

## A Critical Comparative Study of Parasitological and Serological Differential Diagnostic Methods of *Trypanosoma evansi* Infections in Some Farm Animals in Egypt

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**Abstract:** *Trypanosoma evansi* is the most widely geographically distributed pathogenic flagellated haemoprotozoan parasite. However, there is little information about the detection of this blood parasite in equines and bovines in Egypt. The present work aimed at reaching a comparative qualitative analysis of the different parasitological (wet blood smears technique, field stain blood film, haematocrit centrifugation and mouse subinoculation) and serological (CATT and ELISA) diagnostic techniques applied in the detection of *T. evansi* infection at both the individual and farm levels. A total number of 381 blood samples were collected during the period from September 2005 to February 2007, from abattoirs of Giza and Cairo governorates as well as the Giza Zoo abattoir. ELISA revealed that the highest seasonal incidence of infection was in autumn, spring and summer for camels, buffaloes and donkeys, respectively. CATT test detected most seropositive cases at grade 1 whereas out of the 155 positive cases 103 (66.5%) revealed grade 1, followed by 37 (23.9%) grade 2 and 15 (9.6%) grade 3. Generally, the higher seropositive rate against *T. evansi* was observed during autumn among both camels and donkeys. However, the lower rates were noticed during spring among both camels and donkeys. Otherwise, the highest rate was shown during winter among camels and the lowest one was observed during winter among donkeys. The sensitivity of each applied technique was estimated. ELISA recorded 2.50 and 58.33% positives in buffaloes and donkeys samples, respectively. Moreover, CATT test detected 52.50 and 30.08 positive percentages in both buffaloes and donkeys sera. However, camels still kept the highest records in ELISA revealing 63.82% while CATT revealed the moderate records rate in camel, 39%. In conclusion: *T. evansi* is established to infect camels, donkeys and buffaloes in Egypt. Still antibody detection tests are recommended as a useful tool for epidemiological studies of infections. However, our results found that it is recommended to include other antigenic epitopes beside what is already used, that should be originated from local isolates. Notwithstanding a wide array of diagnostic tests for trypanosomiasis, the search for a technique combining a high degree of accuracy and sensitivity should still be a target.

**Key words:** *Trypanosoma evansi* • Isolation • Diagnosis • Seasonal Incidence

### INTRODUCTION

*Trypanosoma evansi* is the most widely geographically distributed pathogenic flagellated haemoprotozoan parasite. It is transmitted mechanically by blood-sucking flies [1] and infects a large number of animals species, including cattle, buffaloes, donkeys, horses and camels, causing trypanosomiasis, commonly known as Surra which evoking significant economic loss [2].

Cases of human trypanosomiasis caused by infection with *T. evansi* have recently been reported in India [3, 4]. This may indicate the role of vectors in the transmission of parasites to humans [5]. The Tabanids are widely distributed all over the world; 12 species are of Mediterranean origin, 4 species of Afro-tropical origin and 2 species of European origin. Moreover, these species are considered as endemic in the Middle East and Egypt [2, 4].

Surra shows a variable clinical course, which ranges from asymptomatic to serious chronic stages characterized by low parasitaemia and involving cardiac and/or gastrointestinal disorders [3]. The most serious disease occurs in horses, camels and dogs causing edema and emaciation [6]. However, infection of donkeys, sheep and goats is usually mild or subclinical [7]. On the other hand, no clinical signs appear in both cattle and buffaloes which are often considered as reservoir hosts [8]. One of the possible causes of such clinical variability has been attributed to the genetic diversity and multiclinality of the natural populations [9]. This has been demonstrated by means of different biochemical and molecular strategies that targeted different genetic markers. Consequently, subdivisions of the parasite have been proposed following different approaches, but their phylogenetic relationships and epidemiological significance have not been disclosed [10-12].

Diagnosis of trypanosomiasis has some problems as commonly used techniques have some limitations. Moreover, the clinical signs are varied and non-specific. Despite many studies have been published to evaluate and compare the sensitivities of the different diagnostic techniques of infection in camels [13], cattle, buffaloes, small ruminants [14], pigs [15] and equines [16], still the discrepancies are predominating.

