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Original Article

Investigation of *Trypanosoma evansi* infection in different animals in Northern Egypt

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Abstract Surra is a chronic disease caused by *Trypanosoma evansi* which is transmitted mechanically by *Tabanus* and *Stomoxys* spp. In the present study, 300 blood samples were collected from six different animal species of cattle, buffaloes, sheep, goats, equines, and dogs from small farmer's holders, medium-sized farms, and from animals slaughtered at abattoirs in Alexandria and Beheira governorates. Blood samples were examined by Giemsa-stained blood smear and Polymerase Chain Reaction (PCR) to detect *T. evansi*. The study revealed that overall prevalence according to microscopy examination and PCR were 0.0% (0.0/300) and 57.78% (104/180), respectively. The infection rates in different animal species were as follows; cattle, 46.67% (14/30); buffaloes, 70% (21/30); sheep, 40% (12/30); goats, 30% (9/30); equines, 66.67% (20/30); dogs, 93.33% (28/30). The results showed that the highest infection rate in cattle, buffaloes, and sheep was recorded in Beheira, while in goats and equines, the highest infection rate was recorded in Alexandria. In dogs, the infection rate was the same in Alexandria and Beheira. Totally the highest infection rate (64.4%) was recorded in Beheira.

Key words: stained blood smears-Trypanosoma evansi-surra -Pcr-Tabanus

Introduction *Trypanosoma evansi* (T. evansi) is a widely distributed haemo protozoan parasite transmitted by Tabanus and Stomoxys species **(Reid, 2002).** According to **Li et al (2009)**, *T. evansi* infects a broad range of animal species like cattle, buffaloes, donkeys, horses, and dogs, causing a disease referred to as 'Surra'. The disease causes significant economic losses due to reduced milk yields, decreased animal market value, and annual mortality rates affecting thousands of animals **(Mekata et al., 2009).**

Surra disease causes variable clinical signs which range from asymptomatic to severe chronic disease **(Joshi et al., 2005).** In horses and dogs, the clinical picture of the disease of *T. evansi* causes fluctuating fever, petechial haemorrhage on the eyelid and vaginal mucosa, abortion, nervous signs, testicular edema, anemia, paresis,

weakness, and lack of appetite **(Desquesnes et al., 2013).**

On the other hand, the disease in sheep, goats, and donkeys is subclinical or mild **(Mahmoud and Gray, 1980).** There are no clinical symptoms in cattle and buffaloes, considering the two animal species as reservoir hosts **(Verma and Gaut, 1978).**

Techniques applied for the detection of *T. evansi* have some limitations **(Davasion et al., 1999).** These include high skills required for microscopic examination (Wet blood film, Field-stained blood film, Haematocrit centrifugation, and Mouse sub inoculation), and false positive, and false negative results of immunological methods **(Viseshakul and Panyim, 1990).**

A high incidence of *T. evansi* was recorded in Egypt, **Zayed et al (2010)** detected a high prevalence of *T. evansi* in Buffaloes and donkeys in Giza. **Elhaig et al (2016)** from Lower Egypt and **Hassan et al (2017)** from Southern Egypt detected a high Prevalence of *T. evansi* in cattle, while in sheep and goats detected by **Ashour et al (2013)** in Cairo.

The objective of the current study was to determine the prevalence of *T. evansi* in different animals (cattle, buffaloes, sheep, goats, horses, and dogs) in the Beheira and Alexandria governorates in Egypt using molecular and light microscopy methods.

Ethical statement. The study was conducted following Alexandria University's Declaration and was approved by the Faculty of Veterinary Medicine's Ethics Committee (protocol no: 118) and no experiments were conducted on the animals in this study.

2. Materials and methods

Study area

A total number of three hundred blood samples were collected from six different animal species of cattle, buffaloes, sheep, goats, equines, and dogs of different ages and sexes from Beheira and Alexandria governorates. Fifty blood samples were collected from each species.

