

Dourine (*Trypanosoma equiperdium* Infection): a Review with Special Attention to Ethiopia

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Abstract: Dourine is a parasitic disease of breeding equids that is transmitted directly from animal to animal during coitus. The causative agent of dourine is *Trypanosoma equiperdium* which is protozoan parasite of family Trypanosomatidae. This organism presents in both genital secretion of male and female equids. *Trypanosoma equiperdium* differs from other trypanosoma in that it's rarely detected in blood rather primary in tissue. Dourine is the only trypanosomal disease which can not be transmitted by biological vectors or which can mostly transmitted venerally. Some times the disease can also transmitted to foals by ingestion of infected colostrum or milk. Historically, dourine has been present in Europe, Asia, Africa and North America. In Ethiopia dourine is restricted to only Arsi-Bale zone of highland area. Depending on virulence of the infecting strain, the nutritional status of the horse and stress factor, the course and clinical signs of dourine are highly variable in manifestation and severity. The disease is characterized mainly by swelling of the genitalia, cutaneous plaques and neurological signs and chronic emaciation. It's difficult to diagnosis this disease as the organism found in tissue parasitism and is also extremely difficult to find and differentiate microscopically from *T. evansi*. The diagnosis should attempt to clinical evidence supported by serology test. Diminazene aceturate and Cymelarsan are currently available drugs for treatment of dourine, although not fully effective in curing. To prevent dourine from being introduced into a herd or dourine-free region, new animals should be quarantined and tested by serology.

Key words: Dourine • Equine • Ethiopia • *Trypanosoma equiperdium* • Serology

INTRODUCTION

Draught animals provide power for the cultivation of the smallholdings and essential modes of transport to take holders and their families' long-distances, to convey their agricultural products to the market places for crop threshing virtually all over the country and bring back their domestic necessities. Ethiopia has 1.91 million horses, 6.75 million donkeys, 0.35 million mules and a bout 0.92 million camels in the sedentary areas of the country [1]. In a country equines have been as animals of hampered for long period of time and still render valuable services mostly as pack animals throughout the country particularly in areas where modern means of transportation are absent [2]. Even though equine species have often been described as sturdy animals; they are exposed to a variety of diseases and a number of other unhealthy circumstances. Among these, parasitic infection is a major cause of illness [3].

Trypanosomiasis is a serious parasitic disease constraint to livestock production and agricultural development in Ethiopia. There are five economically important animal trypanosome species in a country which includes *Trypanosoma congolense*, *T. brucei*, *T. vivax*, *T. evansi* and *T. equiperdium* [4]. Dourine is a chronic or acute contagious disease of equids which is cause by parasitic protozoan organism *Trypanosoma equiperdium* and transmitted by sexually from animal to animal during coitus [5, 6]. The disease can also transmitted from mare to foal can occur via the mucosa, such as the conjunctiva. Trypanosomes were found in the mammary secretions [7] and in skin samples after examination by immunohistochemistry [8].

Unlike other trypanosomiasis dourine is the only trypanosomiasis that is not transmitted by an invertebrate vector and there is no known natural reservoir of the parasite other than infected equids. *Trypanosoma equiperdium* also differs in that it is primarily a tissue

parasite that is rarely detected in the blood [9, 10]. Dourine has a worldwide distribution but currently few cases have been reported owing to the wide use of artificial fertilization technology [10]. Historically, dourine has been present in Europe, Asia, Africa and North America. Following World War I, the disease was eradicated from Western Europe by treatment of some horses with trypanocidas, strict sanitation and serologic screening of all equids [11]. In Ethiopia, the disease is limited to Arsi-Bale highland [12, 13], particularly reported in Ethaya, Sagure, Bekoji and Koffle districts of Arsi- Bale highlands [13]. In those areas, the disease is commonly known as “Dudda Kutaa” or "Laphessa" which means backbone breaker or emaciation in the local language, respectively [12]. The disease is manifested in stallions by developing edema of the prepuce and glans penis and can have a mucopurulent discharge from the urethra and Paraphimosis is also possible, while mares typically develop a mucopurulent vaginal discharge and the vulva becomes edematous [6]. Diagnosis of dourine carried out with the observation of clinical signs and symptoms however, further it’s confirmed by demonstration of the parasite, serological, biochemical and molecular tests [6, 14].