There is little information about the detection of this blood parasite in equines and bovines in Egypt. Only screening studies for the occurrence of *T. evansi* in camels, donkeys and buffaloes were performed by direct examination of blood films and applying the card agglutination test (CATT) [16-24]. Direct blood-film examination revealed the presence of *T. evansi* in camels, but failed to detect the parasite in the blood of donkeys and buffaloes [25, 26].

The present work aimed at reaching a comparative qualitative analysis of the different parasitological (wet blood smears technique, field stain blood film, haematocrit centrifugation and mouse subinoculation) and serological (CATT and ELISA) diagnostic techniques applied in the detection of *T. evansi* infection of camels, water buffaloes and donkeys at both the individual and farm levels. It also aimed at establishing an up-to-date epidemiological study of the seasonal incidence and prevalence of *T. evansi* infection in some Egyptian farm animals (camels, buffaloes and donkeys) with special regards to the blood-sucking insects, considered endemic in Egypt.

## MATERIALS AND METHODS

**Blood Samples:** 381 blood samples were randomly collected from 141 camels (*Camelus dromedarius*), 120 water buffaloes (*Bubalus bubalis*) and 120 donkeys (*Equus asinus*). Samples were collected during the period from September 2005 to February 2007 from camels slaughtered at El-Basateen abattoir, Cairo. Meanwhile, samples from buffaloes were collected from animals slaughtered at El-Warrak slaughter house, Giza governorate, while samples from donkeys were collected from animals slaughtered at the Zoo slaughter house, Giza.

### Parasitological Techniques

**Wet Blood Smears (WBS):** Was carried out according to procedures described by Hoare [27].

**Field Stained Blood film Smears (FSS):** Two blood films were prepared from each sample on two separate clean glass slides, which were air dried at room temperature. Films were fixed with methyl alcohol for 5 minutes, left to dry then stained with Field's stain as follows: Smears were covered with solution 1 (Eosin 0.25 gm, Disodium hydrogen phosphate 5 gm, Potassium dihydrogen phosphate 6.25 gm (Adwic), in 500 ml water) for 3 seconds, followed by rinsing in buffered water (Disodium Hydrogen Phosphate 5 gm, Potassium Dihydrogen Phosphate 6.25 gm (Adwic), 1litter Distilled Water) for 5 seconds. Slides then were stained in solution 2 (Methyl blue 0.4 gm, Azur I 0.25 gm, Disodium hydrogen phosphate 5 gm, Potassium dihydrogen phosphate 6.25 gm (Adwic), in 500 ml water) for 5 seconds. This was followed by rinsing smears in buffered water for 5 seconds. Finally, they were left to dry at room temperature and microscopically examined with a light microscope by an oil immersion lens [28].

### Microhaematocrit Centrifugation Technique (MHCT):

Two microhaematocrit centrifuge tubes were filled from each blood sample, sealed with clay and centrifuged in a microhaematocrit centrifuge at 12000 rpm for 4 minutes. Tubes were then examined by an oil immersion lens at junction of plasma and buffy coats using a capillary tube holder to detect the trypanosomes motility. To confirm the diagnosis, stained blood films were prepared by breaking the capillary tube 1mm below the surface of the buffy coat and a drop of the buffy coat was emulsified with plasma

and expelled on a microscope slide. Slides were fixed and stained with Field's stain then examined by an oil immersion lens [29].

**Mouse Subinoculation (MSI):** A total of 105 blood samples (collected from 63 camels, 21 buffaloes and 21 donkeys) were examined by this technique. Approximately 0.5 ml as an individual dose from each blood sample was intraperitoneally inoculated in two parasites-free Swiss albino mice. The tail blood of each mouse was drawn daily and examined by wet blood smear technique for 21 successive days post inoculation for the presence of *T. evansi* in peripheral blood [30].

