Characterization and Diagnosis of Trypanosomiasis in Equine Species An Updated Review

Asmat Ullah[1,7], Anas Sarwar Qureshi[1,4], Umar Farooq[5], Muhammad Usman Shafi[2], Zaima Umar[6], Mumtaz Hussain[3], Hafiz Muhammad Saif ur Rehman[2], Muhammad Umar Sharif[1], Muhammad Usman[8] and Sarmad Rehan*[1]

[1]Department of Anatomy, University of Agriculture, Faisalabad, Pakistan
[2]Department of Biosciences Faculty of Veterinary Sciences, Bahaudin Zakaryia University, Multan
[3]Department of Anatomy & Histology, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur
[4]Department of Basic sciences, Riphah college of Veterinary Sciences, Riphah International University Lahore, Pakistan
[5]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan 430070, China
[6]Department of Anatomy, The University of Faisalabad, Pakistan
[7]West Carlton Animal Hospital, Ottowa, Canada
[8]Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: drsarmadpk@uaf.edu.pk

Abstract

Equine trypanosomiasis infects horses, donkeys, and mules. It causes a wide array of clinical symptoms ranging from weight loss and fever to neurological impairments, which vary depending on the Trypanosoma species involved such as *Trypanosoma evansi (T. evansi)*, *Trypanosoma equiperdum (T. equiperdum)*. Trypanosomiasis affecting horses, donkeys, and other equine animals is also known as "surra" and "dourine" respectively. Each disease is transmitted either sexually or through vectors. The disease leads to significant economic losses due to reduced productivity and increased mortality. Despite extensive studies, effective control of disease remains a challenging task due to diagnostic limitations and unpredictable pathogenicity of the trypanosome species. Many diagnostic techniques are employed for diagnosis ranging from direct observation of parasite under the microscope to advanced sero-diagnostic procedures, each having its pros and cons. Advanced molecular techniques, like PCR-based diagnostics, have improved sensitivity and specificity in detecting trypanosome infections. Development of the affordable, rapid diagnostic tools and novel treatment options should be the future research focus to mitigate its deleterious impacts.

Keywords: Trypanosomiasis, *T. evansi*, *T. equiperdum*, life cycle, molecular diagnostics, pathogenesis

Cite this Article as: Ullah A, Qureshi AS, Farooq U, Shafi MU, Umar Z, Hussain M, Rehman HMSU, Sharif MU, Usman M, and Rehan S, 2025. Characterization and diagnosis of trypanosomiasis in equine species an updated review. In: Abbas RZ, Akhtar T and Jamil M (eds), Pathways of Infection: Zoonoses and Environmental Disease Transmission. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 166-174. https://doi.org/10.47278/book.HH/2025.34

A Publication of Unique Scientific Publishers

Chapter No: 25-024

Received: 07-Jan-2025
Revised: 21-March-2025
Accepted: 18-Apr-2025

Introduction

Trypanosomiasis is a significant livestock disease, particularly in areas where this parasite is endemic. Affected with trypanosomes, equines—including horses, mules, and donkeys—show a range of clinical symptoms from weight loss and fever to neurological problems. The zoonotic potential of the disease aggravates its complexity as well as the challenges in suitable diagnosis and treatment (Büscher et al., 2019). Especially in Africa, Asia, and some parts of South America, trypanosomiasis is a common problem in equines. Equine trypanosomosis prevalence in Uttar Pradesh, India, was found to be 15.08%; donkeys and mules shown more sensitivity than horses. The observed hemato-biochemical changes in the infected animals draw attention to how the disease affects equine health and productivity (Pal et al., 2021). In Algeria, a seroprevalence of 45.2% for *T. evansi* was observed in horses, indicating considerable environmental and behavioral risk factors (Benfodil et al., 2019).

In Ethopia, about 33 percent animal deaths are caused by trypanosomiasis. The families with livestock afflicted by this illness bear 68 percent increase in animal production costs and a 45 percent decrease in milk yield. There would be a 24.5 times return on investment for every dollar spent on curative strategies for equine trypanosomiasis (Zewdu et al., 2021).

Etiology

The disease trypansomiasis in horses is caused by hemoflagellate parasites of the genus Trypanosoma. These are found in the animals' tissue fluid and blood. Five species from three subgenera are responsible for pathogenesis in horses. The length of the illness and the intensity of the clinical infection are correlated with the virulence of the parasite species and isolates. *T. evansi* produces Surra in equines that are more economically significant in Africa and Asia, high annual animal fatality rate (Eraqat et al., 2020; Aregawi et al., 2019). In

horses, *T. equiperdum* is the cause of dourine, a sexually transmitted disease. While *T. congolense* infects both cattle and donkeys, *T. vivax* and T. brucei infect cattle and, to a lesser extent, horses, causing Nagana (Abbasi et al., 2014).

In Pakistan, the donkey population is projected to be 5.2 million (Anonymous, 2016), with a 2.95 percent annual growth rate. Numerous bacterial, viral, and parasitic infections impact donkey health and can occasionally result in fatalities. As per multiplex PCR results, the most common causative agent of trypanosomiasis in donkeys was found to be *T. evansi* (Khan et al., 2018).

Transmission

In China and Indonesia, *T. evansi* is mechanically spread by the mouthparts of stable flies, horse flies, and blood-sucking flies. The illness is mechanically spread throughout Africa by tsetse flies (Glossina spp.). Because Trypanosoma only survives for a brief period in the flies' mouthpart, the likelihood of infection increases with shorter feeding intervals. Numerous domestic animals are infected, and they could be a potentially dangerous source of the infection's transmission reservoir ((Azzouzi et al., 2025). There have also been reports of *T. evansi* being spread by milk or coitus (Gizaw et al., 2017).

Through the coitus, *T. equiperdum* causes the condition known as dourine in horses. The reproductive tract's mucous membrane and seminal plasma are home to this bacterium. The parasite trypanosomes invade animals through their mucous membranes, which causes an illness. In chronic infections, *T. equiperdum* is found in the peripheral blood despite not being seen in the blood. Although it is extremely uncommon, mechanical transmission is one potential method (Gizaw et al., 2017).

