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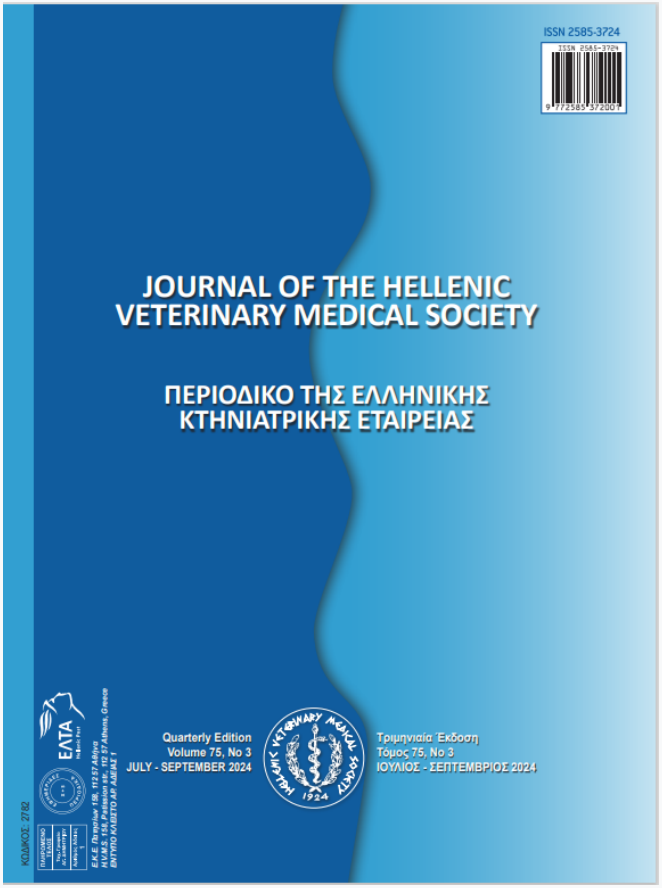

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Research article
Ερευνητικό άρθρο

Seroprevalence and associated risk factors for *Trypanosoma evansi* infection in equine in North of Egypt

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ABSTRACT: This study determined the seroprevalence of *Trypanosoma evansi* infection and its associated risk factors in 200 equines across four Egyptian governorates based on CATT/*T. evansi*. Serological testing revealed *T. evansi* antibodies in 34.5% of the examined animals, exhibiting notable discrepancies among different governorates. Dakahlia exhibited the highest seroprevalence at 42.4%, while the lowest seroprevalence rate (12.5%) was reported in Gharbia. No significant differences were observed in seroprevalence among age groups or equine species. The multivariate regression analysis model identified pivotal risk factors, wherein the presence of flies increasing infection odds threefold and animals lacking routine insecticide treatment having four times higher odds of infection. Equines in poor health condition displayed double the odds of infection. Surprisingly, a history of venereal disease increased the odds of *T. evansi* infection. Notably, there were no substantial differences in infection rates based on the disposal of used syringes or prior mange history. These findings provide essential insights into the transmission patterns of *T. evansi*. Gaining a comprehensive understanding of these identified risk factors is crucial for developing effective methods to reduce the prevalence of *T. evansi* in populations of equids.

Keywords: *T. evansi;* Card agglutination test; venereal diseases; risk factors

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INTRODUCTION

Equine includes horses, donkeys, and mules, play pivotal roles in various socioeconomic domains worldwide. Their contributions are diverse and impactful, extending across agriculture, transport, and traction activities. Equids have historically been indispensable for plowing fields, transporting goods, and serving as work animals in various agricultural settings (de Aluja 1998, Haddy et al., 2020). Owing to their significance, efforts have refocused on tackling infectious diseases that could potentially compromise these animals' welfare and productivity (Stringer 2014, Stringer et al., 2015).

