *Trypanosoma equiperdum* OVI in experimentally infected mice in debre zeit, Ethiopia. *European Journal of Biological Sciences 7 (1)*, 7-13.

Hagos A., Degefa G., Yacob H., Fikru R., Alemu T., Feseha G., Claes F. Goddeeris B.M. (2010b). Seroepidemiological survey of trypanozoon infection in horses in the suspected dourine-infected Bale highlands of the Oromia region, Ethiopia. *Review Science and Technology Office of International Epizootics 29*, 649-654.

Hagos A., Getachew A., Büscher P., Goddeeris B.M., Claes F. (2010a). Serological and parasitological survey of dourine in the Arsi–Bale highlands of Ethiopia. *Tropical Animal Health and Production 42,* 769–776.

Hagos A., Goddeeris B.M., Yilkal K., Alemu T., Fikru R., Yacob H.T., Feseha G., Claes F. (2010c). Efficacy of Cymelarsan® and Diminasan® against *Trypanosoma equiperdum* infections in mice and horses. *Veterinary Parasitology 171,* 200–206.

Hoare C.A. (1972). *The Trypanosomes of Mammals. Zoological Monograph*. Blackwell Scientific Publications, Oxford and Edinburgh, UK.

Igbokwe I.O., Umar I.A., Omage J.J., Ibrahim N.D.G., Kadima K.B., Obagaiye O.K. (1996). Effect of acute *Trypanosoma vivax* infection on cattle erythrocyte glutathione and susceptibility to in vitro peroxidation. *Veterinary Parasitology 63,* 215-224.

IHSES (2007). *Import Health Standard For Equine Semen From The European Union Issued pursuant to Section 22 of the Biosecurity Act 1993*. Dated: 01 August 2007. Available: http://mpi.govt.nz/document-vault/1760 Online source Accessed 5 November 2015.

Kumba F.F., Claasen B., Petrus P. (2002). Apparent prevalence of dourine in the Khomas region of Namibia. *Onderstepoort Journal of Veterinary Research 69,* 295-298.

Lai D.H., Hashimi H., Lun Z.R., Ayala F.J. and Lukes J. (2008). Adaptations of *Trypanosoma brucei* to gradual loss of kinetoplast DNA: *Trypanosoma equiperdum* and *Trypanosoma evansi* are petite mutants of *T. brucei*. In: *Proceedings of the National Academy of Sciences USA 105*, 1999–2004.

Lelli R., Calistri P., Giovannini A., Caporale V. (2012). Evidence of *T. equiperdum* infection in the Italian Dourine outbreaks. 9th ICEID abstracts. *Journal of Equine Veterinary Science 32*, 3-95.

Li F.J., Gasser R.B., Zheng J.Y., Claes F., Zhu X.Q., Lun Z.R. (2005). Application of multiple DNA fingerprinting techniques to study the genetic relationships among three members of the subgenus Trypanozoon (Protozoa: Trypanosomatidae), molecular and cellular probes, application of multiple DNA fingerprinting techniques to study the genetic relationships among three members of the subgenus Trypanozoon (Protozoa: Trypanosomatidae). *Molecular and Cellular Probes 19*, 400-407.

Losos G.J. (1986). *Infectious Tropical Disease of Domestic Animals*. New York: Churchill Livingstone Inc., p 182-318.

Luckins A.G. (1994). Equine Trypanosomosis. *Equine Veterinary Education 6,* 259-262.

Lun Z.R., Lai D.H., Li F.J., Lukes J., Ayala F.J. (2010). *Trypanosoma brucei*: two steps to spread out from Africa. *Trends Parasitology 26*, 424–427.

Nguyen T.T., Zhou M., Ruttayaporn N., Nguyen Q.D., Nguyen V.K., Goto Y., Suzuki Y., Kawazu S., Inoue N. (2014). Diagnostic value of the recombinant tandem repeat antigen TeGM6-4r for surra in water buffaloes. *Veterinary Parasitology 201,* 18–23.

Nguyen T.T., Motsiri M.S., Taioe M.O., Mtshali M.S., Goto Y., Kawazu S., Thekisoe O.M., Inoue N. (2015). Application of crude and recombinant ELISAs and immunochromatographic test for serodiagnosis of animal trypanosomosis in the Umkhanyakude district of KwaZulu-Natal province, South Africa. *The Journal of Veterinary Medical Science 77*, 217–220.

Matthews K.R. (2005). The developmental cell biology of *Trypanosoma brucei. Journals of Cell Science 118,* 283-290.

Metcalf E.S. (2001). The role of international transport of equine semen on disease transmission. *Animal Reproduction Science 68,* 229–237.

OIE (2000). *Report of the Meeting of the OIE Ad Hoc Group on Non-Tsetse Transmitted Animal Trypanosomosis (NTTAT)*. 68/SG/18, 19 May 1999, Paris, pp 14.

OIE (2012). *Trypanosoma evansi* infection (surra). In: *Terrestrial Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

OIE (2013). Dourine. In: *OIE Terrestrial Manual Version adopted by the World Assembly of Delegates of the OIE*. May 2013, Paris, p. 1-6.

Parra N., Jaume M., Boscán K., Hernández A., Mijares A., González M., Alvarado Y., Restrepo J. (2017). *Ex vivo* trypanocidal activity of 1-(2-hydroxybenzylidene) thiosemicarbazide against *Trypanosoma equiperdum. Veterinary Parasitology 245*, 163–167.

Pascucci I., Di Provvido A., Cammà C., Di Francesco G., Calistri P., Tittarelli M., Ferri N., Scacchia M., Caporale V. (2013). Diagnosis of dourine in outbreaks in Italy. *Veterinary Parasitology 193,* 30–38.