The primary vector of *T. vivax*, *T. congolense*, and *T. brucei* infection is Glossina species of tsetse flies. After being ingested from the host, trypanosomes develop in the vector for 8–28 days. When flies feed, they deposit the metacyclic form they have stored in their salivary gland or proboscis into the skin. Both domestic and wild animals serve as reservoir hosts (Abbeele & Caljon, 2016).

Incubation Period

The length of the incubation period is determined by the parasite type, strains, and host susceptibility. The incubation period for *T. evansi* is one to two weeks. Parasites may be found in the blood in the early stages but are not detectable in the later stages. *T. equiperdum* has an incubation period of two to twelve weeks. Parasites may be detected in vaginal or urethral secretions during the early stages of infection. Later on, it is discovered in the subcutaneous tissue and blood. *T. brucei, T. vivax*, and *T. congolense* all require two to three weeks to incubate (Sutcliffe et al., 2014).

Life Cycle

In addition to the tsetse fly, other biting flies such as Tabanus, Haematopota, Stomoxys, Lyperosia, and Chrysops can also mechanically transmit *T. evansi*. All pathogenic trypanosomes, with the exception of T. equiperdum and *T. evansi*, are cyclically transmitted through the bite of a biological vector, such as a tsetse fly (Glossina spp.), which allows the parasite to complete its life cycle (Figure 1). Additionally, *T. congolense* and *T. vivax* can be mechanically transmitted by blood-feeding dipters (Desquesnes and Dia, 2003; Desquesnes and Dia, 2004; Desquesnes et al., 2009).

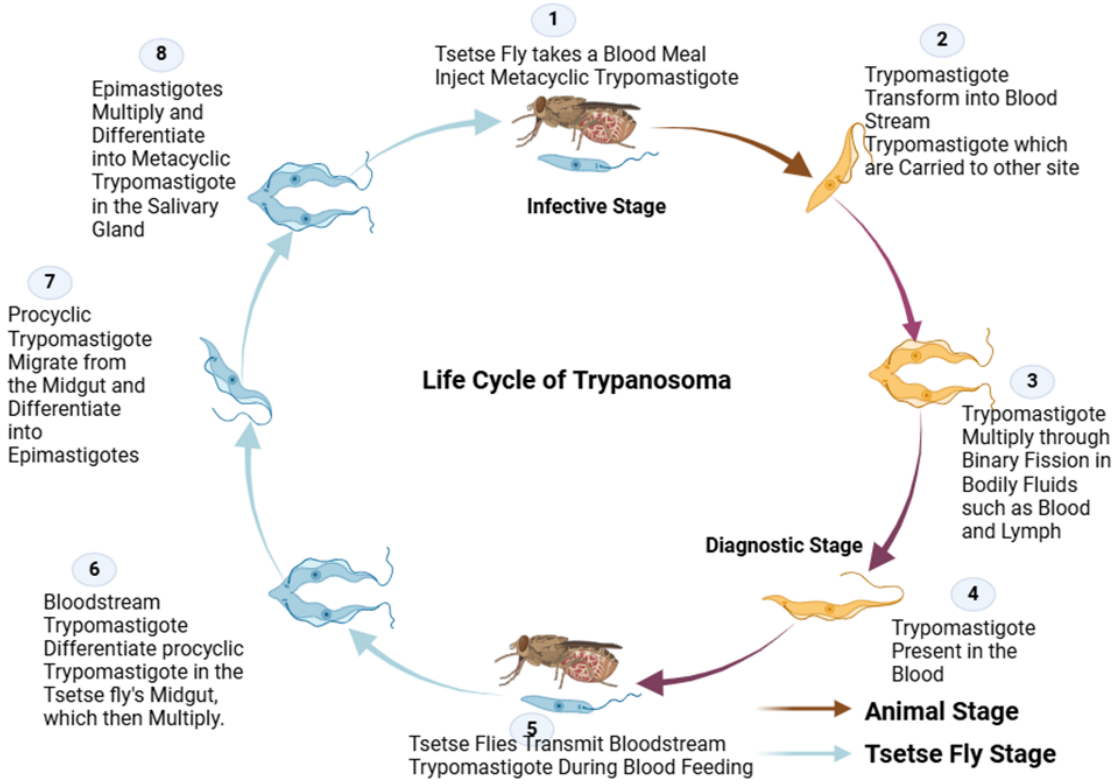

Fig. 1: Life cycle of trypanosomiasis in the vertebrate and invertebrate hosts

Trypanosome-mammalian Host Relationship

During the cyclic transmission, specific morpho-physiological and biochemical changes take place up until the last metacyclic stage, which is an infectious state for the host. Tsetse flies inject the metacyclic form into the animal body while feeding on the host's blood, which subsequently transforms into trypomastigote. Trypomastigotes are released from the lymphatic system into the bloodstream, and the tissue swells at the injection site. Because the surface antigenic coat of parasites varies, the host's immune system is unable to identify them. The host's physiology is altered by the trypanosome infection due to changes in blood pH, hormones, and nutrition concentration (Bakari et al., 2017). Due to the elimination of trypanosome catabolites into the urine, which results in chemo-attraction, a greater number of tsetse flies are drawn to the animals (Getahun et al., 2022; McGettrick et al., 2016).

Trypanosome-tsetse Relationship

Tsetse flies consume the trypanosome parasite by feeding on the blood during the infection in the host (Figure 2). Both *T. brucei* and *T. congolense* change their morphology and metabolism to live in the midgut of tsetse flies. The primary energy source throughout the midgut stage, when transformational changes take place, is proline (Mantilla et al., 2017).

Trypanosomes undergo two changes in order to successfully transmit: establishment and maturation. In the tsetse fly's mouthpart or salivary gland, trypanosomes differentiate into epimastigotes after establishing themselves in the midgut. *T. brucei* matures into the salivary gland, whereas *T. congolense* matures into the mouthparts (Matetovici & Den Abbeele, 2018).

