Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Insight on Surra Disease (*Trypanosoma evansi*) in Horses

Asmaa M. El Nady[1], Aliaa A. Balegh[1*], Asmaa A. Hegab[1], Eman A. El-Kelesh[1], Sahar E. Saba[1] and Momtaz A. Shahein[2]

[1]*Parasitology Department, Animal Health Research Institute, Agricultural Research Center, (ARC), Giza, Egypt.*
[2]*Virology Research Department, Animal Health Research Institute, Agricultural Research Center, (ARC), Giza, Egypt.*

Abstract

THE disease caused by *Trypanosoma evansi*, commonly known as surra, is one of the most economically significant parasitic diseases affecting animals in Egypt, particularly equines and camels. This study aimed to assess the prevalence of surra by comparing various diagnostic methods, including parasitological examinations, CATT/*T. evansi*, ELISA, and molecular techniques. Three hundred eighty-three blood samples were collected from horses in Cairo (158 samples) and Giza (180 samples) districts. Microscopic examination of wet smears and Giemsa-stained thin blood films revealed no positive samples for *T. evansi* (0%). However, 26.33% of the examined samples were positive for *T. evansi* antibodies using the CATT/*T. evansi*, with 4.73% showing strong agglutination and 21.59% demonstrating moderate agglutination. Additionally, out of one hundred examined samples using ELISA 16 samples (16%) were positive. A PCR assay targeting the ITS-1 gene successfully identified a specific band (467 bp) in seven samples, indicating the presence of *Trypanosoma evansi.* Phylogenetic analysis revealed that this strain belongs to *T. evansi* genotype A, and is closely related to strains found in water buffalo in Thailand. The ITS-PCR sequence of *T. evansi* isolates from Egyptian horses demonstrated a high similarity, ranging from 94.9% to 99.7% identity, with various *Trypanosoma species* sequences available in GenBank. Notably, the highest similarity of 99.7% was observed with a strain of *T. evansi* isolated from camels in Egypt. Furthermore, a high positivity was detected with the CATT/*T. evansi* assay indicates that this test is effective for the routine screening of surra in horses.

Keywords: Prevalence, CATT/*T. evansi*, ELISA, PCR, Phylogenetic analysis.

Introduction

Equine vector-borne diseases (EVBD) pose significant challenges to the equine industry and have a notable impact on the health and productivity of horses l [1]. The most economically significant vector-borne blood protozoan diseases in horses are Surra and equine piroplasmosis. Surra is a widespread infectious disease that affects both domestic and wild animals and is caused by the protozoan parasite *Trypanosoma evansi*. This flagellate parasite is pathogenic to camelids and equids [2–3–4]. *T. evansi* is found throughout Africa, extending from the northern regions down to the Middle East, particularly wherever camels are present [4].

In Egypt, *T. evansi* mainly infects camels, especially imported ones; however, it can also cause severe illness in horses, mules, donkeys, and dogs. The disease is transmitted mechanically by blood-feeding flies, particularly horseflies (Tabanus spp.) and stable flies (Stomoxys spp.) [5–6]. Surra manifests in both acute and chronic forms. The acute form primarily affects horses and camels, with an incubation period of 1 to 4 weeks in horses. Acute symptoms include fluctuating fever with high peaks of parasitemia, weakness, lethargy, anemia, severe weight loss, petechial hemorrhages (eyelids and mucosa of the vulva and vagina), abortion, and edema (such as the submaxillary region, legs, brisket, abdomen, testicle sheath, and udder). This may ultimately lead to alterations in locomotion, presenting nervous signs that start in the forelegs and progress to the hind legs [7–8]. In chronic cases, symptoms can include anemia, weight loss, and jaundice, often accompanied by dark yellow urine [4–9–10]. Some horses may carry *T. evansi* without exhibiting clinical signs [11]. Additionally,

*Corresponding author: Aliaa A. Balegh, E-mail: aliaabaleg@yahoo.com, Tel.: 01066324217
(Received 25 August 2025, accepted 28 October 2025)
DOI: 10.21608/ejvs.2025.416857.3078

transplacental transmission has been reported in donkey mares [12].

The impact of Surra can lead to reduced productivity and significant economic losses, with mortality rates in horses potentially exceeding 50% [13]. Diagnosing surra can be challenging due to often non-specific clinical signs associated with the disease, making accurate diagnosis difficult. Therefore, laboratory methods are essential for detecting the parasites. Although microscopic blood examinations can help diagnose surra, their sensitivity is limited and can be improved by various concentration techniques [14]. Serological and molecular tests act as specific diagnostic tools for accurately detecting surra, with polymerase chain reaction (PCR) techniques being the preferred choice routinely used in many laboratories. The sensitivity of PCR is directly related to the amount of DNA present in the sample, which correlates with the level of parasitemia [14].

In 2021, the World Organization for Animal Health (WOAH) recommended the enzyme-linked immunosorbent assay (ELISA) and the card agglutination test (CATT) as standardized methods for diagnosing surra. However, serological tests may lack sensitivity or specificity, and molecular tests do not distinguish between taxa within the Trypanosoma subgenus [5].

Current information on the prevalence of the disease in equines is limited. Therefore, this study aims to assess the prevalence of surra by various diagnostic methods, including parasitological and serological tests such as CATT and ELISA, as well as molecular analysis. Additionally, the study will evaluate and compare the diagnostic performance of these tests.

Material and Methods

Sample collection

From January 2021 to March 2024, 338 blood samples were collected from apparently healthy adult horses in the districts of Cairo (158 samples) and Giza (180 samples) in Egypt.

Blood sampling

Parasitological examination

Two blood samples were collected from the jugular vein of each horse through jugular venipuncture. For serological tests, 3 mL of blood were placed into a serum separator tube. Three mL EDTA-coated blood samples were collected to prepare blood smears and for PCR extraction. The samples were kept in an icebox with ice packs and were transported quickly to the Parasitology Department at the Animal Health Research Institute in Egypt for further analysis.

After centrifugation of the serum separator samples for 10 minutes at 2000 rpm, the serum was aliquoted and stored at –80 °C for subsequent serological tests, including CATT/*T. evansi* and ELISA.

Wet smears

In wet smear preparation, a drop of fresh blood was mixed with a drop of normal saline, and a cover slip was placed over it to create a monolayer. The parasite's movement was observed in 100 microscopical fields at 40x magnification [15].

Thin blood smears

To prepare a blood smear, a drop of collected blood was placed on a clean microscope slide. A second slide was then used to draw the blood at a 45-degree angle, creating a thin smear. After air-drying, the smear was fixed with absolute methyl alcohol and stained with Giemsa stain. Following washing with water, the stained smear was air-dried before being examined under a microscope at 100× using oil immersion to observe the presence of Trypanosoma [16]. A smear is considered positive if at least one trypanosome is observed.

Card agglutination test for T. evansi (CATT/T. evansi)

The CATT/*T. evansi* is a rapid direct agglutination test used for diagnosing *T. evansi* infection in equines by detecting anti-*T. evansi* antibodies (Institute of Tropical Medicine, Antwerp, Belgium). This test utilizes formaldehyde-fixed, Coomassie Blue-stained, freeze-dried trypanosomes of *T. evansi* VAT RoTat 1.2. To conduct the test, approximately 45µl (one drop) of the well-homogenized CATT antigen is placed onto a test card and mixed with 25µl of test serum, diluted at 1:4 with CATT buffer. The card should then be gently rocked for 5 minutes to ensure a constant circular movement by tilting it slowly in one direction, from left-back to right-forth. After this, the reaction is observed under clear light. A reaction is considered positive if blue agglutinates are visible to the naked eye. Reactions are scored as follows: negative (-), indeterminate (±), or positive (+, ++, +++) according to the levels recommended by the manufacturer [17].