Trypanosomes are unicellular extracellular flagellate protozoa within the family *Trypanosomatidae* and the genus *Trypanosoma* (Maslov et al., 2019). Trypanosomiasis is a vector-borne disease poses a significant threat on both animals and human health in tropical and subtropical countries including Egypt, causing substantial economic losses (Mulenga et al., 2020, Putt et al., 1980). Equine trypanosomiasis is a priority disease with a worldwide distribution that necessitates deliberate endeavours to enhance diagnostic procedures, refine management strategies, and develop new treatment modalities (Stringer 2014).

The disease is almost always fatal to horses. If affected horses are untreated, they can die within one week to six months. The two recognized types of *T. evansi* infections are acute and chronic (Nurcahyo et al., 2019). The clinical signs of horse trypanosomiasis include intermittent fever, severe weight loss, progressive weakness, anemia, hemoglobinuria, petechial hemorrhage of mucous membranes, ventral and genital oedema, urticarial plaques, conjunctivitis and keratitis and sever neurological signs (Büscher et al., 2019). Common neurological symptoms of *T. evansi* infection include ataxia and paralysis of the hind quarter and lips usually precede that results death of animal (Aregawi et al., 2019, Ranjithkumar et al., 2014).

Serological agglutination tests have proven effective in diagnosing and studying the prevalence of many diseases in multiple animal species (Laha and Sasmal 2008, Singla et al., 2015). The card agglutination test for *T. evansi* is currently considered to be the standard serum agglutination test endorsed by the World Organization for Animal Health (OIE) for detecting antibodies against *T. evansi* (Reck et al., 2021, Selim et al., 2022a). This test mostly detects IgM antibodies, and the antigen consists of fixed and dyed bloodstream-form *trypanosomes* of the variable surface antigen strain designated as RoTat1.2 (Algehani et al., 2021, Reck et al., 2021).

Many of previous studies evaluate the associated risk factors for *Trypanosoma* infection in horses such as sex, breed, animal species, body condition score (BCS), management system, and age (Elshafie et al., 2013, Raftery et al., 2019, Sumbria et al., 2017). Furthermore, the presence of vectors is influenced by the habitat and seasonality, which are additional risk factors for disease transmission (Golombieski et al., 2023, Yamazaki et al., 2022).

This study aimed to determine the prevalence of *T. evansi* in equines across four Egyptian governorates and assess the associated risk factors for *T. evansi* infection in equine.

MATERIAL AND METHODS

Study area

The study was conducted on equine raising in Four Egyptian governorates which geographically located at the Northern Egypt namely, Dakhalia (31.3° N 31.23° E), Qalyubia (30,41° N 31.21° E), Damiette (31.4° N 31.72° E) and Gharbia (30.86° N 31.02° E), Figure 1. The three governorates (Dakhalia, Qalyubia, and Gharbia) are located in Egypt's Nile Delta and have a hot desert climate (BWh) according to the Köppen climatic classification. This indicates that the region has long, hot summers and moderate winters with minimal rainfall all year. In addition, Damietta's climate is mainly Mediterranean, with dry summers and moderate dry winters. In addition, Damietta's climate is mainly Mediterranean, with dry summers and moderate dry winters.

Study design

A cross-sectional study was performed between June to December 2023, to determine the seroprevalence of *T. evansi* infection in equines in Egypt within the defined study area. The study involved 200 animals, consisting of 151 horses, 46 donkeys, and 3 mules, which were randomly selected from the studied areas. Data of each animal including in the study was collected at time of sampling via questionnaire including location, age (<2 years, 2-4 years and >4 years), presence of flies, use of insecticide, presence of wounds, syringe disposal practices, body condition, and history of venereal disease and mange infection.