Collection of samples

Blood samples were collected at random from animals kept in small farmer's holders and medium-sized farms and from animals slaughtered at abattoirs (agriculture slaughterhouse in Alexandria and Damanhour slaughterhouse) in the period from July 2021 to December 2022. Fifty blood samples (25 from each governorate) were collected from each animal species. Two ml of blood were collected from each animal, into a tube containing ethylene diamine tetra-acetic

acid (EDTA) as anticoagulant (50µl/ml blood) for parasitological examination and DNA extraction. Samples were then kept in a cool box and transported to the Laboratory of Parasitology, Faculty of Veterinary Medicine, Alexandria University.

Blood smears and Giemsa staining

According to Sloss et al (1994), a drop of blood was placed on one end of a glass slide and drawn by another slide to make a thin blood film. The blood smear was air dried, fixed with methanol for 5 minutes, and stained with Hexa-Biotech Giemsa stain (Science For Life) for 30 minutes. Excess stain was removed by gentle washing with tap water and smears were then dried by a filter paper. Stained smears were examined under x40 and x100 power magnifications of the light binocular microscope.

DNA extraction

Whole blood samples were used for extraction of total genomic DNA using whole blood/body fluids GeneJet DNA Purification Mini Kit (Thermo Scientific, Lithuania) according to the manufacturer′s instructions. A final DNA product of 50 µl was eluted. Agarose gel electrophoresis was conducted to confirm the presence of DNA products. Extracted genomic DNA was stored at -20°c till used.

Polymerase chain reaction (PCR) protocol

PCR was conducted for amplification of 164 bp by using a highly repeated sequence of mini chromosome satellite DNA, subgenus trypanozoon specific primers TBR 1/2, with sequences shown in **Table (1)** (Masiga et al., 1992). The primers were used at a concentration of 10 pmol/µl. One hundred and eighty samples (15 samples for each species from each governorate) were tested using the PCR amplification method. The PCR protocol was performed according to the method described by Herrera et al (2005) with some modifications Henidy et al (2019). Briefly, the PCR amplification was performed in a total reaction volume of 25µl containing: 12.5µl Dream Taq Green master mix (Thermo Scientific), 1.5µl DNA template, 1µl of each primer and was completed to 25 µl with nuclease-free water. The samples were thermocycled in a ^{3}Primethermal cycler (TECHNE, UK) with an initial denaturation at 95°C for 3 min. and then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and an extension at 72°C for 30 seconds with a final extension at 72°C for 7 min. PCR products were then analyzed by agarose gel electrophoresis.

Gel electrophoresis

After amplification, 10 µl of PCR products were loaded into 1% agarose gel stained with ethidium bromide (0.5µg/ml). Gels were then electrophorized for 60 minutes in the electrophoresis unit at 100V, with 100 bp DNA ladder at a working concentration of (105 ng/µg) (Jena Bioscience) as a size marker and then visualized by the gel documentation system UVP PhotoDoc-it^{TM} imaging system, analytikjena, USA. Images of the amplified DNA products were acquired and analyzed.

Statistical analysis

Data were statistically analyzed by using the statistical Package for Social Sciences (SPSS) version 25. The data were analyzed using the Chi-Square test (Person Chi-Square and Fisher's Exact test). A probability (p) value ≤ 0.05 was considered statically significant.

3. Results

Microscopic examination-based prevalence

From Alexandria governorate, the microscopic examination of 150 blood smears from six different animal species (cattle, buffalo, sheep, goats, horses, and dogs) revealed that all samples were negative for *T. evansi* infection.

Similarly, the absence of *T. evansi* infection was evident in Beheira samples after the microscopic examination of 150 samples from six different animal species.

Molecular prevalence of *T. evansi* in the studied animals

For the detection of the trypanozoan subgenus-specific gene, 180 samples were examined using PCR-based TBR 1/2 assay. From all samples examined by PCR, 104 (57.8%) out of 180 molecularly positive samples were detected.

Molecular prevalence of *T. evansi* in the examined animals, Alexandria and Beheira.

