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Original Article

# Review on trypanosomiasis and their prevalence in some country on the Red Sea

## Avaliação da tripanossomíase e sua prevalência em alguns países do mar Vermelho

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**Abstract**

Trypanosomiasis is a protozoan infection affecting both human and animals in almost all parts of the world. It can affect a very large range of domestic and wild hosts including camelids, equines, cattle, buffaloes, sheep, goats, pigs, dogs and other carnivores, deer, gazelles and elephants. This review paper was designed to address the effect of this economically important disease in countries on the Red Sea, especially in Egypt, Sudan, Somalia, and Saudi Arabia during the period 2010 to 2020. The prevalence of trypanosomiasis is different between these countries due to different types of diagnostic methods (Giemsa-stained blood smears, Hematocrit centrifugation, Serological test, and molecular analysis PCR) used and differential distribution of vector (Tse tse) flies. In current review, retrospective studies of published literature on distribution and prevalence of *Trypanosoma evansi* infection in the Red Sea Countries was conducted [Google Scholar and PubMed were used to retrieve the published literature from 2000-2020. A total of 77 published articles met the eligibility criteria and were reviewed. A total of 16 reports have been reported on the prevalence and distribution of *Trypnosoma evansi* infection in the Red Sea Countries have been from 2010-2020]. According to the published literature, we can say that trypanosomiasis in camels are more prevalent in Sudan than in other countries, followed by 17% and 51.78% in both clinical and non-clinical cases. Hence, the reliable diagnostic tests should be used for rapid treatment or control of the disease as if not treated appropriately in early-stage, can lead to death of the camels.

**Keywords:** trypanosoma, tse tse flies, PCR.

**Resumo**

A tripanossomíase é uma infecção por protozoário que afeta humanos e animais em quase todas as partes do mundo. Pode afetar grande variedade de hospedeiros domésticos e selvagens, incluindo camelídeos, equinos, gado, búfalos, ovelhas, cabras, porcos, cães e outros carnívoros, veados, gazelas e elefantes. Este artigo de revisão foi elaborado para abordar o efeito dessa doença economicamente importante em países do mar Vermelho, especialmente Egito, Sudão, Somália e Arábia Saudita, durante o período de 2010 a 2020. A prevalência de tripanossomíase é diferente entre esses países devido a tipos distintos de métodos diagnósticos (esfregaços de sangue corados com Giemsa, centrifugação de hematócrito, teste sorológico e PCR de análise molecular) usados e distribuição diferencial de moscas vetoras (tsé-tsé). Na revisão atual, foram realizados estudos retrospectivos da literatura publicada sobre distribuição e prevalência da infecção por *Trypanosoma evansi* nos países do mar Vermelho [Google Scholar e PubMed foram usados para recuperar a literatura publicada de 2000 a 2020. Um total de 77 artigos publicados preencheu os critérios de elegibilidade e foi revisado. E há também 16 relatos sobre a prevalência e distribuição da infecção por *Trypnosoma evansi* nos países do mar Vermelho, de 2010 a 2020]. De acordo com a literatura publicada, podemos afirmar que a tripanossomíase em camelos é mais prevalente no Sudão do que em outros países, seguida por 17% e 51,78% em casos clínicos e não clínicos. Assim, os testes diagnósticos confiáveis devem ser utilizados para o tratamento rápido ou controle da doença, pois, se eles não forem tratados de forma adequada na fase inicial, isso pode levar à morte dos camelos.

**Palavras-chave:** trypanosoma, moscas tsé-tsé, PCR.

## 1. Introduction

Trypanosomiasis is a protozoan disease of man and animals, causes by trypanosomes, affecting cattle, buffaloes, camels, sheep, goats, horses, donkeys, mules, pigs, cats, and dogs throughout the world (Mirshekar et al., 2019; Tamarit et al., 2010; Sumbria et al., 2014). Trypanosomes are unicellular extracellular flagellate protozoa belonging to the family Trypanosomatidae and the genus Trypanosoma (Sobhy et al., 2017). Pathological diseases of livestock reduce

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agricultural output, yield and expected profits up to 30% in net income. This diseases can be lethal resulting total loss and great economic crisis in developing countries, where most family relies on extensive small hold family (twice the impact as in developed countries) (Majekodunmi et al., 2013). Trypanosomiasis is responsible for substantial losses in global production and can be fatal if not diagnosed and proper treatment commence early (Ereqat et al., 2020).

## 2. Trypanosomiasis

Taxonomy, according to Abd-Alla (2009).