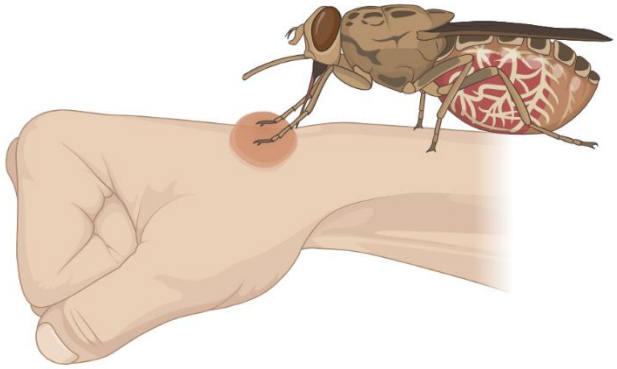

Fig. 2: Tsetse fly feeding on blood

Pathogenesis and Clinical Signs

The disease progression of equine trypanosomiasis often includes an initial acute phase followed by chronic manifestations, including neurological symptoms and reproductive issues. The pathogenic mechanisms involve immune evasion strategies, where parasites change surface glycoproteins to avoid host immune responses (Giordani et al., 2016). Symptoms vary widely, from fever, lethargy, and anemia in early stages to incoordination, paralysis, and other neurological deficits in advanced cases. This variability complicates clinical diagnosis, making laboratory confirmation essential. Animals with acute or chronic *T. brucei* infections experienced intermittent fever, pale mucous membranes, edematous enlargement on the ventral side of the body, and weight loss. The animal had trouble moving in the later phases (Ahmed et al., 2018; Desquesnes *et al.,* 2013).

Vaginal and urethral discharges occur when the reproductive system is dourine-locally infected. The scrotal region, which reaches to the ventral side of the abdomen, has localized edema. The mare develops vulvar mucosal edema and vaginal edema. Anorexia, weakness, anemia, and edema of the limbs and sexual organs are symptoms of *T. congolense* infection in animals (Giordani et al., 2016).

Trypanosomiasis causes anemia and disrupts hematological and biochemical parameters in humans and animals due to factors like trypanosome toxins, intermittent fever, and malnutrition (Stijlemans et al., 2018).

Findings in Rats, Cattle, Camels and Donkeys

Trypanosomiasis in rats caused reduced RBC counts, increased WBCs, and symptoms like splenomegaly and hepatomegaly (Ukpai and Nwabuko, 2014). Donkeys infected with *T. brucei* experienced anemia and elevated neutrophil counts, with significant reductions in hemoglobin and PCV (Hussain et al., 2016). Cattle infected with *T. vivax* exhibited reduced hemoglobin and erythrocyte counts, severe neutropenia, and lymphopenia during acute illness. Altered biochemical markers included decreased lactate dehydrogenase and aspartate aminotransferase levels (Junior et al., 2016).

Infected racing camels demonstrated markedly reduced hemoglobin (Hb), red blood cell (RBC) count, packed cell volume (PCV), as well as serum albumin and iron concentrations. Neutrophil counts were elevated, while other biochemical markers remained steady (Machado et al., 2021). In calves experimentally infected with *T. vivax*, levels of glucose and bilirubin increased, although blood urea nitrogen (BUN) and aspartate aminotransferase (AST) levels considerably decreased (Kadima et al., 2000). Anemia, hyperproteinemia, altered albumin:globulin ratios, and higher liver enzymes were shown by infected dogs (Aquino et al., 2002).

Though albumin levels significantly dropped, naturally infected ruminants had lower RBC counts, higher mean cell volume, and higher bilirubin and protein levels. These results draw attention on how trypanosomiasis affects hematological condition (Ohaeri and Eluwa, 2011). In camels, infection resulted in lower lymphocyte levels and hyperglycemia as well as higher leukocyte count (Padmaja, 2012). Infected pregnant sheep exhibited lowered albumin and total protein levels, which caused subcutaneous edema in aborted fetuses and fetal death.

Alterations in Horses and Donkeys

The naturally *T. evansi* infected horses showed muscle atrophy, pale mucous membranes, and neurological symptoms including ataxia and incoordination. Hematological analysis demonstrated lower erythrocyte counts and packed cell volume as well as higher white blood cell counts (Rodrigue et al., 2009; Shoraba et al., 2023). Reduced erythrocyte counts, hemoglobin concentrations, and serum glucose levels were shown by experimentally infected donkeys. Meningo-encephalitis and splenic enlargement were shown by histopathological studies (Cadioli et al., 2006).

Diagnosis of Trypanosomiasis

Equine trypanosomiasis presents difficult diagnosis because of the ambiguous clinical symptoms and low parasite load in chronic infections. Especially in cases of low parasitemia, conventional diagnostic methods including blood smear microscopy have inadequate sensitivity. Therefore, several molecular diagnostic methods, including polymerase chain reaction (PCR) assays, have been developed to detect trypanosome DNA, offering increased sensitivity and specificity (Alanazi et al., 2018).

Monitoring parasites in lymphatic fluid, blood, and cerebrospinal fluid (CSF) is the most direct and most often used technique. The diseases were identified using these differently beneficial techniques. The fundamental technique was the wet blood film, which entailed examining a small slide containing a drop of blood at its center under a microscope. A thick blood film, necessitating a larger volume of blood for examination, is a more sensitive approach. These techniques utilize Giemsa stain (Jannin and Samaro, 2007).

Wet blood film and thick blood film are more complicated and less sensitive than the widely used enhanced hematocrit centrifugation procedure. Hematocrit tubes in this procedure require a centrifuge machine. Blood and anticoagulant are blended together and then put into the micro-hematocrit tubes, which have one end sealed. The centrifuge machine is used to spin the tubes, which are then viewed under a microscope. Following the spinning, a buffy coat- a region between plasma and blood that contains several trypanosomes appears (Maki et al, 2017).