TBR 1/2-based PCR assay was performed on 180 samples equally distributed between six different animal species from Alexandria and Beheira governorates (cattle, buffaloes, sheep, goats, horses, and dogs), with 15 samples from each animal species in each governorate individually examined. PCR products revealed that prevalence rates in Alexandria were 26.67%, 60%, 13.3%, 40%, 73.3%, and 93.3%, among cattle, buffalo, sheep, goats, horses, and dogs, respectively. While in Beheira, the infection rates were 66.67%, 80%, 66.67, 20%, 60%, and 93.3 among cattle, buffaloes, sheep, goats, horses, and dogs, respectively (**Table 2, 3**).

Agarose gel electrophoresis detected the amplification of PCR products with

tandem bands at a size range of 164 bp and 347 bp, consistent with the tandem repeat nature of the target gene (**Figure 1, 2).**

4. Discussion

In this study, the microscopic examination and polymerase chain reaction (PCR) was used to detect the prevalence of *T. evansi* in different animals (cattle, buffaloes, sheep, goats, equines, and dogs) in two regions of northern Egypt, Alexandria, and Beheira. The overall infection according to microscopy examination and PCR were 0% (0/300) and 57.78% (104/180), respectively. The infection rates in different animal species were as follows; cattle, 46.67% (14/30); buffaloes, 70% (21/30); sheep, 40% (12/30); goats, 30% (9/30); equines, 66.67% (20/30); dogs, 93.33% (28/30).

In the current study, the prevalence of *T. evansi* using blood film examination was (0%) in all different animals. These results agree with those obtained by **Hilali et al (2004)** from Egypt in water buffaloes, **Ravindran et al (2008)** from India in donkeys and dogs, **Zayed et al (2010)** from Egypt in donkeys, and **Benfodil et al (2019)** from Algeria in equines. On the other hand, the prevalence in the current study is lower than those of **Muieed et al (2010)** who reported a prevalence of 5% in horses from Pakistan, and **Ashour et al (2013)** who recorded a percentage of 28.6% and 14.3% in sheep and goats from Egypt, respectively. **Bal et al (2014)** from India and **Setiawan et al (2021)** from Indonesia recorded prevalence rates of 6.9% and 1% in cattle. **Asif et al (2020)** and **Hussain et al (2018)** from Pakistan obtained an infection rate of 3.75% and 4.6% in dogs and water buffaloes, respectively. The low prevalence rate of *T. evansi* using blood film may be due to the chronic nature of *T. evansi* infection in Egypt. Moreover, the blood film is considered an insensitive test unable to detect *T. evansi* in chronic cases **(Nantulya, 1990).**

In this study, the prevalence of *T. evansi* in water buffaloes is 60% and 80% in Alexandria and Beheira governorates, respectively. Our results agree with those of **Zayed et al (2010)** from Egypt, **Sudan et al (2015)** from India and **Alsaad et al (2021)** from Iraq who reported prevalences of 76.23%, 61.25%, and 87.2%, respectively. On the other hand, the prevalence in the current study was higher than rates reported by **Hilali et al (2004)** from Egypt (24%), and **Hussain et al (2018)** from Pakistan (16.15%). The variation in *T. evansi* infection rates may be attributed to different breeds and localities **(Sharma et al., 2018).**

The prevalence of *T. evansi* in cattle from Alexandria is 26.67 % which agrees with those infection rates reported by **Elhaig et al (2016)** from Egypt (30.4%), **Hakeem (2019)** (29.9%), and **Mossaad et al (2020)** from Sudan (24%). On the other hand, lower prevalence of 16%, and 3% were reported by **Muieed et al (2010)** from Pakistan and **Setiawan et al (2021)**, frm Indonesia respectively. The prevalence of infection of *T. evansi* in cattle from Beheira is 66.67% which is higher than the rates of infection recorded by **Bal et al (2014)** from India (46.5%), and **Fereig et al (2017)** from Egypt **(**42.2%). The variation in results may be attributed to the variation in breeds and localities **(Hakeem, 2019)**.