Perrone T.M., Gonzatti M.I., Villamizar G., Escalante A., Aso P.M. (2009). Molecular profiles of Venezuelan isolates of Trypanosoma sp. by random amplified polymorphic DNA method. *Veterinary Parasitology 161*, 194-200.

Radostitis D.M., Gay C.C., Hinchelift K.W., Constable P.C. (2006). *Veterinary Medicine: a Textbook of the Diseases of Cattle, Sheep, Goats, Pigs and Horses*. Tenth edition, London: W.B. Saunders Co. Ltd. p. 1531-1540.

Reid S.A., Husein A. Copeman D.B. (2001). Evaluation and improvement of parasitological tests for *Trypanosoma evansi* infection. *Veterinary Parasitology 102*, 291-297.

Ricketts S., McGladdery A., Crowhurst J., Newton R. (2011). Dourine, an emerging venereal threat to European horses. *Equine Quarterly Disease Surveillance report 6, 7 (2),* 15-18. Accessed on 24/06/2017 from: https://www.aht.org.uk/skins/Default/pdfs/DefraApr-Jun11_Focus2.pdf

Samper J.C., Tibary A. (2006). Disease transmission in horses. *Theriogenology 66,* 551–559.

Sánchez E., Perrone1 T., Recchimuzzi G., Cardozo I., Biteau N., Aso P.M, Mijares A., Baltz T., Berthier D., Balzano-Nogueira L. Gonzatti M.I. (2015). Molecular characterization and classification of *Trypanosoma* spp. Venezuelan isolates based on microsatellite markers and kinetoplast maxicircle genes. *Parasites and Vectors 8,* 536.

Scacchia M., Cammà C., Di Francesco G., Di Provvido A., Giunta R., Luciani, M. (2011). A clinical case of dourine in an outbreak in Italy. *Veterinary Italia 47,* 473-475.

Schnaufer A. (2010). Evolution of dyskinetoplastic trypanosomes: how, and how often? *Trends in Parasitology, 26*, 557–558.

Schulz K. (1935). Dourine or slapsiekte. *Journal of the South African Veterinary Medical Association.* Vol. 6, pp. 4-15, cited in Barrowman et al., 1994.

Sellon D.C., Long M.T. (2007). *Equine Infectious Diseases*. Second Edition, Saunders Elsevier publishing, p. 473-479.

Stephen L.E. (1986). *Trypanosomiasis: A Veterinary Perspective*. Pergamon press Oxford, 551.

Studdert M. (1996). Equine coital exanthema (equine herpesvirus 3). In: Studdert M. (editor). *Virus Infections of Equines*. Elsevier, Amsterdam, pp. 39-46.

Suganuma K., Narantsatsral S., Battur B, Yamasaki S., Otgonsuren D., Musinguzi PS., Davaasuren B., Battsetseg B., Inoue N. (2016). Isolation, cultivation and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia. *Parasites and Vectors 9*, 481.

Swallow B.M. (2000). *Impacts of Trypanosomiasis on African Agriculture*. Nairobi, Kenya: ILRI (International Livestock Research Institute), p. 1-46.

Touratier L. (2000). Challenges of Non-tsetse Transmitted Animal Trypanosomes (NTTAT): An outline and some perspectives. *Annals of the New York Academy of Sciences 916,* 237-239.

Verloo D., Mangus E., Büscher P. (2001). General expression of Ro Tat 1.2 variable antigen type in *Trypanosoma evansi* isolates from different origin. *Veterinary Parasitology 97,* 183-189.

Vulpiani P.M., Carvelli A., Giansante D., Iannino F., Paganico D., Ferri N. (2013). Re-emergence of Dourine in Italy: clinical cases in some positive horses. *Journal of Equine Veterinary Science 33,* 468-474.

Wang Z.L. (1988). The similarities and differences of the characteristics between *T. equiperdum* and *T. evansi. Bulletin of Veterinary College of PLA (China) 8,* 300-303.

Wassal D.A., Gregory R.J., Phipps L.P. (1991). Comparative evaluation of enzyme-linked immunosorbent assay (ELISA) for the sero diagnosis of dourine. *Veterinary Parasitology 39,* 233-239.

Wen Y. Z., Lun Z. R., Zhu X. Q., Hide G., Lai D. H. (2016). Further evidence from SSCP and ITS DNA sequencing support *Trypanosoma evansi* and *Trypanosoma equiperdum* as subspecies or even strains of *Trypansoma brucei. Infection, Genetics and Evolution 41*, 56-62.

Williamson C.C., Stoltsz W.H., Mattheus, A. Schiele G.T. (1988). An investigation into alternative methods for the serodiagnosis of dourine. *Onderstepoort Journal of Veterinary Research 55* (2), 117-119.

Woo P.T.K. (1970). The haematocrit centrifuge technique for the diagnosis of African Trypanosomoses. *Acta Tropica 27,* 384-386.

Wuyts N., Chokesajjawatee N., Panyim S. (1994). A simple and highly sensitivity detection of *Trypanosoma evansi* by DNA amplification. *Southeast Asian Journal of Tropical Medicine and Public Health 25,* 266-271.

Yaeger R. G. (1996). Protozoa: structure, classification, growth, and development. In: Baron S. (editor). *Medical Microbiology*. Fourth edition, Galveston (TX): University of Texas Medical Branch at Galveston. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8325/ (B)

Zhang Z.Q., Giroud C. Baltz T. (1992). *In vivo* and *in vitro* sensitivity of *T. evansi* and *T. equiperdum* to Diminazene, Suramine, Melcy, Quinapyramine and isometamidium. *Acta Tropica 50,* 101-110.