### **Serological Techniques**

**Preparation of *T. Evansi* Crude Antigen:** Six Swiss albino rats; obtained from laboratory animal house, National Research Center propagation of *T. evansi*, each of 100 g body weight was inoculated intraperitoneally with 1ml (1:10) infected blood of previously infected mice at high parasitemic phase; more than 40 parasite/microscopic field. The infected rates were then slaughtered at high parasitemia and the blood was collected in tubes with anticoagulant. Collected blood was centrifuged at 3000 rpm for 15 minutes. The plasma was removed and washed 4 times using phosphate buffer saline. The erythrocytes were lysed using mono distilled water. The suspension was centrifuged at 3000 rpm for 15 minutes and re-washed several times with mono distilled water. The supernatant was then discarded. The sediment (*T evansi* isolate) was sonicated with ultrasonicator on an ice bag for 3 minutes with 30 seconds interval and then centrifuged for 45 minutes at 4°C at 14000 rpm. The supernatant was used as antigen and stored frozen at -20°C until used [20].

**Concentration of Prepared *T. Evansi* Crude Antigen and Determination of Total Protein Contents:** Crude antigen was packed in the dialysis bags and covered with polyethylene glycol, then left overnight at 4°C to avoid protein denaturation. Bags were washed with distilled water and the solutions were collected in tubes and then deposited in a lypholizer to concentrate the final volume to 0.5 ml. The concentrated antigenic material was stored at -20°C till use. The total protein content of the *T. evansi* antigen was calculated from the slope of the standard total protein content according to procedures described by Lowery *et al.* [31].

**Indirect Solid Phase Enzyme Linked Immunosorbent Assay (ELISA):** The optimal antigen concentration was first calculated to be 30 gm/ml as Check Board Titration. ELISA was applied on sera of camels, water buffaloes and donkeys at dilution 1:100 against the prepared *T. evansi* antigens. *T. evansi* antigen and control sera were diluted in carbonate-bicarbonate coating buffer to 1:10. Absorbencies were read using the automated ELISA reader at wavelength 450 nm for alkaline phosphatase and 405 nm for peroxidase, then cut off values were calculated [32].

**Card Agglutination Test (CATT):** On each drawn well on the test card, 25il of diluted sera were added. One drop (45il) of well-homogenized CATT antigen was then added to each well. The mixture was thoroughly mixed and spread out to about 1mm diameter using a stirring rod. The card was agitated in a circular motion using electric rotator at 60-70 rpm at room temperature for 5 minutes. Samples having blue granular agglutination were considered positive. Samples were read in comparison with the control wells and according to supplied instructions [33].

## **RESULTS**

### **Parasitological Examination:**

**Wet Blood Smears Technique:** Examination of 141 camels indicated that 6 (4.25%) were infected with *T. evansi* during the different seasons of the year. The higher infection rate (20%) was observed during winter season followed by summer (6%) and spring (2.08%). However, no camels were found to be infected during autumn season. Unfortunately, the wet blood smear technique failed to detect any *T. evansi* infection in both 120 water buffaloes and 120 donkeys throughout the year.

**Field Stained Blood Smears:** Examination of 141 camels revealed that 8 (5.67%) were positive for *T. evansi* throughout the year. The higher infection rate (20%) was shown during winter season (2 out of 10). However, 5 out of 50 (10%) examined camels were found positive to *T. evansi* during summer season and one out of 48 (2.08%) was positive during spring season. No camels were found positive for *T. evansi* during autumn when examined by this technique. Unfortunately, Field Stained Blood Smears Technique failed to detect any *T. evansi* infection in both 120 water buffaloes and 120 donkeys throughout the period of study.

Table 1: Results of ELISA Applied for Detection of *Trypanosoma evansi* Infection in Camels', Buffaloes', and Donkeys' Samples Collected Seasonally during the Study

Species	Total Number of Samples	Samples Collected Per Season	Cut off Value	ELISA			Total +Ve %	
				+Ve	+Ve%	-Ve		
Camels	141	Winter	10	0.270	6	60	4	63.8
		Spring	48		21	43.7	27	
		Summer	50		32	64	18	
		Autumn	33		31	93.9	2	
Buffaloes	121	Winter	27	0.199	0	0	27	2.47
		Spring	14		1	7.1	13	
		Summer	43		1	32.2	42	
		Autumn	37		1	2.7	36	
Donkeys	120	Winter	40	0.274	18	45	22	58
		Spring	17		12	70.5	5	
		Summer	23		19	82.6	4	
		Autumn	40		21	52.5	19	