The prevalence of *T. evansi* in donkeys from Beheira is 60% which is nearly similar to detection rates obtained by **Zayed et al (2010)** from Egypt (58%); **Abdel-Gawad et al (2019)** from Egypt (53.85%); and **Benfodil et al (2019)** from Algeria (62.1%). While the prevalence of *T. evansi* in horses in Alexandria was 73.3% which is lower than that obtained by **Silva et al (1995)** from Brazil (97.5%). On the other hand, the prevalence rate of the present study was higher than those of **Herrera et al (2004)** from Brazil (34.9%), and **Zahoor et al (2022)** from Pakistan (15.5%). The high prevalence rate of *T. evansi* in donkeys and horses in Egypt may be attributed to bad management, physical work-related stress and exhaustion, and exposure to vectors **(Parreira et al., 2016).**

The prevalence of *T. evansi* in sheep in Alexandria and Beheira is 13.3% and 66.67%, respectively. Our results in Beheira agree with those recorded by **Birhanu et al (2015)** from Ethiopia (12.70%). While our results in Alexandria may be comparable with those reported by **Mossaad et al (2020)** from Sudan (70%). Our results are higher than those reported by **Ereqat et al (2020)** from Palestine (4%), and **Hassan-Kadle et al (2020)** from Somalia (8.3%). However, these results are lower than those reported by **Ashour et al (2013)** from Egypt who recorded an infection rate of 100%. The variation in results may be attributed to a low number of samples (7 samples) collected by **Ashour et al (2013)**, different localities, and breeds **(Musinguzi et al., 2022).**

The prevalence of *T. evansi* in goats in Alexandria and Beheira was 40% and 20%, respectively. Our results may be comparable to those obtained by **Elata et al (2020)** from Philippines who reported a prevalence of 33.9% and **Musinguzi et al (2022)** from Mongolia who reported a prevalence of 35.69%. In the current study,

the prevalence rate is higher than those reported by **Ereqat et al (2020)** from Palestine (13%), and **Hassan-Kadle et al (2020)** from Somalia (17.5%). On the other hand, our results were lower than those of **Ashour et al (2013)** from Egypt (100%) and **Mossaad et al (2020)** from Sudan (86%). The variation in results may be attributed to a low number of samples (7 samples) collected by **Ashour et al (2013)**, different breeds, and localities **(Elata et al., 2020).**

The prevalence of *T. evansi* in dogs in Alexandria and Beheira was 93.3 % and 93.3%, respectively. These results agree with the prevalence obtained by **Silva et al (1995)** of 100% from Brazil and may be comparable with **De Oliveira Porfirio et al (2018)** from Brazil (73%). Our results are much higher than those obtained by **Aquino et al (2010)** from Brazil (29.1%) and **Azhahianambi et al (2018)** from India (19.9%). The remarkably high variation in results may be attributed to the variation in the sampling technique, where most of our samples were collected from stray dogs and dogs having poor management and high vector density exposure **(Alanazi, 2018).**

In the present study, the PCR products showed multiple bands. This might be attributed to the tandem repeat nature of the TBR 1/2 gene. This is in agreement with **Henidy et al (2019)** who reported multiple bands in PCR products after the amplification of *T. evansi* DNA using TBR 1/2. **Ventura et al (2002)** found synapomorphic DNA (termed te664) which is repetitive and not found in other types of trypanosomes and present in intermediate and minichromosomes of *T. evansi*. **Milocco et al (2012)** reported polymeric products after using TBR1/2 primer for the detection of *T. evansi*. The variation in the results between different studies may be attributed to different strains of the parasite, concentration of DNA, different PCR protocols, and differences in primer concentration.

To the best of our knowledge, this is the first confirmation of high levels of infection with *T. evansi* as a causative agent of surra in different animals in Egypt. Our study emphasizes the need for stringent risk assessment studies as prerequisites for control measures. Further investigations focusing on vectors and evaluation of risk factors are needed.