Enzyme-linked immunosorbent assay (ELISA)

The test was conducted using a commercially available kit, the AsurDx™ *T. evansi* antibody (Biostone Animal Health, USA), which is designed to detect IgG antibodies specific to *T. evansi* in horse serum or plasma. Notably, this test does not cross-react with antibodies against *T. brucei*.

The procedure involves adding a diluted serum sample (at a ratio of 1:40) to wells that are coated with purified *T. evansi* protein antigen. If *T. evansi* antibodies are present in the sample, they will bind to the coated antigen. Horseradish peroxidase (HRP)-conjugated antibodies are then introduced to target

the anti- *T. evansi* antibodies. After adding the TMP substrate, the resulting color intensity corresponds to the quantity of anti- *T. evansi* antibodies in the sample. The optical density (OD) is measured at 450 nm.

To calculate the percent positivity (PP) of a sample, the following formula is used:

PP = (OD of test sample / mean OD of positive control) x 100.

If the PP is less than 60%, the sample is negative. Conversely, the sample is positive if the PP is 60% or greater.

Molecular technique (PCR)

Fifteen horse samples were analyzed using PCR to identify the specific type of *Trypanosoma*. Five samples exhibited strong agglutination, and another five showed moderate agglutination for *T. evansi* when tested with CATT/ *T. evansi*. Additionally, five samples tested negative in both parasitological and serological examinations, allowing for a comparison with results from other tests performed on the same samples.

DNA extract

Genomic DNA was extracted from whole horse blood (200 µl of buffy coat) using the QIAamp DNA Blood Mini Kit from Qiagen, Hilden, Germany, according to the manufacturer's instructions. The extracted DNA was stored at -20°C until further analysis.

PCR amplification was conducted using the ITS1 primer sequences as shown in Table 1 [18] from Metabion, Germany. These primers are specifically designed to yield distinct amplified products. The PCR was carried out in a T3 Thermal Cycler (Biometra), with each reaction having a total volume of 25 µl. This volume included 12.5 µl of Emerald Amp GT PCR master mix from Takara, Japan.

The PCR conditions were as follows: primary denaturation at 94°C for 5 minutes, followed by 35 cycles including secondary denaturation at 94°C for 30 seconds, annealing at 65°C for 40 seconds, DNA fragment extension at 72°C for 45 seconds, and a final extension at 72°C for 10 minutes.

The PCR products were analyzed on a 1.5% agarose gel (Appli Chem, Germany, GmbH) prepared in 1x TBE buffer, alongside a 1000 bp DNA ladder as a molecular weight standard. The gels were electrophoresed at 1-5 volts/cm of the tank length for 30 minutes. After staining with ethidium bromide, the gels were photographed using a gel documentation system, and the data was analyzed with computer software. The ITS1-PCR, utilizing the parasite DNA, yielded an amplification product of 467 bp.

DNA Sequencing and Phylogenetic Analysis

We performed partial sequencing of the ITS1 gene from the PCR product isolate using targeted primers. The PCR products were purified with the QIAquick PCR Product Extraction Kit (Qiagen). Sequencing was done with the Big Dye Terminator V3.1 Cycle Sequencing Kit (Perkin-Elmer) and purified using a Centrisep spin column. The DNA sequences were analyzed with the Applied Biosystems 3130 genetic analyzer. Sequence homology to GenBank accessions was determined through BLAST® analysis [19]. Sequence identities were assessed using the Meg Align module of Laser Gene DNA Star [20], and phylogenetic analyses were conducted using maximum likelihood, neighbor-joining, and maximum parsimony methods in MEGA6 [21].

Assessment of diagnostic efficacy

The diagnostic efficacies of parasitological examination, CATT/ *T. evansi*, ELISA, and PCR were evaluated based on the percentage positivity shown by each diagnostic test. Percentage positivity is calculated as the number of positive samples given by a particular diagnostic test divided by the total number of suspected cases, multiplied by 100.

(%) positivity = number of positive samples given by particular diagnostic / total no suspected cases × 100.

Statistical analysis

Statistical analysis was done using IBM© SPSS© Statistics version 26 (IBM© Corp., Armonk, NY, USA). Qualitative data were expressed as frequency and percentage. The chi-square test was used to examine the relation between independent qualitative variables. The McNemar test was used to determine the difference between CATT and ELISA as dichotomous dependent variables. Cohen's Kappa test was used to measure the agreement between CATT and ELISA. All tests were two-tailed. A p-value < 0.05 was considered significant.

Results

Parasitological examination

A total of 338 horse blood samples from Cairo and Giza districts of Egypt were examined by parasitological examination, wet and Giemsa stain blood smears for *Trypanosoma evansi* revealed that all samples were negative (0%).

Serological examination

Results of Card agglutination test for T. evansi (CATT T. evansi)

The CATT/*T. evansi* test results showed that out of 338 examined horses, 89 (26.33%) were found seropositive to *T. evansi* antibodies at the dilution of 1:4. The infection rate in Giza (27.77%) was higher than that recorded in Cairo (24.68%). Among the 89

positive samples, 16 (17.97%) exhibited strong agglutination (++), while 73 (82.02%) showed moderate agglutination (+) (Fig. 1 and Table 2.).

Enzyme-linked immunosorbent assay results (ELISA)

The ELISA results for detecting antibodies to *T. evansi* in horse samples indicated that 16 out of 100 samples (16%) were positive. Among the positive samples, six were from Cairo, and ten from the Giza district. Notably, the samples that tested positive in the ELISA also displayed strong agglutination results when assessed using the CATT/*T. evansi* method (Table 3).

The McNemar test result ($p < 0.001$) highlighted discordance between the two tests. Seventy-three samples showed moderate agglutination but were negative by ELISA (Fig 2 and Table 4.). Furthermore, a Kappa value of 0.046 suggests no agreement between the two testing methods. There was no significant difference between the two governorates regarding the findings of CATT/*T. evansi* and ELISA (p=0.275).

The result of PCR

ITS1-PCR analysis of purified parasite DNA resulted in an amplification product approximately 467 bp in size, among the 15 horse samples examined for *T. evansi* using ITS1 PCR, seven samples tested positive, while the remaining eight were negative. The PCR-positive samples included five with strong agglutination, one with moderate agglutination, and one sample that showed negative with both parasitological and serological results (see Fig. 3 and Table 6).

Genetic characterization results

The nucleotide sequence length of the isolate from the Egyptian horse was amplified, producing a single fragment of the expected size, 467 bp, deposited in the GenBank database under the accession number PP197227.

The phylogenetic analysis of ITS-1 sequences using maximum likelihood, neighbor-joining, and maximum parsimony in MEGA6 rooted tree revealed that the Egyptian horse *T. evansi* isolate (EquiTry1) was found in the same clade with *T. evansi* isolate from water buffalo in Thailand with accession number Mn121260, This clade is contained within a larger clade that includes *T. evansi* isolates identified in Egypt from dromedary camels, with accession numbers MW603779 and MW617067, and from the buffalo in Philippines with accession number DQ472685. The large clade was paralleled to the other clades including *T. evansi* isolates of camel in Egypt, and Buffalo in Philippines. All the previous clades belong to the *T.evansi* genotype A.