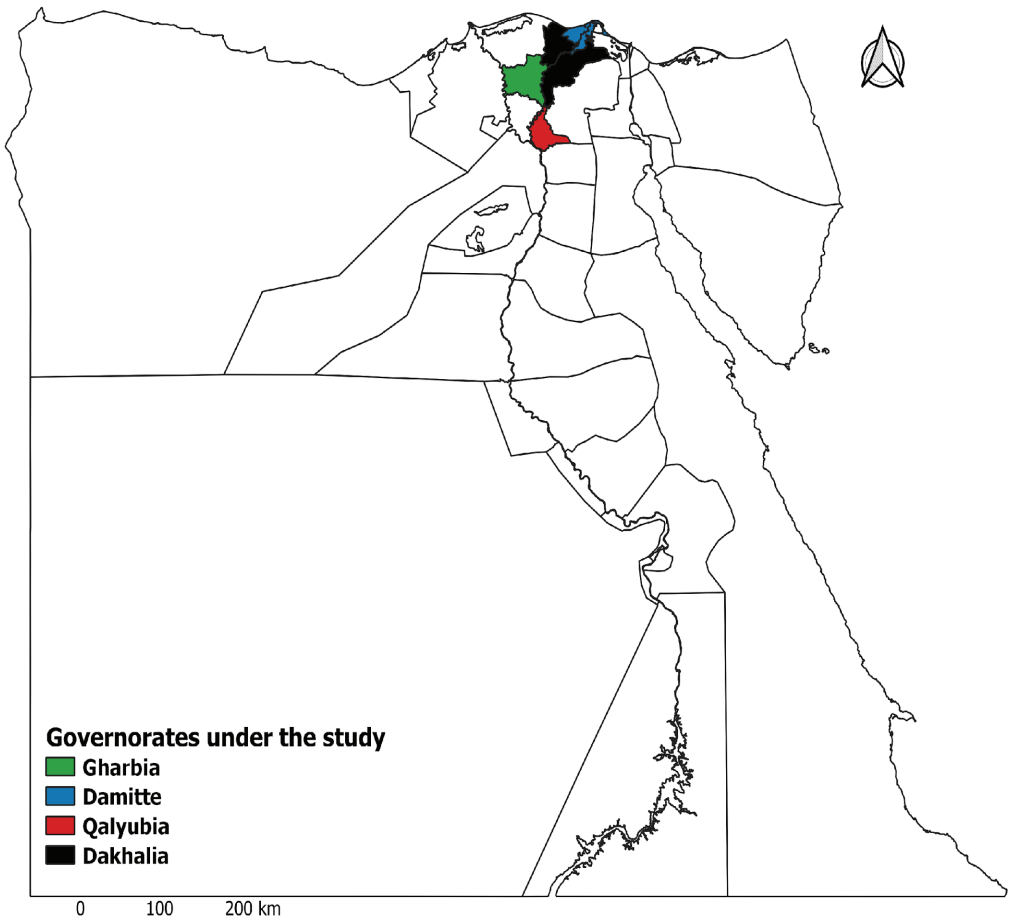

Figure 1: locations of the governorate under the study (MAP generated by QGIS software)

Sampling

Blood sample collection involved aseptically drawing about 5 mL of blood from the jugular vein of each animal, using vacutainer tubes. The sample was centrifuged at 3000 rpm for 10 min to separate the sera which stored at -20°C for subsequent serological examination. The collection of blood samples from animals was executed with meticulous care, and prior consent was obtained from the respective owners.

Card agglutination test (CATT)

T. evansi-specific antibodies were detected using CATT/T. evansi (CATT/*T. evansi*®; Institute of Tropical Medicine Antwerp, Belgium) in serum that had been pre-diluted at a ratio of 1:4 in CATT diluent, following the manufacturer's guidelines. In a concise procedure, 45 µl of the antigen reagent was applied to the card test and combined with 45 µl of equine test sera diluted to 1:4. The resulting mixture was evenly spread on the card using a clean stirring rod and allowed to react with manual rotation for 5 minutes. A positive reaction, indicative of the presence of *T. evansi*-specific antibodies, was visually confirmed by the appearance of blue granular deposits, discernible to the naked eye.