Declaration of Competing Interest

No conflict of interest

Table (1): The sequences of TBR1/2 primers

Primer name	**Primer sequence**
TBR-1 Forward	5´ -GAATATTAAACAATGCGCAG -3´
TBR-2 Reverse	5´ –CCATTTATTAGCTTTGTTGC-3´

Table (2): Prevalence of *T. evansi* in Alexandria Governorate: Data of PCR assay

Animal species	**Number examined**	**Number positives**	**%**	χ^2	**P-value**
Cattle	15	4	26.67	27.04	0.000*
Buffalo	15	9	60		
Sheep	15	2	13.3		
Goat	15	6	40		
Horse	15	11	73.3		
Dog	15	14	93.3		
Total	90	46	51.1		

Significant P< 0.05

* Significant difference between the different species.

Table (3): Prevalence of *T. evansi* in Beheira Governorate: Data of PCR assay.

Animal	**Number examined**	**Number positives**	**%**	χ^2	**P-value**
Cattle	15	10	66.67	20.17	0.001*
Buffalo	15	12	80		
Sheep	15	10	66.67		
Goat	15	3	20		
Horse	15	9	60		

Dog	15	14	93.3		
Total	90	58	64.4		

Significant P< 0.05

* Significant difference between the different species.

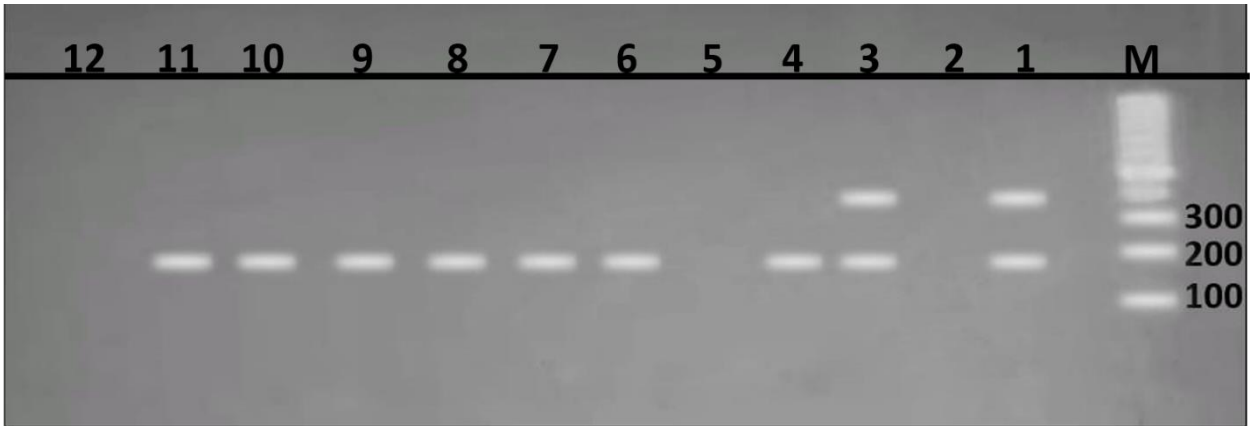

Fig. (1): Ethidium bromide-stained agarose gel of PCR products of *T. evansi* of horse from Alexandria samples showing bands at 164 bp. M= 100 bp DNA ladder, lane 1= control positive, lane 2= negative sample, lanes 3, 4= positive samples, lane 5= negative sample, lanes 6-11= positive samples, lane 12= control negative. Bands at 347 bp were detected due to the repetitive nature of the target gene.