Kingdom: Protista

Phylum: Protozoa

Sub-phylum: Sarcomastigophora

Class: Kinetoplastea

Order: Trypanosomatidae

Family: Trypanosoma

Genus: Trypanosoma

There are 11 different pathogenic trypanosomes causing serious endemic trypanosomiasis in most regions of Africa. Their specific molecular detection and characterization relies on species-specific primers on each sample. Primers ITS1 CF and ITS1 BR, previously designed to amplify the internal transcribed spacer (ITS1) of DNA (Njiru et al., 2005). The main pathogenic Trypanosomes species encountered are *Trypanosoma vivax, T. congolense, T. brucei, T. simiae,* and *T. evansi*, with the first three being the most widespread in cattle (Ahmad et al., 2016). African Animal Trypanosomiasis (AAT) is caused by several trypanosomes species, including *T. congolense*, *T. vivax*, *T. godfreyi*, *T. simiae*, *and T. brucei*. Two of the subspecies of *T. brucei* also cause Human African Trypanosomiasis, although biting flies (Isaac et al., 2016). can mechanically transmit some of them. Trypanosomiasis is considered as haemoparasites. Haemoparasites poses major threat for the development of the cattle industry in Africa, Asia, and Latin America (Salim et al., 2011a). The species has spread far beyond its primary territory (sub-Saharan Africa) and now affects livestock in Northern Africa, Asia, Central America, and South America. This disease is of great concern to many developing countries such as Sudan, where the large camel population (estimated at over 4.6 million heads) is at risk (Salim et al., 2011b). Trypanosomiasis is causing production losses, anemia, weight loss, abortion, and is fatal in a range of domestic and wild species (Desquesnes et al. 2013). Also, induces multiple pathological effects in many organs, including the spleen, liver, brain, and kidney (Ghaffar et al., 2016).

The Knowledge regarding camel's parasite fauna is insufficient. Only some information is available about zoonotic parasites transmitted to humans via contamination or by arthropod vectors (Sazmand et al., 2019). In arid and semi-arid areas in Africa (Somalia, Kenya, Ethiopia, Sudan, Chad, Nigeria and French West Africa), camels are affected significantly by the parasitic diseases. In Central and South America, horses are the main hosts, followed by cattle (Dar, 2016). *Trypanosoma evansi* is one of the most important Trypanosoma spp. Its wide geographic distribution causes the infection of livestock globally, the mode of transmission has zoonotic potential, pathogenicity to several domestic animals, high genetic diversity, and the virulence variation makes it an important parasite. Animal trypanosomiasis caused by *T. evansi* is called surra in camels and is the most lethal disease of 64 of camels worldwide (Getahun et al., 2020). Surra is a disease of camels (*Camelus dromedarius* and *C. bactrianus*) and equids in Africa caused by *T. evansi* and consider as a definitive host and reservoir in camels (Mirshekar et al., 2019).*Trypanosoma evansi* is a serious disease that affects camels and horses in tropical and subtropical countries and often reduces productivity and economic losses (Desquesnes et al. 2013). *Trypanosoma congolense* is a major constraint to animal health in sub-Saharan Africa; the treatment of the disease is impaired by the spread of drug resistance (Chitanga et al., 2011).

### *2.1. Worldwide distribution*

An epidemiological study of *Trypanosoma evansi* infection in dromedaries was conducted in four wilayat (localities) of Southern Algeria: Bechar, El Bayadh, Ouargla, and Tamanrasset. In Bechar, a non-significantly higher prevalence was observed than in El Bayadh (Boushaki et al., 2019). The result of the study by Gerem revealed that camel trypanosomiasis is substantially prevalent in Ethiopia, indicating the need for designing control and prevention strategies (Gerem et al., 2020). Epizootic pathogens are present in camels from northern Kenya. Furthermore, the presence of the same pathogens in camels and camel keds collected from sampled camels suggests the potential use of these flies in xenodiagnoses of haemopathogens circulating in camels (Kidambasi et al., 2020).

According to Hasan *T. evansi*, the infection has a relatively low prevalence in Pakistan's Punjab region (Hasan et al., 2006). A study in Mauritania shown animals in the 5- to 10-yr age group had the highest prevalence, and the study indicated that camel trypanosomiasis was widespread in Mauritania, especially in the wooded areas near waterways (Dia et al., 1997).

According to Tehseen, the prevalence of *T. evansi* in camels from the Cholistan Desert in Pakistan was dependent on gender was six out of the seven parasitological positives were female (Tehseen et al., 2015).