Using the nucleotide basic local alignment search tool (nBLAST), we found that the ITS-PCR sequence of Egyptian horse *T. evansi* isolates exhibited a relatively high sequence similarity, ranging from 94.9% to 99.7% identity with sequences of various Trypanosoma species identified in GenBank. Notably, the highest percentage similarity of 99.7% was found with a strain of *T. evansi* isolated from camels in Egypt (MW603778 and MW603779). This was followed by a 99.4% identity with *T. evansi* from camels in Egypt (MW603773 and MW617067), camels in Iran (JN 896754), as well as strains isolated from buffalo (DQ472684) and water buffalo (Mn121260) in Thailand. Additionally, these sequences displayed 95.5% identity with *T. brucei* (AC159414 and AC159412) and 94.9% to 95.2% identity with *T. equiperdum* (LC386053 and LC386056), as summarized in Table 5 and Figure 4. The identity between the submitted strain and each *T. evansi* genotype were as follows: genotype A ranged from 98.9% to 99.7%, genotype B was 98.6%, and genotype C ranged from 98.9% to 99.1%.

The sequences were aligned, revealing the presence of high similarity among *T. evansi* (genotypes A, B, and C), *T. brucei*, *T. equiperdum*, and the Egyptian horse strain of *T. evansi* (EquiTry1). This alignment highlighted repetitive sequences, as summarized in Figure 5. The sequence of the Egyptian horse isolates under investigation exhibits differences at two amino acid positions (SNPs) specifically, at positions 223 and 268 when compared to genotype A. In genotype B, there were five nucleotide differences at positions 134, 139, 159, 160, and 223, while genotype C exhibited four differences at positions 134, 139, 160, and 223. Additionally, there were thirteen and sixteen nucleotide differences when comparing the current Egyptian horse *T. evansi* with *T. brucei* and *T. equiperdum* respectively.

The positivity rates were presented in Table 6 from parasitological examination, CATT/*T.evansi*, ELISA, and PCR in 15 samples for a comprehensive analysis. The rates were 0%, 66.66%, 33.33%, and 46.666% respectively. CATT/*T. evansi* showed higher positivity.

Discussions

Surra caused by *Trypanosoma evansi* affects a wide range of animals and leads to high morbidity and mortality among various wild and domesticated mammals. In the present study, *T. evansi* was not detected in blood smears during microscopic examinations. Consequently, the prevalence of surra, as determined by wet and Giemsa-stained blood smears, was found to be 0%. This finding is consistent with previous studies: no positive samples using the Micro Hematocrit Centrifugation Test (MHCT), wet blood films (WBF), and Giemsa-stained blood smears (GSBS) [22]. Additionally, horse blood samples to be negative in Indonesia [23]. Similarly, 416 donkeys from various governorates in

Egypt were negative [24]. Also all examined blood smears (177 horses and 29 donkeys) in Algeria to be negative for *T. evansi* when using Giemsa-stained blood films [25].

In contrast, some studies have successfully detected *T. evansi* using parasitological tests. For example, a prevalence of 31.82% and 22.5% in equines in Iraq and Pakistan was reported by microscopic examinations, respectively [26–27]. Similarly, a prevalence of 2% in horses and donkeys was found using thin blood smears in Gujranwala, Pakistan [28]. In addition, a prevalence of 9.1% was reported in horses and donkeys from Gambia [29]. Positivity rate of 0.5% for surra was reported using Woo's test [11]. 9.6% of horses were positive for *T. evansi* in Jordan [30].

The absence of microscopically positive samples for *T. evansi* may be attributed to the low sensitivity in early and acute infections, as it can only detect parasitemia when it exceeds 500,000 trypanosomes/ml of blood [4–15]. Furthermore, during the chronic phase of the disease, parasitemia levels can fluctuate, leading to potential false negatives. Nevertheless, when positive, this examination brings a reliable and subgenus-specific confirmation of the infection.

The CATT/ *T. evansi* is suitable for detecting *T. evansi* -specific antibodies in acute and chronic infections, demonstrating a high positive predictive value and generally high sensitivity in horses [15]. In this study, the CATT/ *T. evansi* test showed that 89 out of 338 examined horses (26.33%) were positive for *T. evansi* antibodies. Similar findings were reported in India, where the positivity rate was 27% [31], and, in Ethiopia, it was 27.6% [32]. This result was higher than those found in other studies; for instance, no positive samples were detected in Indonesian horses [22], while positivity rates were 3.2% in Algeria [33], 13% in Malaysia [34], 13.3% in Indonesia [23], and 14.4% in Pakistan [11]. In contrast, higher positivity rate of 45.2% was recorded in Algeria [25], and a rate of 45% was observed in Indonesia [35].

A significant limitation of the CATT/ *T. evansi* test is that it cannot distinguish between past and current infections [36]. Currently, the CATT/T. evansi is the only available diagnostic kit for Surra, and the World Health Animal Organization recommends it for the serodiagnosis of this disease [35].

Trypanosoma evansi ELISA test is used to examine horse serum samples for the presence of antibodies against soluble antigens derived from whole *T. evansi* lysate. This technique has a high negative predictive value, particularly if the animal has not been recently infected. In this study, 16 out of 100 horse samples (16%) were positive for T. evansi antibodies. Similar survey revealed that 27% of horses in India were positive for *T. evansi* antibodies using the ELISA method [31], while 21.6% of equines in Pakistan tested positive [28]. The lowest positivity rate reported was 1% in Algeria [33].

The current study demonstrates that ELISA effectively detects infections during the chronic stage of the disease. However, it is not optimized for early detection during the prepatent stage, when only small amounts of antibodies (IgG) or IgM may be present [37]. The positive samples from the ELISA test showcased strong agglutination in the CATT/T. evansi test, a testament to the presence of IgG antibodies in sixteen samples. WOAH recommended ELISA alongside CATT/ *T. evansi* to potentially detect antibodies directed against all types of *T. evansi* [15].

The current study demonstrates that out of 15 horse samples tested for *T. evansi* using PCR, 7 were identified as positive (46.6%). Notably, only one of these positive samples yielded a negative result in other test methods, indicating that this animal may be in the early stage of infection before the apparition of specific antibodies. It is important to emphasize that the PCR technique is recognized for its superior sensitivity and specificity of active infection with analytical sensitivities reported as low as 1–20 parasites/mL [38].

The consistent results reported that 40% of horses (10 out of 25) tested positive using TBR PCR, while all horses were negative when tested with RoTat 1.2 VSG PCR [39]. In Iran, 21% of horses (84 out of 400) were positive for *T. evansi* using SSU rDNA PCR [6]. In Pakistan, the positivity rates ranged between 1.3% and 16% in horses, as determined by molecular techniques [11–40]. Additionally, the prevalence of *T. evansi* in horses was 1.75% in Indonesia [22], and 0% using 18S qPCR and ITS1 TD PCR in Algeria [33].

In Sudan, four species of *Trypanosoma* (*T. brucei*, *T. vivax*, *T. simiae*, and *T. congolense*) were identified in the infected horses and donkeys by ITS1-PCR [41]. This variation in species may be due to differences in the types of primers used, the sample processing methods applied including the technique of DNA extraction, and the geographic locations involved.