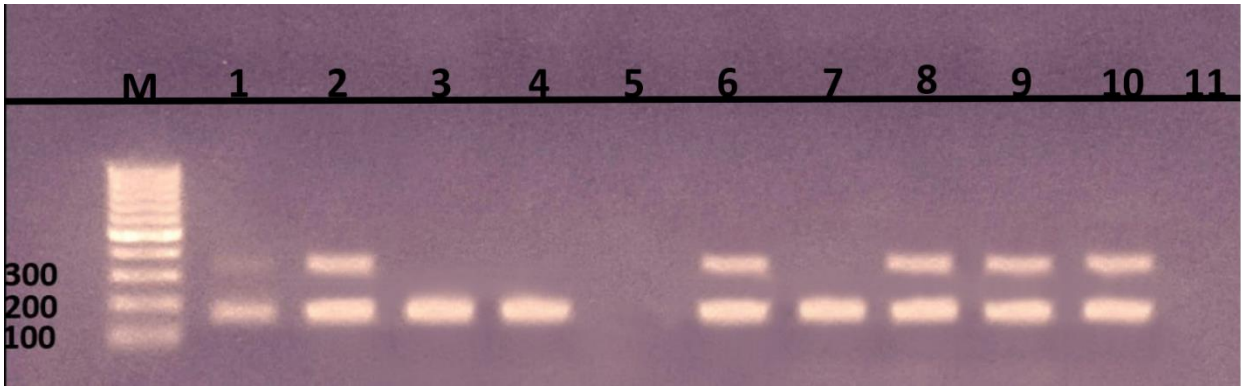

Fig. (2): Ethidium bromide-stained agarose gel of PCR products of *T. evansi* of dogs from Beheira samples showing bands at 164 bp. M= 100bp DNA ladder, lanes 1, 2= control positive, lanes 3, 4= positive samples, lane 5= negative sample, lanes 6-10= positive samples, lane 11= control negative. Bands at 347 bp were detected due to the repetitive nature of the target gene.

References

Abdel-Gawad, A.; Nassar, A. and Abdel-Wahab, A. B. (2019): Evaluation of the card agglutination test (CATT/*T. evansi*) in comparison with PCR for detection of *Trypanosoma evansi* infection

in donkeys in Egypt. J. Egypt. Soc. Parasitol. 49(3): 539-542.

Alsaad, K. M.; Jarad, A. and Lafta, M. H. (2021): *Trypanosomiasis evansi* of buffalo at Basrah, Iraq-clinico-hematobiochemical and diagnostic studies. Biochem. Cell. Arch. 21(2): 2035-2042.

Aquino, L. P. C. T.; Machado, R. Z.; Lemos, K. R.; Marques, L. C.; Garcia, M. V. and Borges, G. P. (2010): Antigenic characterization of *Trypanosoma evansi* using sera from experimentally and naturally infected bovines, equines, dogs, and coatis. Rev. Bras. Parasitol. Vet. 19: 112-118.

Ashour, A. A.; Abou El-Naga, T. R.; Barghash, S.M. and Salama, M. S. (2013): *Trypanosoma evansi*: Detection of *Trypanosoma evansi* DNA in naturally and experimentally infected animals using TBR1&TBR2 primers. Exp. Parasitol. 134(1): 109-114.

Asif, M.; Ashraf, K.; Jamil, T.; Ahmad, N.; Ullah, S.; Hussain, S. Iqbal, M.; Nasrullah, M.; Khan, A. H. and Ghani, M. U. (2020): Canine *Trypanosoma evansi* Infection (Surra) in Lahore, Pakistan-Short Communication. Vet. Ir Zootech. 78(100): 1-4.

Azhahianambi, P.; Jyothimol, G.; Baranidharan, G. R.; Aravind, M.; Latha, B. R. and Raman, M. (2018): Evaluation of multiplex PCR assay for detection of *Babesia spp*, *Ehrlichia* canis and *Trypanosoma evansi* in dogs. Acta Trop. 188: 58-67.

Bal, M. S.; Sharma, A.; Batth, B. K.; Kaur, P. and Singla, L. D. (2014): Detection and management of latent infection of *Trypanosoma evansi* in a cattle herd. Indian J. Anim. Res. 48(1): 31-37.