According to Van Vinh Chau, they report the first laboratory-confirmed case of *T. evansi* in a previously healthy individual in Vietnam, potentially contracted through a raw meat wound during slaughter (Van Vinh Chau et al., 2016).

Statistical analysis showed by El-Naga predict considerable variation in values within locations and age category are highly significant ($p<0.001$), and both sexes were at risk of parasitic infections, particularly females (El-Naga and Barghash., 2016) (Table 1).

### *2.2. Life cycle and vector*

The parasite is found in both intra and extravascular fluids of multiple hosts (Alanazi et al., 2018). It is transmitted

**Table 1.** Prevalence Trypanosome in Some Country on the Red Sea.

| Researcher | The year | Country | Sample | Total of sample | Type of species | method for analysis | percentage |
|---|---|---|---|---|---|---|---|
| Mohamed El Wathig | 2016 | Al-jouf | camel | 195 blood samples and 118 serum samples | *T. evansi* | *ELISA/T. evansi *CATT test *PCR analysis | 25% (49/195) and 3% (4/118) |
| Alanazi et al. | 2018 | Central Region (Riyadh) | camel | 237 | *T. evansi* | *PCR analysis. *Sequencing and phylogenetic trees analysis | 116 (49%) |
| | | Eastern Province | | 221 | | | 106 (48%) |
| | | Al-Qaseem Province | | 156 | | | 79 (50.1%) |
| | | Hail | | 116 | | | 33(28.4%) |
| | | Northern Borders | | 102 | | | 18 (17.6%) |
| Alanazi et al. | 2018 | Saudi Arabia | horses | 368 | *T. evansi* | *RoTat1.2-PCR | 12, 3.3% |
| Alanazi et al. | 2018 | Saudi Arabia | donkeys | 142 | *T. evansi* | *RoTat1.2-PCR | 4, 2.8% |
| Mossaad et al. (2017a) | 2017 | Sudan (Wd-Alhlio, Alshagrab and Khor Wd-Omer) | camels | 189 | *T. evansi* | *conventional parasitological techniques of Giemsa-stained blood smears, wet blood smears, the microhematocrit centrifugation technique (MHCT)and PCR | 7% (13/189), 11% (21/189), 19% (36/189) and 37% (70/189)respectivel |
| | | | | 189 | *T. vivax* | PCR | 25% (47/189). |
| | | | | 189 | *T. evansi* And *T. vivax* | They used a T. evansi-specific PCR (RoTat1.2 VSG gene) to analyze the KIN-PCR-positive samples and a *T. vivax*-specific PCR (Cathepsin L-like gene) to analyse all of the samples | * *T. evansi* was 59% (41/70) * *T. vivax* was 31% (59/189). |

**Table 1.** Continued...

| Researcher | The year | Country | Sample | Total of sample | Type of species | method for analysis | percentage |
|---|---|---|---|---|---|---|---|
| Mossaad et al. (2017b) | 2017 | Sudan(Khartoum State) | German shepherddogs | 50 | *T. evansi T.congolense T. vivaxin* | *serological (CATT/*Trypanosoma evansi*) and molecular (KIN-PCR, RoTat1.2 VSG-PCR and TviCatL-PCR)tests | *CATT/*T. evansi* detected antibodies against *T. evansi* in 15 (30%) dogs, while parasite DNA was detected in 17 (34%) dogs by RoTat1.2 PCR * KIN-PCR detected the subgenus Trypanozoon, *Trypanosoma congolense savannah*, *T. congolense Kenya* and *T. vivaxin* 36(72%),3(6%), 1(2%), and 2 (4%) dogs, respectively. However, a species-specific PCR for *Trypanosoma vivax* was detected 7 (14%) positive cases. |
| Bashir Salim et al. | 2011 | Sudan | camels | *Kassala 50 *Halfa Butana region" 205 *Umshadeeda 67 *South Darfur 365 | *T. evansi* | *diagnosis by a single PCR. Using ITS1 primer-based PCR | * 24.0% (12/50) *57.1% (117/205) * 6.0% (4/67) * 7.1% (26/365) |
| Bashir Salim et al. | 2011 | Sudan (Kurmuk District, Blue Nile State) | cattle + a few samples were also collected from other domestic animals species | 210 Cattle 8Donkeys 2Camels 3Sheep | *T. vivax, T. congolense, T. simiae* and *T. brucei* | *diagnosis by hematocrit centrifugation techniques (HCT) and Giemsa-stained thin blood films were carried out.Also, by ITS1-PCR, which provides a multi-species-specific diagnosis in a single PCR | In Cattle: *70(33.3%) *T. vivax* * 21 (10%) *T. congolense* In Donkey: *3(37.5%)*T.vivax* In Camels: *2(100%)*T.evansi* In sheep: *2(66,7%)*T.vivax* but ITS1-PCR was able to identify four Trypanosoma species namely *T. vivax, T. congolense, T. simiae and T. brucei* in 56.7% (80/141). *T. brucei* showed the highest prevalence of 36.9% (52/141) and the lowest 19% (27/141) was displayed by *T. congolense.* |
| Bashir Salima et al. | 2014 | Sudan | 393horses and 116donkeys | 509 | *T. brucei T. simiae T. vivax T. congolense* | *In horse: *T. brucei* 4.3% *T. simiae*4.1% *T. vivax* 3.6% *T. congolense* 1.5% *In donkeys *T. vivax* 3.4% | using the generic ITS1-PCR diagnostic methods. |
| Ahmed A. Hassan-Kadle et al. | 2019 | Somalia | camel (Camelus dromedarius) | 182 blood samples | *T. evansi* | * All samples were negative for *Trypanosoma spp.* by STDM * 125/182 camels were seropositive for *T. evansi* by CATT/*T. evansi*. | using standard trypanosome detection methods (STDM), serological (CATT/T. evansi) and molecular (ITS1-PCR) methods. |