In the current study, a phylogenetic analysis based on ITS1 (Internal Transcribed Spacer) is crucial for understanding the phylogenetics and taxonomic identities of Trypanosomes, as well as the relatedness of Egyptian horse isolates to *T. evansi* sequences retrieved from GenBank. The phylogenetic tree indicated the presence of three main groups of *T. evansi*, labeled as genotype A, genotype B, and genotype C. The Egyptian equine *T. evansi* isolate is classified within genotype A. These is probably the first report of ITS1 gene sequence for

Egypt isolates of *T. evansi* in horses, but were detected in camels of Egypt [42–43]. The nucleotide basic local alignment search tool (nBLAST) in GenBank indicated that the ITS-PCR sequence of the *T. evansi* isolates found in Egyptian horses showed unique identities ranging from 94.9% to 99.7% with sequences of *Trypanosoma* species available in GenBank. Notably, the highest similarity observed was 99.7%, corresponding to a *T. evansi* strain isolated from camels in Egypt with accession numbers MW603778 and MW603779 [44]. This finding confirms the genetic similarity between *T. evansi* in horses and camels in Egypt, suggesting that camels may be the primary source of infection for horses. The data indicate that horses in contact with dromedaries are 2.49 times more likely to test positive for the disease serologically. Camels are known to exhibit high levels of parasitemia in their blood, and their proximity to horses in areas where the vectors are present likely increases the risk of transmission [25]. The similarities between isolates from various countries and different hosts were notable, including buffalo from Thailand [45], camels from Iran [46], buffalo from China [47], buffalo from Philippines [47], camels from India [46], equines from Mongolia [49]. These findings demonstrated that horses in Egypt are at a high risk of surra infection with potential for interspecies transmission. This situation urgently calls for increased awareness and preventative measures. These sequences also displayed 95.5% identity with *T. brucei*. These results support the view that the ancestral root of *T. evansi* is *T. brucei* [50–51]. The alignment of the amino acid sequences of the Egyptian horse *T. evansi* isolates based on ITS.1 revealed that the divergence was very low with other species and genotypes.

The positivity rates were evaluated across four diagnostic methods parasitological, CATT, ELISA, and PCR in 15 horse samples to provide a comprehensive analysis of these techniques. No clinical symptoms were observed in the examined horses, and all samples tested negative using wet and thin blood smears. However, many samples yielded positive results in the serological and PCR tests, indicating that the horses were likely in the early or chronic phases of infection [22]. The seroprevalence values obtained from the CATT/T. evansi were higher than those from PCR. This difference may be attributed to the potential for false-negative results in parasitological and molecular tests, particularly in animals with chronic illnesses, especially during fluctuating phases when parasites are present in very low numbers or absent from the blood. Additionally, false-positive results in serological tests can occur, as antibodies may persist for several months after animals have been successfully treated with trypanocides [15].

Conclusion

This study identifies the Giza and Cairo districts as high-risk areas for surra and suggests that CATT/ *T. evansi* could be the preferred field test for surveying horses. However, the status of seropositive animals should be confirmed using more sensitive molecular tools. The widespread presence of anti-*Trypanosoma* antibodies highlights the need for control measures and further investigation into multiple animal species, particularly camels in the same area, to determine their potential role as reservoirs to safeguard the health and welfare of our horses.

Acknowledgments

Not appropriate.

Funding statement

This study didn't obtain any funding provision.

Declaration of Conflict of Interest

The authors affirm that there is no conflict of interest.

Ethical statement

The Ethical Research Committee of the Animal Health Research Institute (AHRI), Agricultural Research Center (ARC) approved the animal manipulation and sample collection methods used in this study. (Ethics agreement number; 2525).

TABLE 1. Oligonucleotide primers sequences.

Target gene	Primer sequence (5'-3')	*Trypanosoma* spp.	Length of amplified product (bp)	Reference
Trypanosoma ITS1	CCGGAAGTTCACCGATATTG TTGCTGCGTTCTTCAACGAA	*T. evansi*	467	[18]
		T. brucei	480	
		T. vivax	250	
		T. godfrey	300	
		T. simae	400	
		T. congolense savanna	*700*	
		T. simaetsayo	370	

TABLE 2. Results of the CATT/*T. evansi* examination in horses.

Governorate	Positive		
	No. of strong agglutination (%)	No. of moderate agglutination (%)	No. of Negative agglutination (%)
Cairo	6/158 (3.79)	33/158 (20.88)	119/158 (75.31)
Giza	10/180 (5.55)	40/180 (22.22)	130/180 (72.22)
Total	16 (4.73)	73 (21.59)	249 (73.66)

TABLE 3. Comparison of parasitological examination, CATT/ *T. evansi*, and ELISA.

Governorate	Results					
	Parasitological examination		CATT/*T. evansi*		ELISA	
	Positive	Negative	Positive	Negative	Positive	Negative
Cairo	0/158	158/158	39/158 (24.68%)	119/158 (75.31%)	6/50 (12%)	44/50 (88%)
Giza	0/180	180/180	50/180 (27.77%)	130/180 (72.22%)	10/50 (20%)	40/50 (80%)
Total	0/338	338/338	89/338 (26.33%)	249/338 (73.66%)	(16%) 16/100	84/100 (84%)

TABLE 4. Cross tabulation of the CATT -*T. evansi* and ELISA results based on 100 samples

Discordance between CATT -*T. evansi* and ELISA			ELISA Negative	ELISA Positive	Total
CATT-*T. evansi*	**Negative**	Count	11	0	11
		% within CATT *T. evansi*	100	0.0	100
	Moderate Agglutination	Count	73	0	73
		% within CATT *T. evansi*	100	0.0	100
	Strong Agglutination	Count	0	16	16
		% within CATT *T. evansi*	0.0	100	100
	Total	Count	84	16	100
		% within CATT *T. evansi*	84	16	100

TABLE 5. The identity % between Egyptian horse *T. evansi* identified isolate and the nearest reference *Trypanosoma* spp. on GenBank

No	Accession number	Species/isolate	Host	Location	Identity%	References
1	**Mn121260**	*T. evansi*	buffalo	Thailand	99.4	[45] GenBank
2	**JN896754**	*T. evansi*	Camel	Iran	99.4	[46]
3	**FJ712711**	*T. evansi*	Buffalo	China	98.9	[47]
4	**HQ593644**	*T. evansi*	Buffalo	Philippines	98.9	[48]
5	**HQ593645**	*T. evansi*	Buffalo	Philippines	98.9	[48]
6	**HQ593646**	*T. evansi*	Buffalo	Philippines	98.9	[48]
7	**HQ593642**	*T. evansi*	Buffalo	Philippines	98.9	[48]
8	**DQ472684**	*T. evansi*	Buffalo	Thailand	99.4	[52]
9	**DQ472685**	*T. evansi*	Buffalo	Thailand	98.9	[52]
10	**OQ376675**	*T. evansi*	Buffalo	India	99.1	[53]
11	**MW603778**	*T. evansi*	camel	Egypt	99.7	[44]
12	**MW603773**	*T. evansi*	camel	Egypt	99.4	[44]
13	**MW603779**	*T. evansi*	camel	Egypt	99.7	[44]
14	**MW617067**	*T. evansi*	camel	Egypt	99.4	[44]
15	**kx900449**	*T. evansi*	camel	Iran	98.9	[54] GenBank
16	**JN896755**	*T. evansi*	Camel	Iran	98.6	[46]
17	**KR858268**	*T. evansi*	camel	India	98.6	[55]
18	**LC546905**	*T. evansi*	Buffalo	Philippines	98.6	[56] GenBank
19	**LC521916**	*T. evansi*	goat	Philippines	98.6	[56] GenBank
20	**LC386054**	*T. equiperdum*	Equine	Mongolia	95.2	[49] GenBank
21	**LC386053**	*T. equiperdum*	Equine	Mongolia	94.9	[49] GenBank
22	**LC386056**	*T. equiperdum*	Equine	Mongolia	95.2	[49] GenBank
23	**AC159414**	*T. brucei*	Unknown	Unknown	95.5	[57] GenBank
24	**AC159412**	*T. brucei*	Unknown	Unknown	95.5	[57] GenBank

TABLE 6. Comparison of the positivity rates between parasitological examination, CATT/*T. evansi*, ELISA, and PCR in 15 horse samples

No of samples	Parasitological examinations	CATT/*T. evansi*	ELISA	PCR
5	0/5	5/5++	5/5	5/5
5	0/5	5/5 +	0/5	1/5
5	0/5	0/5	0/5	1/5
Total	0/15(0%)	10/15(66.6%)	5/15(33.3%)	7/15(46.6%)

a **b** **c**

Fig.1. Result of CATT/*T. evansi* in horses a) strong-agglutination (++), b) moderate-agglutination (+) and c) negative.