Mini anion exchange centrifugation

(m-AECT) is another method, which keeps the red blood cell in the filtration matrix because of more negative charge on their surface which later on filters out. Trypanosomes parasites are filtered through the filtration matrix due to less negative charge. Centrifuging the filtrate make it more concentrated and easier for detect under a microscope.

Trypanosomiasis is also diagnosed using fluid aspirates from the lymph nodes, bone marrow, and cerebrospinal fluid. The lumber puncture is used to diagnose chronic disease, bone marrow aspiration can also be used to detect trypanosomes early. This approach requires expertise and experience. Indistinguishable trypanosome features under a microscope are one of the primary issues with microscopic techniques (Picozzi et al., 2002).

Sero-diagnostic Methods

A variety of sero-diagnostic methods are in practice for precise diagnosis of trypanosomiasis in equines.

Card Agglutination Test CATT

It identifies the antibodies from the serum of an animal for infection's diagnosis. A filter paper impregnated with blood shows 91% sensitivity for mass screening program (Chappuis et al., 2002). It is simpler and less expensive, but it doesn't specify the infection stage. Despite its widespread use, many drawbacks have been noted. For instance, it indicates that a significant portion of the population is positive for infections without exhibiting any clinical symptoms. Due to its low sensitivity, some positive results are not discovered, although treated patients continue to test positive for three years (Radwanska et al., 2002).

Enzyme-Linked Immunosorbent Assay (ELISA)

This method is thought to be a diagnostic test for African animal trypanosomiasis and was used to identify the trypanosome infection. To make it more generally accepted and utilized, changes and improvements have been made. The 96-well polystyrene plates absorb the trypanosome antigens. The antigen and antibody complex is created when the serum antibody reacts with antigens from infected cases. When substrate and chromogen are added, the complex's color becomes apparent (Camoin et al., 2019).

Polymerase Chain Reaction (PCR) in Trypanosomiasis Diagnosis

The ability of PCR to detect *Trypanosoma* infections early and more accurately than other methods has been widely demonstrated. In dairy cattle, PCR detected 27 positive cases compared to 13 identified by microscopy. Similarly, card agglutination tests produced results comparable to PCR, confirming its reliability (Aslam et al., 2010). Blood-spotted filter paper combined with Chelex-100 DNA extraction enabled PCR to outperform thin smears in detecting *T. congolense* and *T. brucei* in African trypanosomiasis (Simon et al., 2020).

Hematocrit centrifugation and mini-anion exchange techniques detected 18.9% prevalence of *Trypanosoma* infections in peri-urban cattle. However, PCR revealed a much higher prevalence of 63%, underscoring its greater sensitivity in large-scale epidemiological studies (Baticados et al., 2012). Additionally, experiments on calves infected with *T. vivax* highlighted that PCR and parasitological methods achieved similar sensitivity, with PCR showing higher consistency in mixed infections (Bontempi et al., 2024).

Application in Chronic and Mixed Infections

PCR has shown its effectiveness in cases of ongoing infections when conventional methods prove useless. Later phases of infection failed to identify the parasite, hence mice experimentally infected with Trypanosoma showed earlier identification with PCR than microscopy (Fernández et al., 2008). While microscopy was insufficient, PCR demonstrated to precisely identify mixed infections of *T. congolense, T. vivax*, and *T. brucei* (Kizza et al., 2024).

Field studies on dogs, donkeys, and camels confirm the effectiveness of PCR in infection identification. While microscopic studies turned

up no parasites, PCR successfully identified Trypanosoma in many cases. Moreover, PCR helped to identify mixed infections in animals for whom conventional parasitological techniques were unable to separate (Ravindran, 2008).

Greater incidence of *T. vivax* infections was found in Ethiopia when compared to more limited-scope and sensitive conventional methods (Mehret and Mamo, 2007). Three weeks after infection, PCR found infections in buffaloes; standard parasitological methods called for up to seven weeks (Holland et al., 2001).

Though microscopy found just 6.9%, PCR found *T. evansi* in 46.5% of samples from dairy farms. It also revealed important biochemical changes in infected individuals, including lower hemoglobin levels and higher liver enzymes, hence enhancing diagnostic value (Bal et al., 2014). Constrained to a subset of positive cases, research on equines found that PCR showed better detection rates than microscopy (Mueed et al., 2008).

Innovations and Diagnostic Accuracy

PCR's diagnostic accuracy has been much raised by innovations in diagnostic accuracy and improvements in PCR. Novel primers with TBR1/2 and ESG6/7 have improved detection thresholds, therefore enabling the identification of DNA quantities as lowest as 0.001 ng. Particularly effective in recognizing trypanosomes in experimental settings, these primers have outperformed more traditional primers like ITS1 and 21/22-mer (Fernandez et al., 2009).

Real-world field applications now allow sample collecting for PCR analysis using Flinders Technology Associates cards (FTA cards). Superior sensitivity in a study using FTA-collected buffy coat samples was shown by PCR against microscopy and standard parasitological methods (Picozzi et al., 2002). In identifying *T. evansi*, PCR outperformed Giemsa staining and hematocrit techniques in staining prevalence of 56.9% compared to 4.1% (Abdel-Radey, 2008).

Comparative Studies and Limitations

Real-world field applications now allow sample collecting for PCR analysis using Flinders Technology Associates cards (FTA cards). Superior sensitivity in a study using FTA-collected buffy coat samples was shown by PCR against microscopy and standard parasitological methods (Picozzi et al., 2002). In identifying *T. evansi*, PCR outperformed Giemsa staining and hematocrit techniques, exposing a prevalence of 56.9% against 4.1% by staining (Abdel-Radey, 2008).

Insights from Case Studies

Many species' dependability of PCR is shown by several epidemiological investigations. The main trypanosome species found in Cameroon was *T. vivax*; *T. congolense* and T. simiae followed. The results presented a thorough understanding of species-specific infection rates (Nimpaye et al., 2011). Likewise, compared to merely 6.3% found by microscopy, PCR revealed a 43.6% frequency of infections in clinically healthy animals in Egypt (Radwan and Madaway, 2010). PCR identified *T. evansi* in a trypanosomiasis outbreak in Punjab and suggested notable gross pathological changes including splenomegaly, therefore confirming its diagnostic and pathological efficacy (Kumar et al., 2012).