**Table 1.** Continued...

| Researcher | The year | Country | Sample | Total of sample | Type of species | method for analysis | percentage |
|---|---|---|---|---|---|---|---|
| Adel | 2014 | Egypt | one-humped camel (*Camelus dromedarius*) | 106 | *T. evansi* | *Clinical examination 18 (17%) *Microscopical examination 7(6.6%) *Formol gel test 13 (12.26%) | *Clinical examination *Microscopical examination *Formol gel test |
| | | | | 15 | *T. evansi* | *TBR –PCR 13(86.6%) * RoTat –PCR0(0%) | *TBR –PCR * RoTat –PCR |
| Souzan et al. | 2016 | Egypt | camel | 187 | *T. evansi* | *Giemsa stained blood smears 6(3.21%) *Haematochrite centrifugation 8(4.28%) *CATT 21(11.23%) *PCR 138 | *Giemsa stained blood smears *Haematochrite centrifugation *CATT *PCR |
| Ahmed et al. | 2016 | from Sudan to Egypt | camel | 396 | *T. evansi* | *blood film technique was 12.17% *using TBR 1/2 primer-based PCR 43.3%. | *using thin blood film * PCR techniques |
| Abdel-Rady | 2014 | Egypt | camels (*Camelus dromedaries*) | 460 | *T. evansi* | 9.5% | blood smears |
| Abdullah D. Alanazi(2) | 2018 | Riyadh | Dogs | 117 | *T. evansi* | *smear 5.6% *WBF 3.4% *MHCT3.4% *PCR(ITS1)4.3% *Rotat VSG 1.7% | *using 3 parasitological tests (wet blood film, Giemsa staining, and microhematocrit centrifugation technique) * polymerase chain reaction (PCR) |
| El-Naga and Barghash | 2016 | Northern West Coastal zone of Egypt | camels (*Camelus dromedaries*) | 331 | *T. evansi* | *blood smear20.24%*PCR 67.06% | *Giemsa-stain blood smears (GSBS) *Polymerase chain reaction (PCR) |

mechanically by hematophagous flies (Tabanus, Chrysops, Atylotus, Lyperosia, Haematopota, and Stomoxys). Trypanosome life cycles can be considered relatively complex and divided into two stages in the tsetse fly and inside the mammalian (Dyer et al., 2013).

Firstly, the life cycle of the Trypanosome inside the mammalian host lifecycle begins when the tsetse fly injects the metacyclic forms into the mammalian host and then starts the adaption phase adapted for life the bloodstream inside a tsetse. The metacyclic form is morphologically characterized, including differentiating and proliferating into long, slender bloodstream forms known as trypomastigote forms (infective form) (Dyer et al., 2013) .

From the blood, it can enter into different body fluids, such as lymph and cerebrospinal fluid, and can enter the placenta. The parasite will migrate to the organs from the fluids, particularly the brain and central nervous system (CNS). On the other hand, events occurring inside the vector begin when the tsetse fly takes a blood meal, and the parasites are in bloodstream trypomastigote forms, migrate to the midgut. Next, once they arrive in the midgut, trypomastigote forms start to differentiate; via the esophagus, proboscis, and hypopharynx, they migrate to the salivary gland, where they are able to multiply, and some of them can transform into infectious met acyclic forms (Sharma et al., 2009).