Benfodil, K.; Ansel, S.; Mohamed-Cherif, A. and Ait-Oudhia, K. (2019): Prevalence of *Trypanosoma evansi* in horses (*Equus caballus*) and donkeys (*Equus asinus*) in El-Bayadh district, southwestern Algeria. J. Hellenic Vet. Med. Soc. 70(3): 1631-1638.

Birhanu, H.; Fikru, R.; Said, M.; Kidane, W.; Gebrehiwot, T.; Hagos, A.; Alemu, T.; Dawit, T.; Berkvens, D.; Goddeeris, B. M. and, Büscher P. (2015): Epidemiology of *Trypanosoma evansi* and *Trypanosoma vivax* in domestic animals from selected districts of Tigray and Afar regions, Northern Ethiopia. Parasit. Vectors. 8(1): 1-11.

Davasion, H. C.; Thrusfield, M. V.; Muharsini, S.; Husein, A.; Partoutomo, S.; Rea, P. F.; Masake, R. and Luckins, A. G. (1999): Evaluation of antigen detection and antibody detection tests for *Trypanosoma evansi* of buffaloes in Indonesia. Epidemiol. Infect. 123(1): 149-155.

De Oliveira Portfirio, G. E.; Santos, F. M.; De Macedo, G. C.; Barreto, W. T. G.; Campos, J. B.; Meyers, A. C.;

Andre, M. R.; Perles, L.; De Oliveira, C. E.; Xavier, S. C. D. C.; Andrade, G. B. D.; Jansen, A. M. and Herrera, H. M. (2018): Maintenance of *Trypanosoma cruzi, T. evansi and Leishmania spp*. by domestic dogs and wild mammals in a rural settlement in Brazil-Bolivian border. Int. J. Parasitol.: Parasites. Wild. 7(3): 398-404.

Desquesnes, M.; Holzmuller, P.; Lai, D. H.; Dargantes, A.; Lun, Z. R. and Jittaplapong, S. (2013): *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts and pathogenic effects. Biomed. Res. Int. Article ID 194176.

Elata, A.; Galon, E. M.; Moumouni, P. F. A.; Ybanez, R. H. D.; Mossaad, E.; Salces, C. B.; Bajenting, G. P.; Ybanez, A. P.; Xuan, X.; Inoue, N. and Suganuma, K. (2020): First molecular detection and identification of *Trypanosoma evansi* in goats from Cebu, Philippines using a PCR-based assay. Vet. Parasitol. Reg. Stud. Rep. 21: 100414.

Elhaig, M. M.; Selim, A.; Mahmoud, M. M. and El-Gayar, E. K. (2016): Molecular confirmation of *Trypanosoma evansi* and *Babesia bigemina* in cattle from Lower Egypt. Pak. Vet. J. 36(4): 409-414.

Ereqat, S.; Nasereddin, A.; Al-Jawabreh, A.; Al-Jawabreh, H.; Al-Laham, N. and Abdeen, Z. (2020): Prevalence of *Trypanosoma evansi* in livestock in Palestine. Parasit. Vectors. 13(1): 1-8.

Fereig, R. M.; Mohamed, S. G.; Mahmoud, H. Y.; AbouLaila, M. R.; Guswanto, A.; Nguyen, T. T. Mohamed, A. E.; Inoue, N.; Igarashi, I. and Nishikawa, Y. (2017): Seroprevalence of *Babesia bovis*, *B. bigemina*, *Trypanosoma evansi*, and *Anaplasma marginale* antibodies in cattle in Southern Egypt. Ticks. Tick-Borne Dis. 8(1): 125-131.

Hakeem, M. A. M. (2019): *Seroprevalence of Trypanosoma evansi infection in dairy cattle in Khartoum State, Sudan* (Doctoral dissertation, Sudan University of Science and Technology).

Hassan-Kadle, A. A.; Ibrahim, A. M.; Nyingilili, H. S.; Yusuf, A. A. and Vieira, R. F. (2020): Parasitological and molecular detection of *Trypanosoma spp*. In cattle, goats, and sheep in Somalia. Parasitol. 147(14): 1786-1791.