Treatment with trypanosomal drug is not recommended and ineffective because animals may improve clinically but remain carriers of the parasite [10]. But, one recent study found that bis (aminoethylthio) 4-melaminophenylarsine dihydro-chloride (cymelarsan) was effective in a small number of acutely or chronically infected horses and relapses were not observed up to a year after treatment [6]. Despite this disease is the constraint of equids in area, where its endemic there is not enough information on its treatment and control measures.

Therefore the objectives of this review are to show the available information on epidemiology, diagnosis, control and prevention of disease dourine and also to show its current status in Ethiopia.

Literature Review and Synonyms: First description of the nature of the dourine was established in 1896 following demonstration of the trypanosomes in the blood of infected Algerian horses. Dourine is a chronic or acute contagious disease of equids that is transmitted directly from animal to animal during coitus [15]. The venereal disease of equines or dourine has been known under other names (Arabic "el Dourin", English "Covering disease", German "Beschalseuche", French "Mal de coit", Russian "SlucnajaBolezni" or "Podsedal") [16]. In Ethiopia it’s commonly known as duddakuta or laphessa[12].

Table 1: The taxonomic classification of *Trypanosoma equiperdum*

Kingdom	Animalia
Phylum	Protozoa
Class	Mastogophora
Order	Kinetoplastida
Suborder	Trypanosomatida
Family	Trypanosomatidae
Genera	<i>Trypanosoma</i>
Species	<i>Trypanosoma equiperdum</i>

Source: Urquhart [18].

Etiology: The causal organism of dourine is *Trypanosome equiperdem*, which is protozoan parasite of equids of the subgenus *Trypanozoon* [17, 6].

This subgenus also includes the three subspecies of *Trypanosoma brucei* (*Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei brucei*,) and *Trypanosoma evansi*. *T.b. brucei* causing nagana in domestic animals. *T. b. gambiense* and *T. b. rhodesiense* causing sleeping sickness in human. Further, *T. evansi* causes Surra predominantly in livestock but also in other [17, 19]. It’s difficult to differentiate *Trypanosoma equiperdum* from *Trypanosoma evansi* and of isolating new strains of *T. equiperdum* from clinical cases that have appeared in various parts of the world since 1982 [20]. It’s also difficult to differentiate from *T. brucei* [14]. Like *T. equiperdum* *T. evansi*, is usually monomorphic. However, it sometimes exhibits pleomorphism like *T. evansi* during subpassages in rodents [21]. In *T. equiperdum*, flagellar pocket is relatively more coated vesicles compared with *T. evansi*. It becomes somewhat difficult to differentiate these two species with respect to the ultrastructural properties [17].

Epidemiology

Host Range: *Trypanosoma equiperdum* mainly affects horses, donkeys and mules. These species appear to be the only natural reservoirs for *T. equiperdum* [17, 22]. Zebras have tested positive by serology, but there is no conclusive evidence of infection [23, 6]. The infection may occur in donkeys and mules without obvious clinical signs, but horses usually die from infection without treatment [17]. Rats, rabbits, dogs and mice can be infected experimentally [14].

Distribution: Dourine is endemic in many areas of Asia, Africa, Russia, Eastern Europe; Middle East [24]. It was of great concern in the United States and Canada at the beginning of 20th century. Nowadays, the United States, Western Europe and Australia are considered to be free from dourine [11]. The recent reports of dourine