Table 1: A brief comparison of various detection techniques for diagnosis of trypanosomiasis with their benefits and limitations

Technique	Tissue type	Animal species	Pros	Cons	References
Microscopic examination-blood smear	Blood	All domestic animals	Require less instruments, Easy to conduct in field	More than 50-80% of the infections are undetectable Detects above 10^5 trypanosomes per ml	Wilkowsky Silvina. (2022)
Microscopic examination of the fluid aspirate	lymph nodes, bone marrow, and CSF	cattle	More sensitive than blood smear examination	Less sensitive than DNA detection by PCR	Picozzi *et al.,* 2002
Hematocrit Centrifuge Technique	blood		Sensitivity (100-200 trypanosomes per ml)		Woo, 1969; Maki *et al*, 2017
Mouse inoculation test	Blood	Mouse	Upto 86.23% efficacy		Singh et al., 2003; Jain et al. 2000
Card Agglutination Test and ELISA	Blood	Indian cattle	91 % sensitivity cost effective and simpler	Doesn't describe the stage of infection False positive results	Chappuis *et al.,* 2002; Radwanska *et al.,* 2002; Sudan et al., 2015
DNA detection by PCR	Blood	Equines, buffaloes, camels, dogs	The most sensitive and specific method Can detect as less as 5-10 trypanosomes per ml blood More than 3 times more sensitive than hematocrit centrifugation and mini-anion centrifugation Can detect mixed infections	Requires specialized equipment and expertise Limited use in resource-poor settings	(Holland et al., 2001; Desquesnes and Davila, 2002; Parashar et al., 2015; Sudan et al., 2014; Pruvot et al., 2013; Ravindran, 2008

Molecular Characterization and Phylogenetic Studies

The molecular identification of Trypanosome species has elucidated parasite diversity and host specialization, which are crucial for comprehending disease epidemiology (Table 1). Phylogenetic analyses reveal genetic differences across *T. evansi* strains, potentially affecting pathogenicity and therapeutic responsiveness. These investigations contribute to the advancement of targeted diagnostic tools and vaccinations; however, the development of vaccines for horse trypanosomiasis is a formidable challenge due to the antigenic diversity of the parasite (Aregawi et al., 2019; Giordani et al., 2016).

Control and Treatment Strategies

Controlling the vector populations forms the main preventative measure against trypanosomiasis. In the endemic locations, efficient vector control covers environmental sanitation, insecticidal treatment, and tsetse fly traps. These strategies help to control disease and lower the transmission chances (Halder et al., 2019). Currently, there are few therapy choices for equine trypanosomiasis and most of them are useless in the neurological phases of the disease (Asrar et al., 2022). The medications reduce parasitemia but might not clear the infection. Moreover, medication resistance is a new problem that emphasizes the need for continuous research and the development of new treatment choices (Ahmed et al., 2018).

Controlling the spread of trypanosomiasis depends on good animal husbandry techniques including separating the sick animals and preserving hygienic stabling conditions. Research in endemic areas underlines the need of community-based interventions involving public awareness campaigns and major vector control initiatives (Giordani et al., 2016; Le Roux, 2020).

New treatment targets have come from studies looking at host-pathogen interactions. Variability in disease characteristics among infected equines suggests the possibility for individualized treatment plans. Moreover, the creation of molecular inhibitors aiming at parasite-specific pathways promises great possibilities for the next pharmaceutical development (Raftery et al., 2020).

Conclusion

Equine trypanosomiasis is a neglected but serious illness with continuous diagnostic and therapeutic difficulties. Modern developments in molecular diagnostics have enhanced detection capacity, although actual field applications are limited because of cost and resource availability. Given the significant financial gains associated with the control of trypanosomiases, future studies should concentrate on developing cost-effective, quick diagnostic methods and new treatment approaches as well as investigating the possibility for an efficient vaccination for horse trypanosomiasis.

References

Abbasi, I. H. R., Sahito, H. A., Sanjrani, M. I., Abbasi, F., Memon, M. A., Menghwar, D. R., Kaka, N. A., Shah, M. N., & Memon, M. (2014). A disease complex pathogen "Trypanosoma congolense" transmitted by tsetse fly in donkeys. *Herald Journal of Agriculture and Food Science Research, 2*(1), 44–48.

Abdel-Radey, A. (2008). Epidemiological studies (parasitological, serological and molecular techniques) of *T. evansi* infection in camels (*Camelus dromedarius*) in Egypt. *Veterinary World, 1*(11), 325–328.

Ahmed, Y., Hagos, A., Merga, B., Van Soom, A., Duchateau, L., & Goddeeris, B. (2018). *T. equiperdum* in horses: A neglected threat. *Vlaams Diergeneeskundig Tijdschrift, 87*, 66–75.

Alanazi, A. D., Puschendorf, R., Salim, B., Alyousif, M. S., Alanazi, I. O., & Al-Shehri, H. R. (2018). Molecular detection of equine trypanosomiasis in the Riyadh Province of Saudi Arabia. *Journal of Veterinary Diagnostic Investigation, 30*(6), 942–945. https://doi.org/10.1177/1040638718798688

Anonymous. (2016). *Pakistan Economic Survey* (Vol. 24, pp. 45).

Aquino, L. P. C. T. D., Machado, R. Z., Alessi, A. C., Santana, A. E., Castro, M. B. D., Marques, L. C., & Malheiros, E. B. (2002). Hematological, biochemical and anatomopathological aspects of the experimental infection with *T. evansi* in dogs. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 54*(1), 8–18.