During this migration from the midgut to the salivary glands, the parasite population size experiences a pronounced reduction (Oberle et al., 2010).

### *2.3. Symptoms*

*T. evansi* that causes equine trypanosomiasis, surra or heyam also known as derrengadera *T. evansi* caused a chronic disease with undulant parasitemia alternating with some cryptic periods of at least 54 days, with no clinical signs, *T. equiperdum*, never described as infectious to ruminants, and also caused a chronic disease with low undulant parasitemia. *T. vivax* caused an acute infection with severe anemia showing a drop of more than 70% of the hematocrit value, high fever, and rapid deterioration of physical condition (Parra-Gimenez and Reyna-Bello, 2019). *T. equiperdum*, a tissue parasite adapted to sexual transmission and the dourine's causative agent, a specific disease that affects only Equidae (Parra-Gimenez and Reyna-Bello, 2019).

*T. vivax* causes a critical and often fatal disease in ruminants such as cattle, buffalos, sheep and goats, due to the high fever and induced anemia (Gonzatti et al., 2014) . Clinical signs include fever, emaciation, anorexia, immunosuppression thrombocytopenia, microthrombi formation, and hemorrhage suggestive of disseminated intravascular coagulation, have also been demonstrated (Silva et al., 2016).

In camels and horses, trypanosomiasis is found in acute and chronic forms, and clinical signs include intermittent fever, lacrimation, conjunctival petechiae, anemia, edema, enlarged lymph nodes, abortion, decreased fertility, and loss of body weight, which can result in death (Alanazi et al., 2018). The clinical signs of *T. evansi* infection include anemia, recurrent fever, weight loss, emaciation, swelling of the hind limbs, and hemostatic abnormalities (Desquesnes et al., 2013). The syndromes associated with *T. evansi* infection are severe and fatal, especially in the disease's late stage. The disease varies from chronic to lethal acute, accompanied by progressive weakness, depletion, enlarged lymph nodes and death (Saleh et al., 2009; Herrera et al., 2002).

According to Herrera's microscopic examination, animals infected by *T. evansi* produce lymphoid hyperplasia of both lymph nodes and spleen, varying degrees of necrosis with associated inflammation in the liver (Abd El-Baky and Salem, 2011). Biochemical changes were studied in both naturally infected camels and experimentally infected rats with *T. evansi*; the results revealed significant increases in the activities of antioxidant enzymes and prolonged increases in the values of prothrombin time (P.T.) and activated partial thromboplastin time (APTT), which were seen in both camels and rats. In addition, anemia and significant leucocytosis were observed in both camels and rats. Serum biochemical parameters showed significant increases in the activities of hepatic enzymes in both of them. In contrast to camel results, there was an increase in blood urea nitrogen (BUN) and serum creatinine concentrations in experimentally infected rats. Different cytological changes associated with *T. evansi* infection were observed in both camels and rats (Hilali et al., 2006).

According to Hilali, the liver function tests revealed a significant elevation in the activity of lactate dehydrogenase enzyme (LDH), globulin, total bilirubin and indirect bilirubin, while alkaline phosphatase enzyme showed a significant decrease (Baldissera et al., 2017).

Liver samples from mice infected by *T. evansi* showed increased lim ($P < 0{\cdot}05$) reactive oxygen species (ROS), thiobarbituric reactive acid substances (TBARS), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels and superoxide dismutase) SOD (activity, and decreased non-protein thiols) NPSH (levels and catalase) CAT(activity ($P < 0{\cdot}05$) compared with uninfected animals death (Bilheiro et al., 2019).

The infection by *T. evansi*, typically exhibit weakness, fever, anemia, edema, uveitis, hepatomegaly, splenomegaly, and paraplegia, which can lead to death (Amin et al., 2020) .

Amin concluded that *T. evansi* infection in dromedary bulls causes severe damage to the testicular tissue and decreases the reproductive hormone levels associated with severe morphological disorders in sperms due to oxidative stress resulting from the infection. These findings indicate that *T. evansi* can cause reproductive failure and fertility damage (Dargantes et al., 2005).

Findings indicated immunosuppression in the lymph nodes, spleen, and bone marrow during the the third month after infection in *T. evansi* (Aquino et al., 2002).