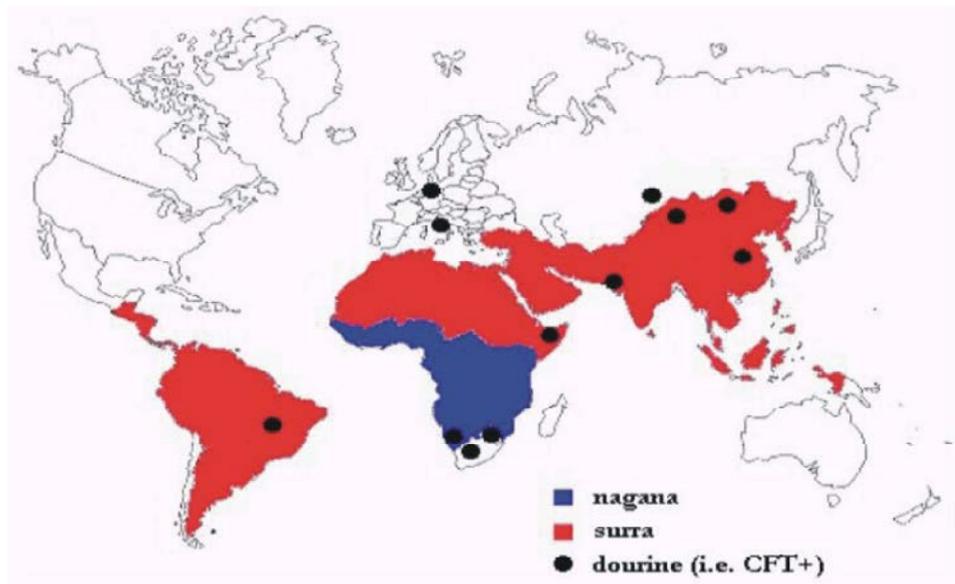


Fig. 1: • Map showing the global Distribution of Dourine and other trypanosomal disease
• Adopted from: Claes [11]

(i.e. CFT positive cases) were in China, Kyrgyzstan, Kazakhstan, Botswana, Pakistan, Ethiopia, Namibia, Italy, South Africa, Brazil and Germany. However, due to possible cross-reactions in the complement fixation test (CFT) it is difficult to conclude that seropositive animals are real *T. equiperdum* cases [20]. In Ethiopia, the disease is located in highland of arsi-bale zone [25]. Disease was widely spread in Ethaya, Sagure, Bekoji and Koffle districts of Arsi- Bale highlands [12].

Transmission and Source of Infection: Transmission from stallions to mares is more common, but mares can also transmit the disease to stallions. *T. equiperdum* can be found in mucous exudates, the seminal fluid of the penis and sheath of stallions and the vaginal secretions of infected mares [6]. Unlike other trypanosomal infections, dourine is transmitted almost exclusively during coitus. Dourine is the only trypanosomosis that is not transmitted by an invertebrate vector. *T. equiperdum* differs from other trypanosomes in that it is primarily a tissue parasite that is rarely detected in the blood [6, 10].

Other means of transmission may also be possible, but there is no evidence that arthropod vectors play any role in transmission. Foals born from mares infected with *T. equiperdum* may be infected during parturition or in utero. Transmission to foals by ingestion of infected colostrum or milk is considered rare. Foals that ingest colostrum from infected mares will become infected due to passive transfer of antibodies;

these foals are usually sero negative by 4 to 7 months of age [26]. The presence of trypanosomes in the mammary secretions may support that the infection can occasionally pass to foals during suckling [7].

Clinical Signs: The incubation period of *T. equiperdum* infection is highly variable; it may be as short as 1 to 2 weeks or as long as several years [26]. The clinical course can vary considerably under different conditions, depending on the virulence of the infecting strain, the nutritional status of the infected animal and the presence of other stress factors [27, 28]. Generally the disease in horses is chronic, persists for one or two years and is divided into three stages: stage 1 (genital edema), stage 2 (cutaneous signs) and stage 3 (nervous signs) [14].

Stage 1 involves swelling and, genital edema manifesting 1-2 weeks after infection. In stage 2, typical cutaneous plaques ("silver dollar" plaques) appear, with thickening of the skin, considered pathognomonic by some authors. Stage 3 is characterized by progressive neurological disorders and paresis of the hindquarters, anemia and often ending in death [10, 16, 24, 29]. Generally, the initial lesions of dourine often involve the genitalia. Mares typically develop a mucopurulent vaginal discharge and the vulva becomes edematous. , vaginitis with polyuria, vulvitis and signs of discomfort may be seen. There may also be raised and thickened semitransparent patches on the vaginal mucosa. Abortion may also some times be able to occur [6, 24].



Fig. 2: Selected serologically positive clinical cases of dourine in horses from the Arsi-Bale highlands. a. Photo showing oedematous swelling over the ventral abdomen and penis. b. Photo showing depigmentation over the external genitalia. c. Photo showing depigmentation over the external genitalia and udder. d. Photo showing poor body condition in horse infected with *T. equiperdum*
Source: Hagos [30].