Aregawi, W. G., Agga, G. E., Abdi, R. D., & Büscher, P. (2019). Systematic review and meta-analysis on the global distribution, host range, and prevalence of *T. evansi*. *Parasites & Vectors, 12*(1), 67. https://doi.org/10.1186/s13071-019-3320-3

Aslam, A., Chaudhary, Z. I., Rehman, H., Ashraf, K., Ahmad, N., Yaqub, T., & Shakoori, A. R. (2010). Comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural trypanosomal infection in equines. *Pakistan Veterinary Journal, 30*(1), 42–46.

Asrar, R., Farhan, H. R., Sultan, D., Ahmad, M., Hassan, S., Kalim, F., & Ali, W. (2022). Review Article Bovine Babesiosis; Review on its global prevalence and anticipatory control for one health. *Continental Veterinary Journal, 2*, 42-49.

Azzouzi, C., Boucheikhchoukh, M., Mechouk, N., Sedraoui, S., & Benakhla, A. (2025). Checklist of medico-veterinary important biting flies (*Ceratopogonidae, Hippoboscidae, Phlebotominae, Simuliidae, Stomoxyini, and Tabanidae*) and their associated pathogens and hosts in Maghreb. *Parasitologia, 5*(1), 1. https://doi.org/10.3390/parasitologia5010001

Bakari, S. M., Ofori, J. A., Kusi, K. A., Aning, G. K., Awandare, G. A., Carrington, M., & Gwira, T. M. (2017). Serum biochemical parameters and cytokine profiles associated with natural African trypanosome infections in cattle. *Parasites & Vectors, 10*(1), 312. https://doi.org/10.1186/s13071-017-2255-9

Bal, M. S., Sharma, A., Ashuma, B., Kaur, P., & Singla, L. D. (2014). Detection and management of latent infection of *T. evansi* in a cattle herd. *Indian Journal of Animal Research, 48*(1), 31–37.

Baticados, W. N., Castro, D. L., & Baticados, A. M. (2012). Parasitological and PCR detection of *Trypanosoma evansi* in buffaloes from Luzon, Philippines. *Ceylon Journal of Science (Biological Sciences), 40*(2), 141–146. https://doi.org/10.4038/cjsbs.v40i2.3930

Baylis, M., & Mbwabi, A. L. (1995). Feeding behaviour of tsetse flies (*Glossina pallidipas Austen*) on Trypanosoma-infected oxen in Kenya. *Parasitology, 110*(3), 297–305. https://doi.org/10.1017/S0031182000063824

Benfodil, K., Ansel, S., Mohamed-Cherif, A., & Ait-Oudhia, K. (2019). Prevalence of *T. evansi* in horses (*Equus caballus*) and donkeys (*Equus asinus*) in El-Bayadh district, southwestern Algeria. *Journal of Hellenic Veterinary Medical Society, 70*(4). https://doi.org/10.12681/jhvms.21786

Bontempi, I. A., Arias, D. G., Castro, G. V., Peverengo, L. M., Díaz, G. F., Allassia, M., Greif, G., & Marcipar, I. (2024). Improved serodiagnosis of *Trypanosoma vivax* infections in cattle reveals high infection rates in the livestock regions of Argentina. *PLOS Neglected Tropical Diseases, 18*(6), e0012020. https://doi.org/10.1371/journal.pntd.0012020

Brun, R., Hecker, H., & Lun, Z. R. (1998). *T. evansi* and *T. equiperdum*: Distribution, biology, treatment and phylogenetic relationship (a review). *Veterinary Parasitology, 79*(2), 95–107. https://doi.org/10.1016/S0304-4017(98)00146-5

Büscher, P., & Lejon, V. (2004). Diagnosis of human African trypanosomiasis. In I. Maudlin, P. Holmes, & M. A. Miles (Eds.), *Trypanosomiasis Textbook* (pp. 203–218). CABI Publishing.

Büscher, P., Gonzatti, M. I., Hébert, L., Inoue, N., Pascucci, I., Schnaufer, A., Suganuma, K., Touratier, L., & Van Reet, N. (2019). Equine trypanosomosis: Enigmas and diagnostic challenges. *Parasites & Vectors, 12*(1), 234. https://doi.org/10.1186/s13071-019-3484-x

Cadioli, F. A., Marques, L. C., Machado, R. Z., Alessi, A. C., Aquino, L. P. C. T., & Barnabé, P. A. (2006). Experimental *T. evansi* infection in donkeys: Hematological, biochemical and histopathological changes. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 58*(5), 749–756.

Camoin, M., Kocher, A., Chalermwong, P., Yangtarra, S., Kamyingkird, K., Jittapalapong, S., & Desquesnes, M. (2019). The indirect ELISA *Trypanosoma evansi* in equids: Optimisation and application to a serological survey including racing horses, in Thailand. *BioMed Research International, 2019*, 2964639. https://doi.org/10.1155/2019/2964639

Chappuis, F., Stivanello, E., Adams, K., Kidane, S., Pittet, A., & Bovier, P. A. (2002). Card agglutination test for trypanosomiasis (CATT) end-dilution titer and cerebrospinal fluid cell count as predictors of human African Trypanosomiasis (*T. brucei gambiense*) among serologically suspected individuals in southern Sudan. *American Journal of Tropical Medicine and Hygiene, 71*, 313–317.

Clausen, P. H., Wiemann, A., Patzelt, R., et al. (1998). Use of a PCR assay for the specific and sensitive detection of *Trypanosoma* spp. in naturally infected dairy cattle in peri-urban Kampala, Uganda. *Annals of the New York Academy of Sciences, 849*, 21–31.

Desquesnes, M., Biteau-Coroller, F., Bouyer, J., Dia, M. L., & Foil, L. D. (2009). Development of a mathematical model for mechanical transmission of trypanosomes and other pathogens of cattle transmitted by tabanids. *International Journal of Parasitology, 39*, 333–346.

Desquesnes, M., & Dia, M. L. (2003). *T. vivax*: Mechanical transmission in cattle by one of the most common African tabanids, *Atylotus agrestis*. *Experimental Parasitology, 103*, 35–43.

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

Desquesnes, M., & Dia, M. L. (2004). Mechanical transmission of *T. vivax* in cattle by the African tabanid *Atylotus fuscipes*. *Veterinary Parasitology, 119*, 9–19.