Aquino's experiment showed infected animals in *T. evansi* showed a progressive decrease in red blood cell count and hemoglobin concentration, leading to anemia, which persisted, from the third week post-infection until the end of the study. Leucopenia and neutropenia were observed between weeks 2 and 5 of the infection. The infected dogs developed hyperproteinemia and a decrease in albumin (Biswas et al., 2010).

Biswas's experiment on rats affected in *T. evansi* showed damage in the brain tissue and choroid plexus shows and this similarity with the cases of African trypanosomiasis (Rodrigues et al., 2009).

Neurologic signs have been described occasionally in the terminal phase of natural infection by *T. evansi* in horses, cattle, deer, and buffaloes, but in horses have not been well-documented (El-Metanawey et al., 2009).

### *2.4. Diagnosis*

Early detection of *T. evansi* plays an important role in epidemiology and animal health (Salim et al., 2011a) . Clinical signs of trypanosomiasis may be absent in camels, and thus, laboratory diagnosis should be carried out for confirmation of infection and several methods with varying degrees of sensitivity and specificity may be used for the diagnosis of trypanosomiasis. Standard Trypanosoma detection methods, such as microscopical examination of fresh or stained blood-smears, used in the identification of Trypanosoma spp. they may lack sensitivity and specificity (Salim et al., 2011b).

Trypanosoma can be detected initially in the blood by using a light microscope at 40 X magnification, a microscopic examination by using Giemsa-stained smears of the blood (Mafie et al., 2018).

The card agglutination test is a serological assay, for *T. evansi* (CATT/ *T. evansi*), is a rapid diagnostic test and is currently recommended by the World Organization for Animal Health (Salim et al., 2011b). Sera were also tested, for the presence of anti- *T. evansi* antibodies using the card agglutination test for *T. evansi* (CATT/*T. evansi*). Approximately 45 µl of the antigen were transferred onto the test card and mixed with 25 µl of the test sera diluted at 1/4 with PBS pH 7.2 as per ' 'manufacturer's instructions. The card was agitated for 5 min, and the reaction was checked in the clear light. The positive reaction was confirmed on recording agglutinations (blue agglutinates) (Songa and Hamers, 1988).

Also, the formal gel test (FGT) was carried by adding two drops of concentrated formalin solution (37% formaldehyde) to 1 ml of serum; the test was considered positive if the serum coagulated immediately and turned white (Jacobson, 2004).

Diagnosis has also been done by the preparation of antigen for enzyme linked immunosorbent assay (ELISA). ELISA Procedure for each trypanosome isolate: an ELISA plate was used all under the same conditions, with each sample in duplicate. Negative serum samples were obtained from each sample two weeks prior to infection and used as negative controls. During experimental infection samples were taken at days -1, 2, 10, 18, 24, 30, 39, 47 and 61 sensitized plates with 20 g/well of antigen were washed five times with washing buffer (W.B.) and blocked with 200 µl/well of 5% skim milk in PBS for an hour at 37C. Plates were washed five times with W.B., and 100 µl/well of each serum diluted 1:200 in W.B. was added. Positive and negative reference sera were included in each ELISA plate. After incubation for an hour at 37C, plates were washed five times with W.B., and 100 1of the conjugate (Rabbit peroxidase-conjugated anti-(any sample) IgG Pierce. ImmunoPure antibody diluted 1:5000 with W.B. was added, and plates were incubated for 60 minutes at 37C. After incubation, the plates were washed three times, and 100 µl of the substrate solution, ABTS 2% H2O was added and incubated at 37 °C for 45 min. Absorbance was measured photometrically at 405 nm on an ELISA reader (Parra-Gimenez and Reyna-Bello, 2019).

To detect Trypanosoma infection with a low level of parasitemia in the chronic stage, DNA-based techniques have been developed (Alanazi et al., 2018). These techniques, including polymerase chain reaction (PCR), have been widely used in diagnosing trypanosomiasis infection in camels, horses, cattle, and pets, given its sensitivity and specificity in detecting all stages of parasitic infection (Alanazi et al., 2018).

Molecular tools like conventional PCR especially, are useful when a large number of animals need to be sampled during field studies (Behour et al., 2015). DNA-based methods have the advantage of being more sensitive and able to identify trypanosomes to the subspecies level and to detect mixed infections (Isaac et al., 2016). Molecular diagnosis includes the use of species-specific primers in single and nested PCR to amplify the internal transcribed spacer (ITS) regions of ribosomal DNA (Konnai et al. 2009).