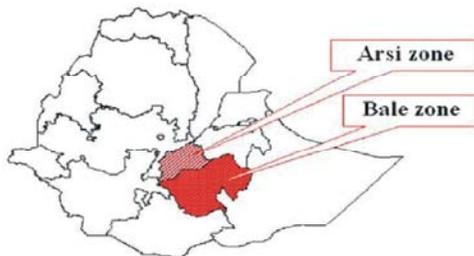


Fig. 3: Map of Ethiopia showing Arsi and Bale zones where dourine is endemic
Source: Hagos [30]

Stallions develop edema of the glans penis (Figure 3a) and, prepuce can have a mucopurulent discharge from the urethra. Paraphimosis is possible. Genital edema can disappear and reappear in both stallions and mares; each time it resolves, the extent of the permanently thickened, indurated tissue becomes greater. Vesicles or ulcers may also be detected; when they heal, these ulcers can leave permanent white scars called leukodermic patches. In addition, the genital region, perineum and udder may become depigmented (Figure 3b and 3c). In some horses, edema can spread to involve the ventral abdomen and perineum, including the scrotum in stallions and mammary gland in mares [6, 10].

Pathological Lesion of Dourine: Dourine is characterized by cachexia, muscular hypotrophy, anemia, ataxia and lack of coordination of the hindquarters, genital lesions and

skin edematous plaques [7]. In contrast to other trypanosomes the presence of nervous signs without sensory alterations seems to confirm the tropism of *T. equiperdum* for the peripheral rather than the central nervous system [27, 31].

Diagnosis: Diagnosis of dourine may difficult due to the conditions like, limited knowledge about the parasite and host-parasite interaction following infection but, in practice diagnosis is based on clinical evidence supported by serology [25, 30]. Dourine can be diagnosed by identification of the parasite; however, *T. equiperdum* are extremely difficult to find and differentiate microscopically from *T. evansi*. A small number of trypanosomes may be present in the lymph, vaginal or preputial washings or scrapings (collected soon after infection), edematous fluids of the external genitalia, mammary gland exudates or fluid content of plaques. The organisms are more likely to be detected soon after the edema or plaques first appear and they only occur for a few days in plaques [6].

Wet and Thick Blood Films: In thin and thick blood film 5 to 10 μ l of blood is placed on a slide and examined microscopically at X400 magnification under a cover slip. The parasite (trypanosoma) are observed moving between the erythrocytes in infected animals. Although it's still in use it has very low sensitivity, with detection limit as high as 10, 000 trypanosomes/ml. Giemsa or Field's-stained thin blood films have a similarly low sensitivity. It is time consuming (10-20 minutes per slide) and requires expertise to recognize the parasite [32, 33].

Serology: It is extremely difficult to detect the parasite in the body fluids of infected horses; therefore, diagnosis of *T. equiperdum* infection is based on serological evidence. Despite the development of antibody and antigen enzyme-linked immunosorbent assays (ELISA) for *T. equiperdum* Katz [34], Complement fixation test (CFT) remains the only internationally recommended test and has been used successfully in eradication program [6].

There are also several other alternative serological tests that are used, such as the arrayed immunodiffusion method, the agar gel immunodiffusion test Hagebock [35] and the competitive immunoassay (cELISA). The cELISA method has several advantages over the CFT: it can be performed in less time than the corresponding CFT procedure, it is reproducible, results are objectively measured and calculated and the method is amenable to automation [36]. While serological tests can be the method of choice for mass screening of populations, their

main limitation will remain the failure to demonstrate the parasite due to lack of sensitivity, especially for the detection of *T. equiperdum*, which is considered to be a tissue parasite rather than a blood parasite [17].

Molecular Technique: Currently, a highly sensitive real-time PCR for *Trypanozoon* subgenus was applied on fluid and tissues samples and fluid from a naturally dourine-infected horse, enabling the detection of low numbers of parasites [7, 8]. PCR and other related DNA amplification methods have been used to examine exudates or tissue samples, taking into account their failure on blood samples after the initial phase of the infection [37]. Direct diagnosis based on molecular techniques can be highly sensitive for parasite detection in body fluids such as blood [38].

Treatment: Pharmaceutical therapy is not recommended because animals may recover clinically but remain carriers of the parasite [10]. Following in vitro drug sensitivity tests a relative efficacy of Diminazeneaceturate on *T. equiperdum* isolates was yet, observed [39, 40]. In contrast, it was shown by Tuntasuvan [41] that Diminazeneaceturate was ineffective in curing and preventing relapses of *T. evansi* infections in horses and mules. Despite this knowledge, local veterinarians and veterinary assistants in the highlands of Ethiopia still use diminazene to treat suspected trypanosome infections. Horses are treated against dourine only irregularly when trypanocidal drugs are available, but even such treated animals show frequent relapse and generally, treatment is not able to cure clinical cases. Some of the trypanocidal drugs used in the area, whenever available, include Veriben(diminazeneaceturate) and quinapyriminesulphate (Triquin-S®; Wockhardt Veterinary Ltd., India) [42].