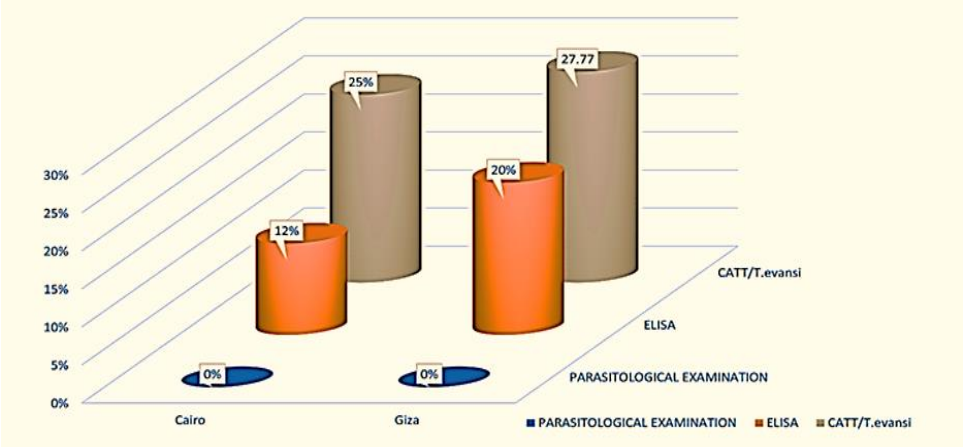

Fig.2. Comparison of parasitological examinations, CATT/ *T. evansi* and ELISA.

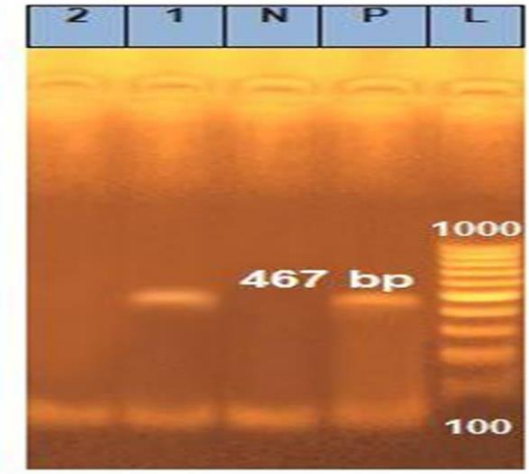

Fig.3 Agarose gel electrophoresis (1.5%) of amplified PCR products from Egyptian horse stained with ethidium bromide showing successful 467-bp DNA fragment compared with 100-bp DNA ladder lane 1: positive control; lane 2: negative control; lane 3 natural positive infected samples; lane 4 negative samples.

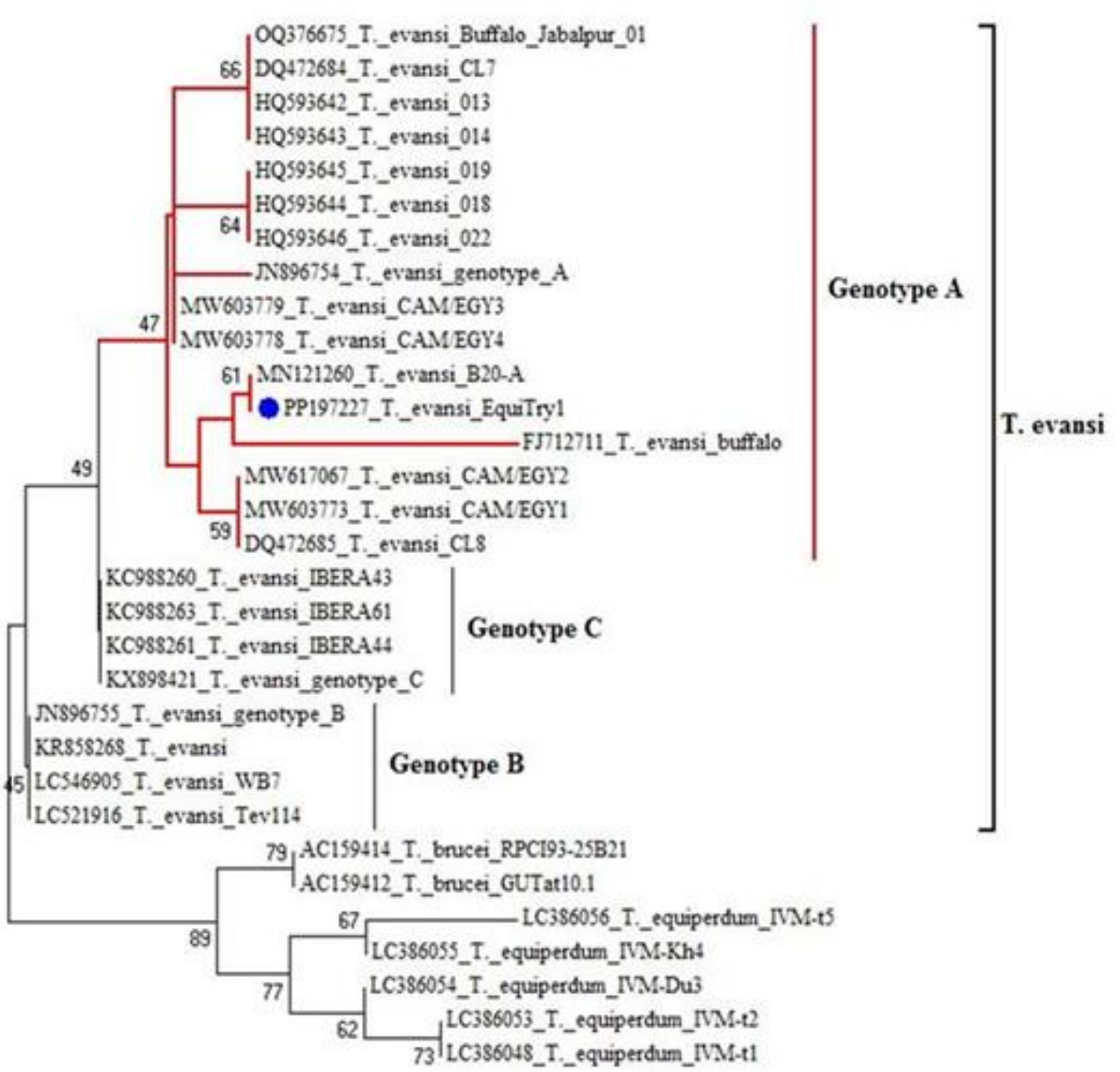

Fig. 4. Phylogenetic analysis of Egyptian horse *T. evansi* isolates of the present study with other *T. evansi* isolates sequences collected from GenBank based on 467 bp of the ITS-1 gene sequenced. The sequence obtained in this study is highlighted with a blue square.