### *2.5. Prevention*

The number of disease control methods within the vertebrate host is limited; moreover, the evolution of resistance to trypanocidal drugs makes chemotherapy difficult to sustain for AAT control. For these reasons, vector control remains a very important part of the integrated management of AAT (Behour et al., 2015). Both mechanical transmissions by vectors and animal movement contribute to trypanosomosis distribution (Holmes, 2013).

These recent and future control initiatives require reliable information on the geographic distribution of (AAT) and its vectors in Sudan. Even though various epidemiological studies were conducted over the years, geo-referenced and harmonized data on tsetse and (AAT) occurrence at the national level are lacking. This type of information is crucial for evidence-based planning, execution, and monitoring of field interventions. It is to fill this gap that the Veterinary Research Institute of Sudan (VRI) performed an exercise on a national level mapping, aimed at building a geo-referenced database of tse tse flies and bovine trypanosomosis for Sudan. The initiative is technically supported by the Food and Agriculture Organization of the United Nations (FAO), in particular in the framework of the Atlas of tsetse and AAT (Holmes, 2013).

### *2.6. Vaccination and treatment*

Trypanosoma infection in humans or animals can be fatal if left untreated. Chemotherapy is a major means of controlling the infection; however, the available treatment options have various shortcomings, including limited efficacy, toxicity, and the emergence of resistant strains of trypanosomes (Ahmed et al., 2016).

*Trypanosoma evansi* has gained resistance to most drugs used; therefore, it requires alternative medicines)

(Adeyemi et al., 2018). Trypanosomiasis medicines produce serious side effects, and the parasite becomes resistant to the drugs used) (Dkhil et al., 2020). Diminazene aceturate is commonly used to treat domestic animals infected with *T. evansi*, but it causes some toxicity to the host (Kirchhoff and Rassi Junior, 2011).

Nigella sativa oil (NSO) in the experiment for Nassef et al. (2018), NSO showed a trypanocidal effect, however, it was not as effective as cisplatin or diminazene (Carmo et al., 2015).

It is, therefore, necessary to search for new safe drugs against trypanosomiasis (Adeyemi et al., 2018). The infected mice were treated with *Indigofera oblongifolia* and has decreased the number of the parasites in the blood significantly compared with their number in the control blood samples. The protective effect can be due to active compounds such as phenol, quinines, saponins and coumarin (Nassef et al., 2018). In a study in most of the blood samples tested for Trypanosoma evansi, neither of the treatment was successful in most clinical cases using the known effective anti-trypanosomes such as Naganol, Cymelarsan and Antrycide (Shahjahan et al., 2005).

The prevalence of disease has increased to epidemic proportions, lack of a mammalian vaccine and affordable and effective drugs have hindered disease control (Aksoy, 2003).

A necessity for *T. evansi* infection control is the availability of reliable and sensitive diagnostic tools. At the same time, DNA-based PCR detection techniques meet these criteria (Aksoy, 2003).

To date, several drugs have been used for the treatment of *T. evansi* infection, yet their efficacy and toxicity, particularly towards the kidneys and liver, have proven to be problematic. Moreover, in some instances, drug resistance has also been reported. Thus, it is important to investigate alternative therapies for the treatment of *T. evansi* (Li et al., 2020).

Six of the compounds were capable of curing *T. evansi*-infected mice at drug doses as low as 0.5 and 0.25 mg/kg of body weight administered for four consecutive days, and they were more effective than the standard drugs suramin, diminazene, and quinapyramine. After all selection criteria were applied, three diamidine compounds (D.B. 75, DB 867, and DB 1192) qualified as lead compounds and were considered to have the potential to act as preclinical candidates against *T. evansi* infection (Gillingwater et al., 2009).

In vivo tests showed an increase of longevity in groups treated with diminazene aceturate associated with sodium selenite. When combined with chemotherapy, the sodium selenite may represent an alternative in treating trypanosomosis (Tonin et al., 2011). Diminazene aceturate is commonly used to treat domestic animals infected with *T. evansi* but it causes some toxicity to the host (Kirchhoff and Rassi Junior, 2011).

Another study evaluated the therapeutic efficacy and safety of using 30 deoxyadenosine (Cordycepin e adenosine analogue) combined with deoxycoformycin (Pentostatin an adenosine deaminase inhibitor) in mice infected with *Trypanosoma evansi*. We show that the combination of Cordycepin (2.0 mg kg1) and Pentostatin (0.2, 0.5, 1.0, 2.0 mg kg1) is effective in the clearance of T. evansi, although at the higher concentrations of Pentostatin 2 mg kg1 some toxicity was observed in the liver and kidney. Since the Cordycepin 2.0 mg kg1 and Pentostatin 0.2 mg kg1 combination were effective and had low toxicity, we recommend this as a therapeutic option for a *T. evansi* mouse model (Li et al., 2020).