Statistical analysis

Data were analyzed statistically using SPSS software ver. 24 (IBM, USA). Chi square was used to analyse the relationship between seroprevalence of *T. evansi* and risk variables. The Results have *P* values less than 0.05 considered significant. All variables with a $P < 0.25$ in the univariate analysis were submitted to the multivariate logistic regression model to analyse the independent risk factors of each variable (Selim et al., 2022b, Selim et al., 2021c). The odds ratio (OR) and 95% confidence interval (CI) were calculated using multivariate logistic regression.

RESULT

In the present study the antibodies against *T. evansi* were detected in 69 (34.5%) of examined equines using CATT. The seroprevalence varied significantly

among the governorates ($P \leq 0.05$), where Dakahlia governorate had the highest seroprevalence rate of 42.4% (95% CI: 33.54-51%), and Gharbia governorates exhibited the lowest seroprevalence rates of 12.5% (95% CI 2.24-47.09%), Table 1.

Furthermore, there were no notable significant ($P>0.05$) variations in the seroprevalence of *T. evansi* among different age groups or species of equines. The seroprevalence rates for horses, donkeys, and mules were 31.1%, 43.5%, and 66.7%, respectively. The seroprevalence rates in equids were 34.8% for those < 2 years, 30.8% for those aged 2-4 years, and 36.0% for those >4 years.

In addition, the results reveled that the flies play an important role in spreading of *T. evansi* infection where the seroprevalence of *T. evansi* increased significantly in presence of flies (48.2%, 95%CI: 35.66-60.99) and in absence of use of insecticides (44.4%, 95%CI: 35.42-53.84). Interestingly, the body condition and history of venereal disease were significantly associated with *T. evansi* infection in equine, where it was higher in poor condition animals (49.3%, 95%CI: 37.65-60.93) and animals had history of venereal diseases (71.4%, 95%CI: 35.89-91.78), Table 1.

Table 1: Seroprevalence of *T. evansi* in equines in relation to different factors

Variable	Total examined animal	No of positive	%	95%CI	*P* value
Locality					
Dakhalia	118	50	42.4	33.54-51.00	0.002*
Qalyubia	32	12	37.5	22.93-54.75	
Damiette	42	6	14.3	6.72-14.29	
Gharbia	8	1	12.5	2.24-47.09	
Age					
<2	23	8	34.8	18.81-55.11	0.8
2 -4	52	16	30.8	19.92-44.27	
> 4	125	45	36.0	28.12-44.72	
Species					
Horse	151	47	31.1	24.29-38.91	0.151
Donkey	46	20	43.5	30.21-57.75	
Mule	3	2	66.7	20.77-93.85	
Presence of flies					
Yes	56	27	48.2	35.66-60.99	0.011*
No	144	42	29.2	22.36-37.06	
Use of insecticide					
Yes	92	21	22.8	15.45-32.39	0.001*
No	108	48	44.4	35.42-53.84	
Presence of wound					
Yes	57	17	29.8	19.53-42.66	0.294
No	143	52	36.4	28.93-44.51	
Syring disposal					
Yes	197	68	34.5	28.23-41.4	0.966
No	3	1	33.3	6.15-79.33	
Body condition					
Poor	67	33	49.3	37.65-60.93	0.002*
Good	133	36	27.1	20.24-35.19	
History of venereal disease					
Yes	7	5	71.4	35.89-91.78	0.036*
No	193	64	33.2	26.42-39.54	
History of manage					
Yes	11	3	27.3	9.74-56.56	0.604
No	189	66	34.9	28.49-41.96	
Total	200	69	34.5	28.26-41.32	

*Result considered positive if $P<0.05$

Table 2: Multivariate logistic regression of risk factors associated with *T. evansi* infection in equines.