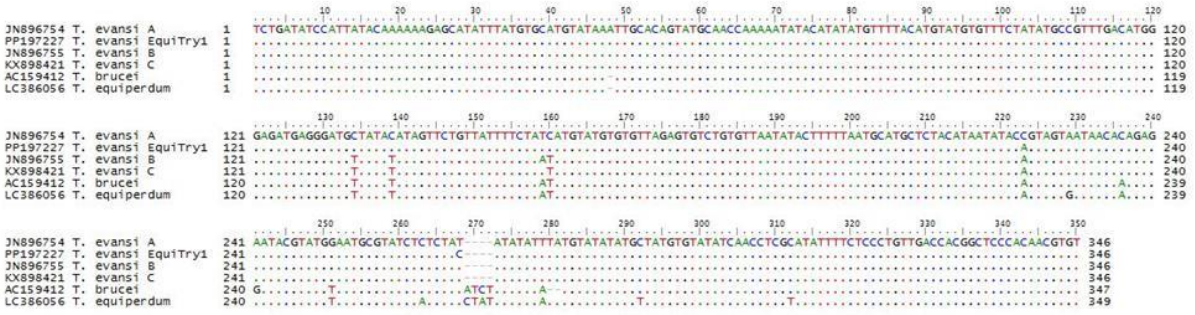

Fig. 5. Multiple sequence alignments of *T. evansi* genotypes (A, B &C), *T. brucei*, *T. equiperdium* and *T. evansi equi* Try1.

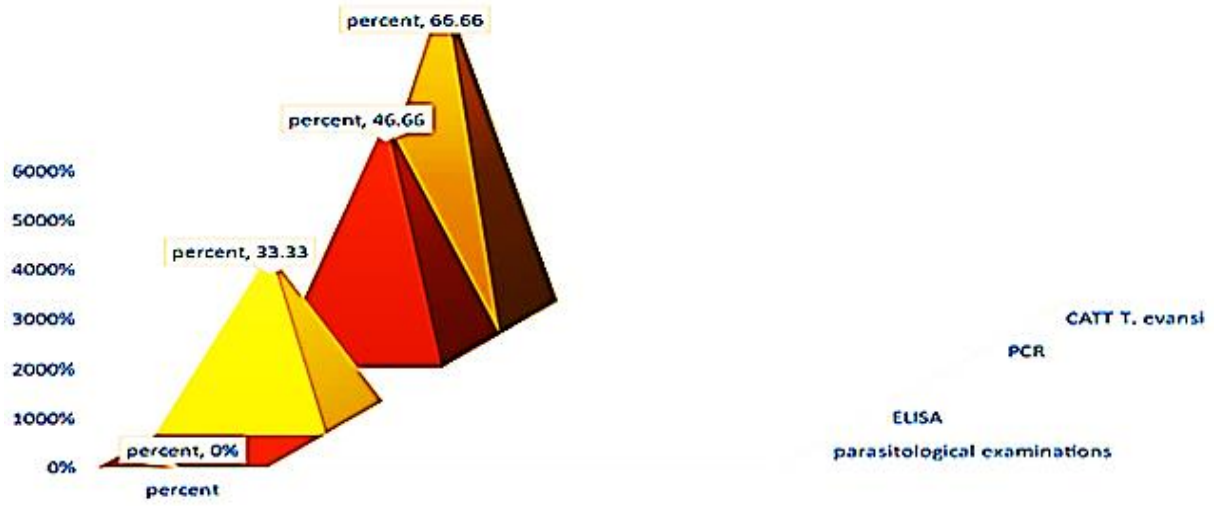

Fig. 6 The positivity rates between microscopical examinations, CATT/*T. evansi*, ELISA and PCR.

References

1. Onmaz, A.C., Beutel, R.G., Schneeberg, K., Pavaloiu, A.N., Komarek, A. and Van den Hoven, R. Vectors and vector-borne diseases of horses. *Veterinary Research Communications*, **37**(1), 65-81 (2013).

2. Brun, R., Hecker, H. and Lun, Z.R. *Trypanosoma evansi* and *T. equiperdum*: distribution, biology, treatment and phylogenetic relationship (a review). *Veterinary Parasitology*, **79**, 95-107 (1998).

3. Gutierrez, C., Desquesnes, M., Touratier, L. and Büscher, P. *Trypanosoma evansi*: recent outbreaks in Europe. *Veterinary Parasitology*, **174**, 26–29 (2010).

4. Desquesnes, M., Holzmuller, P., Lai, D.H., Dargantes, A., Lun, Z.R. and Jittaplapong, S. *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomedical Research International*, (2013). Doi: 10.1155/2013/194176. Epub 2013 Aug 19.

5. Büscher, P., Gonzatti, M.I., Hébert, L., Inoue, N., Pascucci, I., Schnaufer, A., Suganuma, K., Touratier, L. and Van Reet, N.. Equine trypanosomosis: enigmas and diagnostic challenges. *Parasites and Vectors*, **12**, 234 (2019).

6. Javanshir, A., Tavassoli, M. and Esmaeilnejad, B. Morphological, serological, molecular detection and phylogenetic analysis of *Trypanosoma evansi* in horses of different regions in Iran. *Parasitology Research*, **122**(8), 1873-1881 (2023).

7. Mohd Rajdi, N.Z.I., Mohamad, M.A., Tan, L.P., Choong, S.S., Reduan, M.F.H., Hamdan, R.H. and Zalati, C.W.S. First case report on the occurrence of *Trypanosoma evansi* in a Siam B Mare in Kelantan, Malaysia. *Veterinary Medicine Science*, **7**(2), 303–309 (2021). Doi: 10.1002/vms3.379. Epub 2020 Nov 7. PMID: 33161648; PMCID: PMC8025636.

8. Stephen, L.E. Trypanosomiasis: a veterinary perspective. 414–551 (1986).

9. Rodrigues, A., Fighera, R.A., Souza, T.M., Schild, A.L. and Barros, C.S. Neuropathology of naturally occurring *Trypanosoma evansi* infection of horses. *Veterinary Pathology*, **46**(2), 251–258 (2009). doi: 10.1354/vp.46-2-251. PMID: 19261636.

10. Laha. R., Bera, A.K., Panja, P. and Sikdar, A. Clinical and haemato-biochemical studies in natural trypanosomosis of equines. *Indian Journal of Animal Sciences*, **74**, 339–340 (2004).

11. Tehseen, S., Jahan, N., Desquesnes, M., Shahzad, M. I. and Qamar, M. F. Field investigation of *Trypanosoma evansi* and comparative analysis diagnostic tests in horses from Bahawalpur, Pakistan. *Turkish Journal of Veterinary & Animal Sciences*, **41**(2), 20 (2017).

12. Pathak, K. and Kapoor. M. Transplacental transmission of *Trypanosoma evansi* in a donkey. *Indian Veterinary Journal*, **76**, 179 (1999).

13. Silva, R.A.M.S., Arosemena, N.A.E., Herrera, H.M., Sahib, C.A., and Ferreira, M.S.J. Outbreak of trypanosomosis due to *Trypanosoma evansi* in horses of Pantanal Mato-grossense, Brazil. *Veterinary Parasitology*, **60** (1-2), 167–171 (1995).

14. WOAH. *Trypanosoma evansi* infection (Surra) (Chapter 2.1.21). In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Paris, France*, 314-328 (2012).

15. WOAH. Terrestrial Manual Chapter 3.1.21. Surra in all species (*Trypanosoma evansi* infection). (2021).

16. Thabet, N.F., Kandil, O.M. and Fararh, K.M. E. Impact of *Theileria equi* Infection on Arabian Stallion Fertility: Serological and Hormonal Perspectives. *International Journal of Veterinary Science*, **13**(6), 1002-1008 (2024).

17. Bajyana, S.E. and Hamers, R. A. card agglutination test (CATT) for veterinary use based on an early VAT RoTat 1-2 of *Trypanosoma evansi*. *Annual Society Belgium Medicine Tropical*, **68**, 233–240 (1988).

18. Musaya, J. Chisi, J. Senga, E. Nambala, P. Maganga, E. Matovu, E. and Enyaru, J. Polymerase chain reaction identification of *Trypanosoma brucei rhodesiense* in wild tsetse flies from Nkhotakota Wildlife Reserve, Malawi. *Malawi Medical Journal*, **29** (1), 5-9 (2017).

19. Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.*, **215**(3), 403-410 (1990).