Ereqat, S., Nasereddin, A., Al-Jawabreh, A., Al-Jawabreh, H., Al-Laham, N., & Abdeen, Z. (2020). Prevalence of *Trypanosoma evansi* in livestock in Palestine. *Parasites & Vectors, 13*(1), 21. https://doi.org/10.1186/s13071-020-3894-9

Fernández, D., González-Baradat, B., Eleizalde, M., González-Marcano, E., Perrone, T., & Mendoza, M. (2009). *Trypanosoma evansi*: A comparison of PCR and parasitological diagnostic tests in experimentally infected mice. *Experimental Parasitology, 121*(1), 1–7. https://doi.org/10.1016/j.exppara.2008.09.013

Getahun, M. N., Ngiela, J., Makwatta, J. O., Ahuya, P., Simon, T. K., Kamau, S. K., Torto, B., & Masiga, D. (2022). Metabolites from trypanosome-infected cattle as sensitive biomarkers for animal trypanosomosis. *Frontiers in Microbiology, 13*, 922760. https://doi.org/10.3389/fmicb.2022.922760

Giordani, F., Morrison, L. J., Rowan, T. G., De Koning, H. P., & Barrett, M. P. (2016). The animal trypanosomiases and their chemotherapy: A review. *Parasitology, 143*(14), 1862. https://doi.org/10.1017/S0031182016001268

Gizaw, Y., Megersa, M., & Fayera, T. (2017). Dourine: A neglected disease of equids. *Tropical Animal Health and Production, 49*(5), 887–897.

Halder, B., Dhara, S., & Ghosh, A. (2019). Trypanosomiasis in equines: A brief discussion. *International Journal of Livestock Research.* https://doi.org/10.36062/ijah.60.1.2021.49-57

Herder, S., Simo, G., Nkinin, S., & Njiokou, F. (2002). Identification of trypanosomes in wild animals from southern Cameroon using the polymerase chain reaction (PCR). *Parasite, 9*(4), 345–349.

Hilali, M. A. A., Nassar, A., & Abdel-Wahab, A. (2006). Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *T. evansi*. *Veterinary Parasitology, 139*, 237–243.

Holland, W. G., Claes, F., My, L. N., Thanh, N. G., Tam, P. T., Verloo, D., Büscher, P., Goddeeris, B., & Vercruysse, J. (2001). A comparative evaluation of parasitological tests and a PCR for *T. evansi* diagnosis in experimentally infected water buffaloes. *Veterinary Parasitology, 97*(1), 23–33.

Hussain, M., Saeed, Z., Gulsher, M., Shaikh, R. S., Ali, M., Akhtar, M., & Iqbal, F. (2016). Molecular detection and seasonal prevalence of *T. brucei* and its effect on hematobiochemical parameters in donkeys from Dera Ghazi Khan District in Southern Punjab, Pakistan. *Pakistan Journal of Zoology, 48*, 1781–1786.

Jannin, J., & Simarro, P. (2007). Protozoan diseases: African trypanosomiasis. In *International Encyclopedia of Public Health* (pp. 331–335).

https://doi.org/10.1016/B978-012373960-5.00486-X

Kadima, K. B., Gyang, E. O., Saror, D. I., & Esievo, K. A. (2000). Serum biochemical values of *T. vivax*-infected cattle and the effects of lactose in saline infusion. *Veterinarski Archive, 70*, 67–74.

Khan, A. U., Qureshi, A. S., Hassan, M. F., Rehan, S., & Sarfraz, A. (2018). Molecular identification of trypanosomes and their effects on hematological and biochemical parameters in donkeys in Punjab, Pakistan. *International Journal of Agriculture and Biology, 20*, 1607–1612.

Kizza, D., Azuba, R., Wampande, E., Okwasiimire, R., Owembabazi, L., Mandela, W., Waiswa, C., & Odoi, A. (2024). Comparative evaluation of rapid diagnostic test and PCR-based diagnostic assay for identification of trypanosomes in cattle of Apac and Kiryandongo districts, Uganda: A cross-sectional study. *BMC Veterinary Research, 20*, 570. https://doi.org/10.1186/s12917-024-04436-7

Kouadio, I. K., Sokouri, D., Koffi, M., Konaté, I., Ahouty, B., Koffi, A., & N'Guetta, S. P. (2014). Molecular characterization and prevalence of *Trypanosoma* species in cattle from a northern livestock area in Côte d'Ivoire. *Open Journal of Veterinary Medicine, 12*, 312–314.

Kumar, H., Gupta, M. P., Sidhu, P. K., Mahajan, V., Bal, M. S., & Kaur, K., et al. (2012). An outbreak of acute *T. evansi* infection in crossbred cattle in Punjab, India. *Journal of Applied Animal Research, 40*(3), 256–259.

Lai, D. H., Hashimi, H., Lun, Z. R., Ayala, F. J., & Lukes, J. (2008). Adaptations of *T. brucei* to gradual loss of kinetoplast DNA: *T. equiperdum* and *T. evansi* are petite mutants of *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences, 105*(6), 1999–2004. https://doi.org/10.1073/pnas.0801537105

Maki, A., Abdalla, H. S., & Mustafa, M. (2017). The prevalence of *T. evansi* in camels (*Camelus dromedarius*) in South Darfur State. *Journal of Veterinary Medicine and Animal Production, 7*(1), 10–13.