Variables	B[a]	S.E.[b]	OR[c]	95% CI[d] for OR		P value
				Lower	Upper	
Locality						
Dakhalia	1.987	1.329	7.30	0.54	98.65	0.135
Qalyubia	1.807	1.371	6.09	0.41	89.52	0.188
Damiette	0.528	1.403	1.70	0.11	26.52	0.706
Presence of flies						
Yes	1.095	0.365	2.99	1.46	6.11	0.003
Use of insecticides						
Yes	0.811	0.350	2.25	1.13	4.46	0.020
Body condition						
Poor	0.602	0.350	1.83	0.92	3.63	0.086
History of venereal						
Yes	2.218	1.184	9.19	0.90	93.62	0.061

Logistic regression coefficient
Standard error
Odds ratio
Confidence interval

The multivariate logistic regression analysis for the significant factors revealed that the odds of *T. evansi* infection in equines were seven times higher in Dakhalia governorate (OR= 7.29, 95%CI: 0.54-98.65), three times higher in presence of flies (OR=2.99, 95%CI: 1.46-6.11) and two times higher in case of insecticides use (OR= 2.25, 95%CI: 1.13-4.46). In addition, the probability of *T. evansi* infections increased significantly in poor conditioned animals (OR= 1.83, 95%CI: 0.92-3.63) and in animals have history of venereal disease (OR=9.19, 95%CI: .90-93-62), Table 2.

DISCUSSION

T. evansi infection has been reported in horses, mules and donkeys all over the world (Aregawi et al., 2019). It is currently classified as a new zoonotic parasite (Fong 2017). In Egypt, *T. evansi* infection have been reported among camels, cattle, buffaloes and donkeys (Elhaig and Sallam 2018, Selim et al., 2022a, Zayed et al., 2010) but the epidemiological data about the presence of infection in horses are very scarce. Therefore, the aim of present study was to determine the presence of antibodies against *T. evansi* in equine and evaluate the associate risk factor for the infection.

The current study demonstrated that the overall seroprevalence rate of *T. evansi* in equines cross the studied governorates was 34.5% by CATT/*T. evansi*, which was lower than those previous reported rates in seroprevalence in equines in El-Bayadh, Algeria district 47.6% (Benfodil et al., 2019) but higher than this reported among horses in Peninsular Malaysia 13.9% (Elshafie et al., 2013) and donkeys in Egypt 30.08% (Zayed et al., 2010). In addition, the seroprevalence rate of *T. evansi* was reported in other species in Egypt by CATT/*T. evansi,* it was 52.50% in buffaloes (Zayed et al., 2010), 18.9% to 39% in camels (Selim et al., 2022a, Zayed et al., 2010).

This difference in seroprevalence between various studies and countries may be attributed to the difference in the management and husbandry regimens of equines or differences in the sampled populations, variations in diagnostic methodologies (Elshafie et al., 2013, Hilali et al., 2004, Laha and Sasmal 2008, Marzok et al., 2023, Selim and Khater 2020, Selim et al., 2021a, Selim et al., 2021b, Sumbria et al., 2017).

Moreover, the results revealed significant variation in the seroprevalence rates across the studies governorates, where Dakhalia and Qalyubia governorates showed the significant higher seroprevalence than Damietta and Gharbia. These findings come in accordance with those found by Benfodil et al., (2019) who revealed that prevalence rate was higher in localities near to water source as Watering points and vegetation constitute a favorable environment to the survival of vectors. Environmentally, the Nile Delta region where Dakhalia and Qalyubia are located sees abundant water sources and vegetation that promote higher densities of tabanid flies, vectors known to transmit *T. evansi* mechanically between equids (Desquesnes et al., 2013).

Interestingly, the current study found no statistically significant differences in *T. evansi* seroprevalence between equine age groups or species which come in agreement with findings of Elshafie et al., (2013). The seropositivity rates were comparable across young (<2 years), adult (2-4 years) and old (>4 years) animals, ranging from 30.8% to 36%. The seropositivity rates were comparable across horses, donkeys and mules ranging from (31.1% to 66.7%) and this result was contrast to other results which revealed higher prevalence rate of infection in Algeria, Jordon and Egypt in horses and donkeys (Benfodil et al., 2019, Zayed et al., 2010).