Table 2: Results of CATT Applied for the Detection of *Trypanosoma evansi* Infection in Camels', Buffaloes', and Donkeys' Samples Collected Seasonally during the Study

Species	Total number of samples	samples Collected per Season	CATT			Total +Ve %	
			+Ve	+Ve %	-Ve		
Camels	141	Winter	10	6	60	4	81.78
		Spring	48	7	14.5	41	
		Summer	50	26	52	24	
		Autumn	33	16	48.48	17	
Buffaloes	121	Winter	27	14	51.8	13	76.23
		Spring	14	8	57.14	6	
		Summer	43	22	51.16	21	
		Autumn	37	19	51.35	18	
Donkey	120	Winter	40	6	15	34	44.4
		Spring	17	3	17.6	14	
		Summer	23	8	34.7	15	
		Autumn	40	20	50	20	

**Microhaematocrit Centrifugation:** The same results estimated by Field Stain Blood Smear technique examination for *T. evansi* in camels were recorded, with a total infection rate of 5.67% (8/141). Seasonally, the infection rate reached 2.08, 10 and 20.0% during spring, summer and winter seasons, respectively. It was also found that no camels were found infected with *T. evansi* during the autumn season. As recorded by Field Stain Blood Smear, this technique did not reveal *T. evansi* infection in water buffaloes and donkeys throughout the year.

**Mouse Sub Inoculation:** This method revealed that 15 out of 141 samples (10.63%) of camels were infected with *T. evansi* during the different seasons of the year. Seasonally, 7 out of 10 (70%) were found infected during winter season as the highest infection rate. Moreover, 12% (6/50) were found infected during summer versus 4.16% (2/48) during spring. However, no camels were found infected during autumn. MIT also failed to detect any *T. evansi* infection in both 120 water buffaloes and 120 donkeys.

**Serological Examination**

**Indirect Solid Phase Enzyme Linked Immunosorbent Assay:** ELISA results indicated that there were seasonal variations in seropositive rates for all examined animal species, whereas the highest seasonal incidence of infection with *T. evansi* was in autumn, spring and summer for camels, buffaloes and donkeys, respectively (Table 1).

**Card Agglutination Test (CATT):** Out of 381 examined camels (141), buffaloes (120) and donkeys (120) by CATT, 155 (40%), were found to be seropositive for *T. evansi*. The reaction of seropositive animals showed at 3 grades according to anti-*trypanosoma* antibody titers. Grade 1, 2 and grade 3. Out of 155 seropositive sera, 103 (66.5%) were shown at grade 1, followed by 37 (23.9%) grad 2 and 15 (9.6%) grade 3.

CATT test recorded that out of 141 examined camels, 55 (39.0%) were found to be seropositive to *T. evansi*, while out of 120 donkeys examined, 37 (30.8%) were found to be seropositive to *T. evansi*. CATT test demonstrated

a higher seropositive rate among water buffaloes, compared to camels and donkeys. It was detected that out of 120 examined buffaloes, 63 (52.5%) were seropositive for *T. evansi*. The seropositive rate varied from season to season. Generally, the higher seropositive rates of *T. evansi* were detected by CATT test was observed in autumn among both camels and donkeys. However, the lower rates were noticed in spring among both camels and donkeys. Otherwise, the highest rate was shown in winter among camels and the lowest one was observed in winter among donkeys (Table 2).

On the other hand, the three grades of CATT positive results were noticed in the tested sera of all examined host species. Grade 1(+) was the most common grade, in all species; 42 (76.3%), 42 (66.7%) and 19 (51.4%) cases in camels, buffaloes and donkeys, respectively.

Grade +2 was recorded as 18.2% (10 cases), 19% (12 cases) and 40.5% (15 cases) in camels, buffaloes and donkeys, respectively. Grade +3 was the less in prevalence, revealing 5.5% (3 cases), 14.3% (9 cases) and 8.1% (3 cases) in camels, buffaloes and donkeys, respectively (Table 2).