20. Thompson, J.D., Higgins, D.G. and Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. *Nucl. Acids Res*., **22**(22), 4673-4680 (1994).

21. Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol*., **30**(12), 2725-2729 (2013).

22. Sawitri, D. H. and Wardhana, A. H. Detection of Trypanosoma evansi in healthy horses, cattle and buffaloes in East Sumba: eight years after outbreak. *IOP Conf. Ser.: Earth Environ*, (2024).

23. Nurcahyo, W., Yowi, M.R.K., Hartati, S. and Prastowo, J. The prevalence of horse trypanosomiasis in Sumba Island, Indonesia, and its detection using card agglutination tests. *Veterinary World*, **12**(5), 646-652 (2019).

24. Abdel-Gawad, A., Nassar, A. Abdel-Wahab, A. Evaluation of the Card Agglutination Test (CATT/T. Evansi) in comparison with PCR for detection of Trypanosoma evansi infection in donkeys in Egypt. *Journal of the Egyptian Society of Parasitology*, **49**(3), 539–542 (2019).

25. Benfodil, K., Ansel, S., Mohamed-Cherif, A. and Ait-Oudhia, K. Prevalence of Trypanosoma evansi in horses (Equus caballus) and donkeys (Equus asinus) in El-Bayadh district, southwestern Algeria. *Journal of the Hellenic Veterinary Medical Society*, **70**(3), 1631–1638 (2019).

26. Basima, A. A., Wisam, S. K. and Osama M.A. Electrocardiographic Changes Associated with Trypanosomiasis in Horses. *Egyptian Journal of Veterinary Sciences*, **55**(2), 345-357 (2024).

27. Zahoor, J. M., Kashif, A., Nasir, M., Bakhsh, M.F., Qamar, A. and Sikandar, A. R. Molecular detection and therapeutic study of Trypanosoma brucei evansi from naturally infected horses in Punjab, Pakistan. Polish *Journal of Veterinary Sciences*, **25**(3), 429–435 (2022).

28. Aslam, A., Chaudhary, Z.I., Ur Rehman, H., Ashraf, K., Ahmad, N., Yaqub, T., Maqbool, A. and Shakoori, A.R. Comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural trypanosomal infection in equines. *Pakistan J Zool*., **42**, 371-376 (2010).

29. Pinchbeck, G.L., Morrison, L.J., Tait, A., Langford, J., Meehan, L., Jallow, S., Jallow, J., Jallow, A. and Christley, R.M. Trypanosomosis in The Gambia: prevalence in working horses and donkeys detected by whole genome amplification and PCR, and evidence for interactions between trypanosome species. *BMC Vet Res*., **4**, 7 (2008).

30. Abo-Shehada, M.N., Anshassi, H., Mustafa, G. and Amr, Z. Prevalence of Surra among camels and horses in Jordan. *Prev Vet Med*., **38**, 289–293 (1999).

31. Laha, R., and Sasmal, N.K. Endemic status of Trypanosoma evansi infection in a horse stable of the eastern region of India – a field investigation. *Trop Anim Health Prod*., **40**, 357–361 (2008).

32. Gari, F.R., Ashenafi, H., Tola, A., Goddeeris, B.M. and Claes, F. Comparative diagnosis of parasitological, serological, and molecular tests in dourine-suspected horses. *Tropical Animal Health and Production*, **42**, 1649-1654 (2010).

33. Benfodil, K., Büscher, P., Abdelli, A., Van Reet, N., Mohamed-Sherif, A., Ansel S, et al. Comparison of serological and molecular tests for detection of *Trypanosoma evansi* in domestic animals from Ghardaïa district, South Algeria. *Vet. Parasitol*., **1**, 280:109089. (2020).

34. Elshafie, E.I., Sani, R.A., Hassan, L., Sharma, R., Bashir, A. and Abubakar, I.A. Seroprevalence and risk factors of *Trypanosoma evansi* infection in horses in Peninsular Malaysia. *Res. Vet. Sci*, **94**, 285-289 (2013).

35. Dewi, R.S., Wardhana, A.H., Soejoedono, R.D. and Mulatsih, S. Evaluation of Surra treatment strategies for horses and buffaloes in East Sumba District, Nusa Tenggara Timur Province of Indonesia (2010-2016). *Jurnal IImu Ternak dan Veteriner*, **4**(1), 39-48 (2019).

36. Tong Q., Chen, R., Kong, Q., Goossens, J., Radwanska, M., Lou, D., Ding, J., Zheng, B., Fu, Y., Wang T., Stefan, M. and ShaoHong, L. DNA Detection of *Trypanosoma evansi*: Diagnostic validity of a new assay based on loop-mediated isothermal amplification (LAMP). *Vet. Parasitol.*, **250**, 1–6 (2018). doi: 10.1016/j.vetpar.2017.12.006.

37. Ramírez-Iglesias, J.R., Eleizalde, M.C., Gómez-Piñeres E. and Mendoza M. *Trypanosoma evansi*: A comparative study of four diagnostic techniques for trypanosomosis using rabbit as an experimental model. *Exp. Parasitol*., **128**, 91–96 (2011).

38. WOAH. Compendium of Standard Diagnostic Protocols for Animal Trypanosomoses of African Origin. (2017).

39. Elhaig, M.M., Sallam, N.H. Molecular survey and characterization of *Trypanosoma evansi* in naturally infected camels with suspicion of a *Trypanozoon* infection in horses by molecular detection in Egypt. *Microb. Pathog*., **123**, 201–205 (2018).

40. Muieed, M.A., Chauhdry, Z.I. and Shakoori, A.R. Comparative studies on the sensitivity of polymerase chain reaction (PCR) and microscopic examination for the detection of *Trypanosoma evansi* in horses. *Turk. J. Vet. Ani. Sci.*, **34**, 507-512 (2010).

41. Salim, B. Mohammed, A.B. and Sugimoto, C. Molecular detection of equine trypanosomes in the Sudan. *Veterinary Parasitology*, **200**, 246-250 (2014).

42. Selim, A., Alafari, A., Attia, K., AlKahtani, M.D.F., Albohairy, F.M. and Elsohaby, I. Prevalence and animal level risk factors associated with *Trypanosoma evansi* infection in dromedary camels. *Sci Rep*. **27**, **12**(1), 8933 (2022). doi: 10.1038/s41598-022-12817-x. PMID: 35624132; PMCID: PMC9142598.

43. Elhaig, M.M., Youssef, A.I. and El-Gayarc, A.K. Molecular and parasitological detection of *Trypanosoma evansi* in camels in Ismailia, Egypt. *Vet. Parasitol*, **198**, 214–218 (2013).

44. Alfaleh, F.A. and Elhaig, M.M. Molecular prevalence, associated risk factors and genetic characterization of *Trypanosoma evansi* in camels. *Microb. Pathog*., **175**, 105967 (2023).

45. Saiyasombat, R., Sajjapongse, S., Mohkaew, K., Jiratanh, M. and Thiptara, A. Comparing genetic characteristics of Internal transcribed spacer (ITS) regions of *Trypanosoma evansi* infecting rusa deer (Cervustimorensis) and water buffalo (*Bubalus bubalis*) in Thailand, unpublish, GenBank. (2019).

46. Pourjafar, M., Badiei, K., Sharifiyazdi, H., Chalmeh, A., Naghib, M., Babazadeh, M., Mootabi Alavi, A., Hosseini Joshani-Zadeh, N. Genetic characterization and phylogenetic analysis of *Trypanosoma evansi* in Iranian dromedary camels. *Parasitol. Res*., **112** (2), 899-903 (2013). Doi: 10.1007/s00436-012-3121-5. Epub 2012 Sep.