Moreover, the present results aligned with previous studies which showed higher prevalence rate in donkey and mules (Sumbria et al., 2017), and these results was attributed to donkeys and mules are kept mainly outdoors under poor conditions during daily work, their chance of exposure to vectors increases, resulting in an increased risk of haemoparasitic infection in these species (Kouam et al., 2010, Sumbria et al., 2015). This lack of age-species predilection suggests that once *T. evansi* transmission is established within a population, all animals are equally susceptible regardless of their life stage or type. Further, horses, donkeys and mules exhibited similar seroprevalence without any species seeming particularly vulnerable to infection over others.

In this investigation, equids exhibiting poor body condition demonstrated an elevated susceptibility to *T. evansi* infection in comparison to those with good body conditions. Moreover, *T. evansi* was diagnosed in several horses with poor body condition using microscopy, serological and molecular techniques (Berlin et al., 2012). A parallel pattern was identified in camels within Egyptian governorates, as reported by Selim et al., (2022a). Also, comparable findings have been reported in Eastern Ethiopia and Nigeria (Fikru et al., 2015, Takeet et al., 2013). This association may be attributed to the interconnection between animal immunity and nutritional status, as elucidated in previous research (Eyob and Matios 2013). The compromised condition of equines with poor body condition may render them more susceptible to infection. Additionally, prior studies have established a correlation between *T. evansi* infection and clinical manifestations such as fatigue, wasting, and performance decline due to anemia (Podaliri Vulpiani et al., 2013).

The present study elucidates a noteworthy variation in the seroprevalence rate based on the utilization of insecticides and the presence or absence of flies. Specifically, the group characterized by the non-use of insecticides exhibited the highest prevalence of *T. evansi* infection at 44.4%, while the presence of flies correlated with a prevalence of 48.2% (Baldacchino et al., 2014). The observed higher prevalence in the absence of insecticide use and the presence of flies may be attributed to several factors. Firstly, inadequate insecticide application might result in ineffective control of the vector population, allowing for increased transmission of *T. evansi*. Additionally, the presence of flies in the environment can serve as mechanical vectors, contributing to the tramsmission of *T. evansi* such as *tabanids* and *Stomoxys* which considered the most important mode of transmission (Abera 2016, Algehani et al., 2021, Desquesnes et al., 2022, Radwan et al., 2022, Selim et al., 2021d).

The present study revealed a significant finding that animals with a confirmed history of venereal disease showed a sustained increase in the probability of being infected with *T. evansi*. This association suggests a possible connection between venereal diseases and *T. evansi* infection, supporting the concept that *T. evansi* may be transmitted through sexual means, as evidenced by Büscher et al., (2019). Moreover, we hypothesize that venereal diseases might create favorable conditions for *T. evansi* infection.

Our investigation has yielded a non-significant result regarding the presence of skin wounds or abrasions. This finding contradicts the proposed concept, which consider wounds as potential conduits for trypanosome transmission via biting insects as wounds could serve as attractive feeding locations for these insects (Desquesnes et al., 2013, Powar et al., 2006). Moreover, the present findings showed non - difference in infection rates associated with the disposal of used syringes (p=0.966). These findings contradict those presented by Dávila and Silva (2000), who suggested that the use of non-sterile surgical instruments or needles could play a role in the transmission of infection, particularly during vaccination campaigns and mass treatments.

CONCLUSION

This study highlights the prevalence of *T. evansi* as a serious parasite infection among equids in Egypt, with considerable geographical variations and recognized risk factors such as the presence of flies, the absence of insecticide use, poor physical health, and history of venereal disease. Based on these data,

it appears that focused insect management and comprehensive veterinarian care could be viable strategies for reducing the infection load caused by this zoonotic parasite. Additional sudies are essential to build upon these findings and establish effective, evidence-based control measures.

CONFLICT OF INTEREST

There are no conflicts of interest declared by the authors.

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