Grade 1 recorded a higher rate 75.0% (6/8) in summer, followed by 66.7% (2/3) in spring, 50.0% (3/6) in winter and 40.0% (8/20) in autumn. Grade 2 showed a higher rate 50.0% (10/20) in autumn. This was followed by 33.3% (2/6) in winter, 33.3% (1/3) in spring and 25.0% (2/8) in summer. Grade 3 recorded a relatively low rate 16.7% (1/6) in winter and a lower rate 10.0% (2/20) in autumn. However, no donkeys were found seropositive at grade 3 in summer and spring. (Charts 1 and 2).

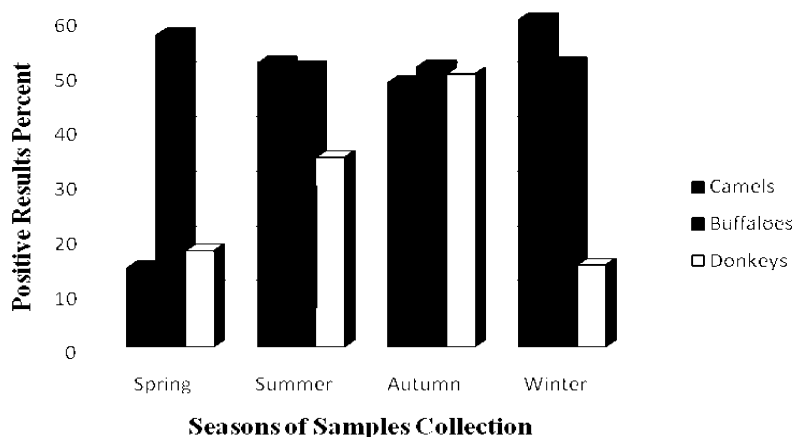


Chart 1: Comparison between Positive Results Percent of CATT Applied for Detection of *Trypanosoma evansi* Infection in Camels', Buffaloes', and Donkeys' Samples Collected Seasonally during the Study.

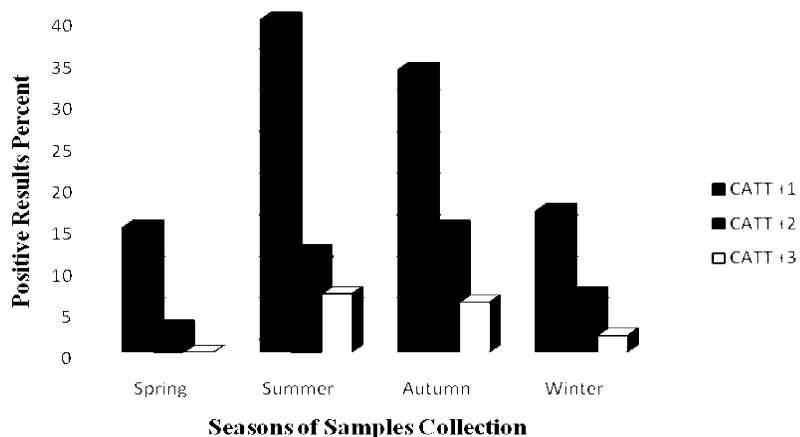


Chart 2: Results of CATT Applied for the Detection of *Trypanosoma evansi* Infection in Egyptian Farm Animals' Samples Collected Seasonally during the Study Showing Different Grades of Positives Results.

Table 3: Comparison between the Results of Parasitological and Serological Techniques Employed in the Diagnosis of *Trypanosoma evansi* Infection in Camels, Buffaloes, and Donkeys' Samples Collected Seasonally during the Study

Animal Species	Total Exam. No.	W.B.S.		F.S.S.		M.H.C.T.		M.I.T.		ELISA		CATT	
		+Ve No.	%	+Ve No.	%	+Ve No.	%	+Ve No.	%	+Ve No.	%	+Ve No.	%
Camels	141	7	4.96	8	5.67	8	5.67	15	10.63	90	63.8	55	39
Buffaloes	120	0	0	0	0	0	0	0	0	3	2.50	63	52.50
Donkey	120	0	0	0	0	0	0	0	0	70	58.33	37	30.08
Total	381	7	1.83	8	2.14	8	2.14	15	4.098	163	42.78	158	41.47