According to Behour, the PCR could detect *T. evansi* in different organs in the chronic stage (i.e., the disappearance of parasite from blood) (Bilheiro et al., 2019).

Major obstacles to the control of equine trypanosomosis are the lack of vaccines, the inability of drugs to cure the disease's neurological stage, the inconsistent case definition, and the limitations of current diagnostic (Büscher et al., 2019).

Excessive use of trypanocidal drugs can lead to cases of drug resistance. Multiple resistance cases have been widely reported for drugs such as isometamidium chloride and diminazene aceturate (Nuryady et al., 2019).

## 3. Discussion

A total of 16 articles published on the prevalence and distribution of Trypnosomiasis in animals in different countries of the Red Sea. In this review 16 articles was reviewed and summarized current status of trypnosomiasis and rates of its spread among different countries of the Red Sea including Egypt, Sudan, Somalia, and Saudi Arabia. The current review suggested that difference in the prevalence and distribution of *Trypnosoma evansi* in different countries is due to the variation in geographical location; different animals management system, seasons and animals' breed, sex and age. The countries where seems more concerned about control measures, has displayed the lower infection rates. Trypnosomiasis were highest in camels in Sudan (%), followed by 17% and 51.78%, in both clinical and non-clinical cases. Sudan is the second camel's rearing country in the world with camel population estimated at over 4.6 million heads (Elamin et al., 1998). The prevalence values within each species depend on the diagnostic method used and the geographical region covered by the reports, with a high heterogeneity observed among countries. Species-wise, higher estimated prevalence values were observed in camel followed by Dogs, donkeys and horses. Camels are one of the main sources of income and food for millions of pastoralists in Red Sea countries. The regions with the lower prevalence of the *Trypnosoma evansi* infections in the camels markets could possibly explained as due to better awareness towards better feeding, management, use of medicines and dependence of camel keepers to sell them with good prices. The *Trypnosoma evansi* infections were more common in camels in comparison with dogs, donkeys and horses, might be due to the reason that Trypanosomosis in camels is of a chronic nature, with animals becoming progressively weaker and emaciated, while in equines and dogs it is of the acute fatal type (Gill, 1991). Even though donkeys, dogs and horses are exposed to similar vector challenges as camels, they are significantly less infected with trypanosomes, possibly because of the greater feeding preference of vectors for camels or the

better ability of donkeys to deter the flies from feeding by skin rippling, head movements and other behavioral avoidance mechanisms (Faye et al., 2001).

Similarly, the prevalence of trypanosomiasis is different between these countries due to different types of diagnostic methods including Giemsa-stained blood smears, Hematocrit centrifugation, Serological test, and molecular analysis PCR used and differential distribution of vector (Tse tse) flies. The diagnostic method used has a major impact on reported distribution of parasitic infection, with studies using parasitological methods reporting a very low prevalence in all the species compared to the other detection methods. This is due to the fact that a large proportions of infections in the field are chronic, and do not develop detectable levels of parasitemia (Killick-Kendrick, 1968).Antigen detection tests are expected to be poorly sensitive for the same reasons as parasitological tests but also due to the presence of antigen-antibody complexes. Molecular tests are considered superior to parasite and antigen detections due to their ability in detecting pre-patent and chronic infections. However, sensitivity and specificity of molecular tests vary as a function of the target sequence, primers and probes (Tehseen et al., 2015).

In general, this review showed that *Trypnosoma evansi* infections are endemic in Red Sea countries however scarce information is available from wildlife and humans. *Trypnosoma evansi* infect many mammalian hosts through the flies' bites, exhibiting a broad spectrum of pathogenicity in different species with different Epidemiology, economic impact and various reservoirs.

As with any review, limitations associated with potential publication bias should be considered in this meta-analysis. Statistical evaluation of publication bias was not undertaken for various reasons where variability was obviously expected within and among diagnostic test categories, geography, breed of animals sampled, period of study etc.

## 4. Conclusions

This systematic review and meta-analysis study provides comprehensive information on the geographical distribution, host range and prevalence of *Trypnosoma evansi* infections in Red Sea countries. According to this review it is concluded that trypanosomiasis in camels was prevalent in Sudan more than in other countries, either by clinical and non-clinical cases. Therefore, the reliable diagnosis should be used for rapid treatment or control of the disease, because the animals can lead to death if not treated at early stage.

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