Cymelarsan® was found to be quite effective in curing horses at both 0.25 mg/kg and 0.5 mg/kg in acute as well as chronic form of dourine and relapses were not observed up to a year after treatment. The sensitivity of the particular trypanosome strain to Cymelarsan® was also supported by the relative improvement in the mean PCV levels of horses following treatment [42].

Prevention and Control: There is no vaccine available for dourine. As dourine is primarily a venereal disease, prevention of artificial insemination with infected horses (stallions or mares) or natural mating or infected stallion semen is the most important means of control. Testing blood for the presence of antibodies against *T. equiperdum*, which is more reliable than testing for the

presence of the protozoan parasite itself is very important for establishment of freedom from infection. Any introductions of horses from endemic areas or areas of incursion should be isolated and blood tested for antibodies by complement fixation test to prevent the occurrence of disease in area where it's free of disease [28].

When this disease is found in an area, quarantines and the cessation of breeding Stallions can prevent transmission while infected animals are identified. Dourine can be eradicated from a herd, using serology to identify infected equids. Infected animals are typically euthanized. While *T. equiperdum* does not survival for long in the environment, good hygiene and sanitation are nevertheless advisable at assisted mating to avoid any potential for fomite-mediated transmission [6].

Status of Dourine in Ethiopia: The first official report of dourine in Ethiopia was made in 1980 to investigate a persistent disease problem in horses in the administrative regions of Arsi and Bale [12]. Since then, dourine has been found to be prevalent throughout the highlands of Ethiopia, particularly in the Arsi and Bale zones [25]. Because of diagnostic problem, lack of effective trypanocidal drugs, unrestricted movement and uncontrolled breeding dourine is remains a potential threat to the life and productivity of the high equine population in Ethiopia [42]. The problem of dourine in Ethiopia has been recognized by local farmers for many years and it has been found to be a threat to the life and productivity of the equine population in the Arsi-Bale highlands [12].

According to this report, the disease was widely spread in Sagure, Ethaya, Bekoji and Koffle districts of Arsi- Bale highlands. The dourine endemic foci, the Arsi-Bale highlands of Ethiopia are situated outside the tsetse-infested belt (2400-3400 meters above sea level[13]. According to Melke [43] the disease was also found in Dodola, Assassa and Adaba district of Arsi-Bale highland.

Similarly the prevalence of dourine in the arsi-bale highlands has been reported by different researches using different diagnostic test. Accordingly, Clausen [44] reported 28.3% seroprevalence using CFT. Hagos [30] reported seroprevalence of 28%, 24.81% and 19.26% using CATT/*T. evansi*, Latex/*T. evansi* and ELISA/*T. evansi*, respectively. Fikru [45] reported the prevalence of dourine to be 4.6% (11 out of 237) parasitologically using Woo test and relatively higher prevalence using serology CATT/*T. evansi* test (27%) and molecular tests

(36.7% RoTaT 1.2 PCR and 47.6% 18S PCR. Interestingly, this is for the first time that trypanosomes causing dourine were parasitologically demonstrated by Woo test in Arsi-Bale highlands from horses suspected of dourine and showing clinical signs. The findings of the study disclosed that dourine is highly prevalent and one of the major diseases of horses in the area [45].

CONCLUSION

Conclusively, Dourine is a serious, often chronic, venereal disease of horses and other equids. This protozoal infection can result in neurological signs and emaciation and case fatality rate is high. No vaccine is available and the long-term efficacy of treatment is uncertain. Unlike other trypanosomal infections, dourine is transmitted almost exclusively during breeding. The problem of dourine in Ethiopia has been to be a threat to the life and productivity of the equine population in the Arsi-Bale highlands. To prevent dourine from being introduced into a herd or dourine-free region, new animals should be quarantined and tested by serology. Stallions should be castrated in an attempt to prevent disease transmission. Furthermore, serological test should be done in all ages of equine and infected animals are typically euthanized to eradicate the disease in area where it's endemic.

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