**Comparative Analysis of the Investigated Diagnostic Techniques:**

This study clearly demonstrated that all used parasitological techniques succeeded to detect *T. evansi* in the blood of infected camels, but never from blood of water buffaloes and donkeys. Although no *T. evansi* was detected from the blood of buffaloes and donkeys in this study, ELISA and CATT succeeded to detect anti-*T. evansi* antibodies in their sera. The sensitivity of the applied techniques was evaluated by estimating the percentage of positive sera (Table 3).

**DISCUSSION**

The conventional parasitological techniques, especially the direct smear examination, frequently fail to detect the parasite in the chronic phase and are recommended only in the acute phase whereas parasites are colonized in the blood [32]. While detection of trypanosome antigens lacks sensitivity and specificity [33], assays for the detection of anti-trypanosome circulating antibodies have high measures of validity and are economical and applicable at large-scale screening [34].

This study clearly demonstrated that all used parasitological techniques succeeded to detect *T. evansi* infection in the blood of infected camels, indicating 4.25, 5.67, 5.67 and 10.63% prevalence rates, respectively, during the different seasons of the year, despite of the endemic status of *T. evansi* in Egypt [24] but never from the blood of water buffaloes and donkeys. The low percentage of infection agrees with previous publications which recorded that examination by blood films or wet preparations is successful during the acute phase of infection with high parasitaemia when trypanosomes concentrations in peripheral blood are above the detection limits (2,500,000 trypanosomes/ml) [35]. However, chronic trypanosomiasis reveals only a small percentage of infections when the parasite is frequently extra vascular, or is present in very low undetectable numbers [20]. In other words, both techniques are missing

considerable percentages of actual infected animals reaching 50-80% of positive cases [35]. The outcome of this study concerning *T. evansi* detection of natural infections in farm animals nearly does not differ from most previous worldwide or Egyptian trials [36] though disagrees with some others [16, 25].

Consequently, when MHCT and MIT were applied, with a complementary parasite concentration method, the percentage of positive cases increased and the number of false negative cases declined. This is in line with previous publications, favoring the concentration of the scanty parasites (25 to 85 trypanosomes/ml) rendering them to be seen via thin smears after capillary centrifugation [29]. On the other hand, our results are in contrast to the results obtained by El-Sawalhy and Ebeid in [20].

Despite the practicability of the direct detection by parasitological methods, a need for a more sensitive and rapid diagnostic test for individual cases having undetectable concentrations has been a must [13]. Such technique should overcome the discrepancy resulting from the difference in parasite infectivity and virulence between species making infections pass without being noticed making the infected hosts act as reservoirs for the parasite, as the case with buffaloes and donkeys showing continuous fluctuation of *T. evansi* infection during the propagation of parasites and their rapid disappearance from the blood circulation to cryptic sites [13], acquiring tolerance to trypanosomosis by natural selection, possessing genetic capacity to better control parasitaemia [37] and/or other behavioral avoidance mechanisms [36].

ELISA and CATT succeeded to detect anti-*T. evansi* antibodies in the sera of each cases. The sensitivity of each applied technique is evaluated by estimating the percentage of positive sera. Ab-ELISA detected incidence rates of 2.50% in buffaloes and 58.33% in donkeys. Moreover, the CATT test recorded 52.50% in buffaloes and 30.08% in donkeys' seroprevalence for *T. evansi*. However, camels still kept the highest records of infection even in serological diagnosis revealing 63.82 and 39% by ELISA and CATT positive cases, respectively.

This can be explained by the fact that antibodies to *T. evansi* could persist even after the elimination of the parasite from the blood circulation [25] which does not necessarily mean the presence of the parasite antigens in the sera of the animals. Therefore, these methods can not be used for the diagnosis of patent or sub-patent infections [26], neither for the differentiation of infected individuals from cured ones, nor for assessing the effectiveness of applied drugs or disease progression because they detect the antibodies that are long lasting after treatment [38].