47. Tian, Z., Liu, G., Xie, J., Shen, H., Zhang, L., Zhang, P. and Luo, J. The internal transcribed spacer 1 (ITS-1), is a controversial marker for the genetic diversity of Trypanosoma evansi. *Exp. Parasitol*., **129**(3), 303–306 (2011).

48. Villareal, M., Mingala, C. and Rivera, W. Molecular characterization of *Trypanosoma evansi* isolates from water buffaloes (*Bubalus bubalis*) in the Philippines. *Acta Parasitologica*, **58** (1), 6-12 (2013). https://doi.org/10.2478/s11686-013-0110-5.

49. Suganuma, K., Naransatsral, S., Batter, B., Yamasaki, S., Otgonsuren, D., Musinguzi, P., Davaasuren, B., Battsetseg, B. and Noboru, I. Isolation, cultivation, and molecular characterization of a new *Trypanosoma equiperdum* strain in Mongolia (GenBank). (2018).

50. Carnes, J., Anupama, A., Balmer,O., Jackson, A., Lewis, M., Brown, R., Cestari, I., Desquesnes, M., Gendrin, C., Hertz-Fowler,C., Imamura, H., Ivens, A., Kořený, L., Lai, D., MacLeod, A., McDermott, S.M., Merritt, C., Monnerat, S., Moon, W., Myler, P., Phan, I., Ramasamy, G., Sivam, D., Lun, Z., Lukeš, J., Ken Stuart, K. and Schnaufer, A. Genome and phylogenetic analyses of *Trypanosoma evansi* reveal extensive similarity to *T. brucei* and Multiple Independent Origins for Dyskinetoplasty. *PLoS Neglected Tropical Diseases*, **9** (1), e3404 (2015). doi:10.1371/journal. and.0003404 [PMC free article] [PubMed].

51. Metwally, D.M., Al-Turaiki, I.M., Altwaijry, N., Alghamdi, S.Q. and Alanazi, A.D. Molecular identification of *Trypanosoma evansi* isolated from Arabian camels (*Camelus dromedarius*) in Riyadh and Al-Qassim, Saudi Arabia. *Animals*, **11**, 1149 (2021). https://doi.org/ 10.3390/ani11041149.

52. Khuchareontaworn, S., Singhaphan, P., Viseshakul, N. and Chansiri K. Genetic diversity of *Trypanosoma evansi* in buffalo based on internal transcribed spacer (ITS) regions. *Journal of Veterinary Medical Science*, **69**, 487–493 (2007). DOI: 10.1292/jvms.69.487.

53. Verma, R., Punia, V., Das, G., Kumar, S., Nath, S. and Swamy, M. Assessment of genetic diversity of *Trypanosoma evansi* in the domestic animal populations through ITS-1 gene sequence analysis. *Parasitology Research*, **123**(1) (2024). doi: 10.1007/s00436-023-08024-w. PMID: 38047956.

54. Zanguee, F. and Ganjali, M. Trypanosoma evansi 18S ribosomal RNA gene of camel in Iran. GenBank. (2016).

55. Sarkhel, S.P., Gupta, S.K., Kaushik, J., Singh, J., Gaur, D.K., Kumar, S. and Kumar R. Molecular characterization of the internal transcribed spacer 1 (ITS 1) region of different *Trypanosoma evansi* isolates of India. *J Parasit Dis*., **41**(2), 527-533 (2017). Doi: 10.1007/s12639-016-0843-9. Epub 2016 Sep 29. PMID: 28615873; PMCID: PMC5447620.

56.Suganuma, K., Elata,A. and Mossaad, E. Epidemiological studies on trypanosomosis in different animal species in the Visayas region of the Philippines. Unpublished, GenBank. (2020).

57. Ghedin, E., Blandin, G., Bartholomeu, D., Caler, E., Haas, B., Hannick, L., Shallom, J., Hou, L., Djikeng, A., Feldblyum, T., Hostetler, J., Johnson, J., Jones, K., Koo, H.L., Larkin, C., Pai, G., , J., Khalak, H.G., Salzberg, S., Simpson, A.J., Peterson Tallon, L., Van Aken, S., Wanless, D., White, O., Wortman, J., Fraser, C.M. and El-Sayed, N.M.A. Direct submission to GenBank with no title (unpublished) (2005).

نظرة عامة عن مرض السرا (التريبانوسوما ايفانساى) فى الخيول

أسماء محمد كمال النادى[١]، علياء عبدالرحمن عبدالله بالغ[١]، أسماء عبدالودود محمد حجاب[١]، إيمان أحمد محمد القلش[١]، سحر علوان رزق سبع[١] و ممتاز عبدالهادى عفيفى شاهين[٢]

[١] قسم الطفيليات، معهد بحوث الصحة الحيوانية، مركز البحوث الزراعية، الجيزة، مصر.

[٢] قسم الفيروسات، معهد بحوث الصحة الحيوانية، مركز البحوث الزراعية، الجيزة، مصر.

الملخص

تسبب التريبانوسوما ايفانساى مرضا شائعا يعرف بالسرا، وهو واحد من أكثر الأمراض الطفيلية المؤثرة إقتصاديا والتى تصيب الحيوانات فى مصر وبخاصة الفصيلة الخيلية والجمال. هدفت هذه الدراسة تحديد مدى إنتشار مرض السرا بالطرق التشخيصية المختلفة والتى تشمل الفحص الميكرسكوبى، إختبار التلازن بالبطاقة، الإليزا والتقنيات الجزيئية. تم تجميع عدد ٣٣٨ عينة دم من ١٥٨حصان من محافظة القاهرة و ١٨٠ حصان من محافظة الجيزة. بالفحص الميكرسكوبى لمسحات دموية غيرمصبوغة ومصبوغة بصبغة الجيمسا، تبين سلبية جميع العينات لطفيل التريبانوسوما ايفانساى. بإجراء إختبار التلازن بالبطاقة، ثبتت إيجابية ٣٣,٢٦% من الحيوانات لطفيل التريبانوسوما ايفانساى والتى تراوحت درجة التلازن فيما بينهم بين ٤,٧٣% شديد التلازن و ٥٩,٢١% متوسط التلازن. تم إجراء إختبار الإليزا على عدد ١٠٠ عينة سيرم خيول وثبتت إيجابية ١٦% منها. تم إستخدام إختبار تفاعل البلمرة المتسلسل للجين ITS-1 وثبتت إيجابية ٧عينات لطفيل التريبانوسوما ايفانساى عند الحزمة الخاصة بالتريبانوسوما ايفانساى (٤٦٧). كشف فحص الشجرة الجينية أن العترة المعزولة للطفيل تنتمى للطرز الجينى A للتريبانوسوما ايفانساى والذى يتقارب جدا مع العترة الموجودة فى الجاموس فى تايلاند. كشف التعدد النيوكلوتيدى للعترة المعزولة من طفيل التريبانوسوما ايفانساى من الخيول المصرية عن تقارب كبير يتراوح بين ٩,٩٤% و ٧,٩٩% مع الأنواع الأخرى للتريبانوسوما المسجلة فى بنك الجينات.

كشف التعدد النيوكلوتيدى عن تشابه بنسبة ٧,٩٩% بين العترة المعزولة من الخيول ومثيلتها فى الجمال المصرية. بالإضافة إلى ذلك، فلقد سجلت نسبة إيجابية كبيرة بإستخدام إختبار التلازن بالبطاقة بما يؤكد أن هذا الإختبار فعال فى الكشف الدورى عن مرض السرا فى الخيول.

الكلمات الدالة: مدى إنتشار، إختبار التلازن بالبطاقة، الإليزا، تفاعل البلمرة المتسلسل، تحليل الشجرة الجينية.