Mantilla, B. S., Marchese, L., Casas-Sánchez, A., Dyer, N. A., Ejeh, N., Biran, M., & Silber, A. M. (2017). Proline metabolism is essential for *T. brucei* survival in the tsetse vector. *PLOS Pathogens, 13*(1), e1006158. https://doi.org/10.1371/journal.ppat.1006158

Matetovici, I., & Den Abbeele, J. V. (2018). Thioester-containing proteins in the tsetse fly (*Glossina*) and their response to trypanosome infection. *Insect Molecular Biology, 27*(3), 414. https://doi.org/10.1111/imb.12382

McGettrick, A. F., Corcoran, S. E., Barry, P. J., McFarland, J., Crès, C., Curtis, A. M., Franklin, E., Corr, S. C., Mok, K. H., Cummins, E. P., Taylor, C. T., Liew, A. A., & Nolan, D. P. (2016). *Trypanosoma brucei* metabolite indolepyruvate decreases HIF-1α and glycolysis in macrophages as a mechanism of innate immune evasion. *Proceedings of the National Academy of Sciences, 113*(48), E7778–E7787. https://doi.org/10.1073/pnas.1608221113

Nimpaye, H., Njiokou, F., Njine, T., Njitchouang, G. R., Cuny, G., Herder, S., & Simo, G. (2011). *T. vivax, T. congolense* "forest type" and *T. simiae*: Prevalence in domestic animals of sleeping sickness foci of Cameroon. *Parasite, 18*(2), 171–179. https://doi.org/10.1051/parasite/2011182171

Ohaeri, C. C., & Eluwa, M. C. (2011). Abnormal biochemical and haematological indices in trypanosomiasis as a threat to herd production. *Veterinary Parasitology, 177*(3), 199–202. https://doi.org/10.1016/j.vetpar.2010.11.001

Padmaja, K. (2012). Haemato-biochemical studies of camels infested with trypanosomiasis. *Veterinary World, 5*, 356–358.

Pal, V., Singh, A., Singh, H., & Sethi, K. (2021). Prevalence, relative risk factors, and hemato-biochemical changes associated with equine trypanosomosis in eastern Uttar Pradesh. *Indian Journal of Animal Health, 60*(1). https://doi.org/10.36062/ijah.60.1.2021.49-57

Picozzi, K., Tilley, A., Fevre, E. M., et al. (2002). The diagnosis of trypanosome infections: Applications of novel technology for reducing disease risk. *African Journal of Biotechnology, 1*(2), 39–45.

Radwan, M. E., & El-Madawy, R. (2010). Diagnosis and epidemiological studies of bovine trypanosomiasis in Kaliobia Governorate. *Journal of American Science, 6*(11).

Radwanska, M. M., Chamekh, L., Vanhamme, F., Claes, S., Magez, E., Magnus, P., & Baetselier, P. (2002). The serum resistance-associated gene as a diagnostic tool for the detection of *T. brucei rhodesiense*. *American Journal of Tropical Medicine and Hygiene, 67*, 684–690. https://doi.org/10.4269/ajtmh.2002.67.684

Raftery, A., Jallow, S., Coultous, R. M., Rodgers, J., & Sutton, D. (2020). Variation in disease phenotype is marked in equine trypanosomiasis. *Parasites & Vectors, 13*, Article 89. https://doi.org/10.1186/s13071-020-04317-4

Ravindran, R., Rao, J. R., Mishra, A. K., Pathak, K. M. L., Babu, N., Satheesh, C. C., & Rahul, S. (2008). *T. evansi* in camels, donkeys, and dogs in India: Comparison of PCR and light microscopy for detection. *Veterinarski Arhiv, 78*(1), 89–94.

Samuel, F. U., Adamu, S., Mohammed, B., Chiezey, P. N., Mohammed, A. K., Ereke, S. O., & Ibrahim, M. A. (2018). Effect of experimental *T. congolense* infection on serum profiles of lipid and cholesterol in pack donkeys. *Nigerian Veterinary Journal, 39*(1), 57–65.

Shoraba, M., Shoulah, S. A., Arnaout, F., & Selim, A. (2023). Equine trypanosomiasis: Molecular detection, hematological, and oxidative stress profiling. *Veterinary Medicine International, 2024*(1), Article 6550276. https://doi.org/10.1155/2024/6550276

Simon, N., Shallat, J., Wietzikoski, C. W., & Harrington, W. E. (2020). Optimization of Chelex 100 resin-based extraction of genomic DNA from dried blood spots. *Biology Methods & Protocols, 5*(1), bpaa009. https://doi.org/10.1093/biomethods/bpaa009

Stijlemans, B., De Baetselier, P., Magez, S., Van Ginderachter, J. A., & De Trez, C. (2018). African trypanosomiasis-associated anemia: The contribution of the interplay between parasites and the mononuclear phagocyte system. *Frontiers in Immunology, 9,* 218. https://doi.org/10.3389/fimmu.2018.00218

Sutcliffe, O. B., Skellern, G. G., Araya, F., Cannavan, A., Sasanya, J. J., Dungu, B., Van Gool, F., Munstermann, S., & Mattioli, R. C. (2014). Animal trypanosomosis: Making quality control of trypanocidal drugs possible. *Revue Scientifique et Technique de l'OIE, 33*(3), 813–830. https://doi.org/10.20506/rst.33.3.2320

Taylor, K., & Authié, E. M. L. (2004). Pathogenesis of animal trypanosomiasis. In I. Maudlin, P. H. Holmes, & M. A. Miles (Eds.), *The Trypanosomiases* (pp. 331–350). CAB International.

Ukpai, O. M., & Nwabuko, O. P. (2014). Effects on haematological parameters and pathology of internal organs of *T. brucei*-infected albino rats. *Nigerian Journal of Biotechnology, 27,* 8–13.

Van Den Abbeele, J., & Caljon, G. (2016). Tsetse fly saliva proteins as biomarkers of vector exposure. In *Arthropod Vector: Controller of Disease Transmission, Volume 2* (pp. 195–208). https://doi.org/10.1016/B978-0-12-805360-7.00011-3

Wilkowsky, S. (2022). Trypanosomiasis in animals. In *MSD Veterinary Manual.* https://www.msdvetmanual.com/circulatory-system/blood-parasites/trypanosomiasis-in-animals

Zewdu Ayalew, A., Gebeyehu Manie, F., Menale, K., & Tigist Mekonnen, M. (2021). The economics of trypanosomiasis: Empirical evidence on its impacts on livestock production and welfare. *International Centre of Insect Physiology and Ecology*