The low positive incidence rate in buffaloes revealed by Ab-ELISA, which is in contrast to their CATT records, indicated the occurrence of false negative results that can be explained by the presence of low undetectable levels of immunoglobulins during the course of trypanosomiasis. Moreover, some of the 46.5% apparently negative cases of the examined buffaloes might have been infected only a few days prior to sampling, since no seroconversion was detected in their specimens even by CATT. This is in agreement with previous results [40]. However, other researchers had strongly considered the role of major variant surface (VSG) cross-reaction in buffalo's sera as a main cause of recorded higher prevalence rates by CATT in this species; thus positive samples by CATT should be then checked by Ag-ELISA for confirmation [40].

In contrast, the higher false negative reaction of CATT in some samples of donkeys and camels could be a result of antigenic variation due to periodical switch in major VSG which is considered a common feature in trypanosomes [41]. Also, the discrepancy in the serological incidence in our results between Ab-ELISA and CATT concerning donkeys and camels might be due to the evolution of new *T. evansi* isolates that lack RoTat 1.2 gene and/or do not express RoTat 1.2 VSG due to development of genetic changes that have different protein profiles during infection as recorded elsewhere in the world [39, 42- 44]. Moreover, we may add that the noticed differences in the degree of sensitization of immune systems between the examined animal species [45] and the weakness in the immune reaction revealed by serological latency which usually accompanies the chronic phase of infection [46]. Finally, the difference in virulence between *T. evansi* strains and subsequently, host susceptibility to infection and protozoan propagation was proposed [41]. In fact most of the examined animals in the present investigation seem to be in a chronic state suffering declined humoral immune response [47].

CATT is a quick and easy test which can be performed under field conditions. The sensitivity of CATT related to early expression of the antigen VAT RoTat 1.2 after infections, thus anti-*Trypanosoma* spp. antibodies are first detected at day 4 to 7 from infection and persist long after [43]. The tendency for lower reaction grade in most samples could be a result of antigenic variation due to periodical switch in major variant surface glycoprotein which is considered a common feature in trypanosomes [41]. Also, it reflects the decrease in antibody titers and subsequently, the reactivity against one particular predominant VAT, which does not mean decrease in antibody titer against other *T. evansi* specific antigens. This highlights the importance of other *T. evansi* antigens to be involved in such diagnostic technique as CATT [26].

The highest infection rate was observed during the winter season, which confirms that acute infections occur during the rainy season of the year when the biting vectors are most active [7]. On the other hand, the highest seasonal seroprevalence of *T. evansi* in autumn, spring and summer for camels, buffaloes and donkeys, respectively confirmed that seroconversion post infections occur during seasons other than winter; the acute infection time and seasonal difference in antibodies titers is a host-parasite interaction and immune-system-related rather than climate or vector dependent [32].

In Egypt, *T. evansi* prevalence varied in studies related to diagnostic techniques, localities and hosts. Rather similar incidence rates were recorded in Egypt [14, 19, 20, 22, 48]. The differences between our results and those obtained in previous studies [24, 28, 39, 42, 43] might be attributed to different factors such as the variation in the breeding methods and activities of the infected flies with the variation in the climatic conditions such as the length of the rainy season, difference in the number of collected samples and difference in breeds included in the study, in addition to differences in the immune systems in the examined animals species [29, 32].

Despite the disagreement of some researchers [28, 46], the application of nucleic acid techniques can overcome the low sensitivity of the previous discussed diagnostic techniques, especially the parasitological protocols since it offers higher sensitivity in the detection as low as a single trypanosoma/sample. In addition, it can differentiate between active and cured infections [48].

Finally, as serological tests can be the methods of choice for mass screening, their main limitation will remain the failure to demonstrate the parasite. The only way to tackle this problem will be the improvement of

parasitological tests or the development of molecular tests. In addition, two or more techniques should be used to diagnose reservoir hosts and seronegatives animals should be re-tested after 30 days from the first examination due to serological latency. Qualitative and quantitative identification of common and potential *T. evansi* immune reactive antigens among variant isolates from different species is of interest for universal immunodiagnosis of infection in different hosts.

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