Henidy, S. A.; Ashmawy, K. I.; Abou-Akkada, S. S.; Elshanat, S. and Deweir, A. W. (2019): Field investigation and comparative evaluation of diagnostic tests of *Trypanosoma evansi* in camels in Egypt. Egypt. Vet. Med. Soc. Parasitol. J. 15(1): 1-10.

Herrera, H. M.; Dávila, A. M. R.; Norek, A.; Abreu, U. G.; Souza, S. S.; D'andrea, P. S. and Jansen, A. M. (2004): Enzootiology of *Trypanosoma*

evansi in pantanal, Brazil. Vet. Parasitol. 125(3-4): 263-275.

Herrera, H. M.; Norek, A.; Tatiana, P. T.; Freitas; Rademaker, V.; Fernandes, O., and Jansen, A. M. (2005): Domestic and wild mammals infection by *Trypanosoma evansi* in a pristine area of the Brazilian Pantanal region. Parasitol. Res. 96: 121-126.

Hilali, M.; Abdel-Gawad, A.; Nassar, A.; Abdel-Wahab, A.; Magnus, E. and Buscher, P. (2004): Evaluation of the card agglutination test (CATT/*T. evansi*) for detection of *Trypanosoma evansi* infection in water buffaloes (*Bubalus bubalis*) in Egypt. Vet. Parasitol. 121(1-2): 45-51.

Hussain, R.; Khan, A.; Qayyum, A.; Abbas, T.; Ahmad, M.; Mohiuddin, M. and Mehmood, K. (2018): Clinico-hematological and oxidative stress in Nili Ravi buffaloes infected with *Trypanosoma evansi*. Microb. Pathog. 123: 126-131.

IBM Corp. Released (2017): IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.

Joshi, P. P.; Shegokar, V. R.; Powar, R. M.; Herder, S.; Katti, R.; Salkar, H. R.; Dani, V. S.; Bhargava, A.; Jannin, J. and Truc, P. (2005): Human trypanosomiasis caused by *Trypanosoma evansi* in India: the first case report. Am. J. Trop. Med. Hyg. 73(3): 491-495.

Li, S. Q.; Yang, W. B., Lun, Z. R.; Ma, L. J.; Xi, S. M.; Chen, Q. L.; Song, X. W.; Kang, J. and Yang, L. Z. (2009) Immunization with recombinant actin from *Trypanosoma evansi* induce protective immunity against *T. evansi*, *T. equiperdum*, and *T. b. brucei* infection. Parasitol. Res. 104(2): 429-435.

Mahmoud, M. M. and Gray, A. R. (1980): Trypanosomiasis due to *Trypanosoma evansi* (Steel, 1885) Balbiani, 1888. A review of recent research. Trop. Anim. Health Prod. 12(1): 35-47.

Masiga, D. K.; Smyth, A. J.; Hayes, P.; Bromidge, T. J. and Gibson, W. C. (1992): Sensitive detection of trypanosomes in *tsetse* flies by DNA amplification. Int. J. Parasitol. 22(7): 909-918.

Mekata, H.; Konnai, S.; Witola, W. H.; Inoue, N.; Onuma, M. and Ohashi, K. (2009): Molecular detection of trypanosomes in cattle in South America and genetic diversity of *Trypanosoma evansi* based on expression-site-associated gene 6. Infect. Genet. Evol. 9(6): 1301-1305.

Milocco, C.; Kamyingkird, K.; Desquesnes, M.; Jittapalapong, S.; Herbreteau, V.; Chaval, Y.; Douangboupha, B. and Morand, S. (2012): Molecular demonstration of *Trypanosoma evansi* DNA in wild rodents from Cambodia, Lao PDR and Thailand. Transbound Emerg. Dis. 60(1): 17-26.

Mossaad, E.; Ismail, A. A.; Ibrahim, A. M.; Musinguzi, P.; Angara, T. E.; Xuan, X.; Inoue, N. and Suganuma, K. (2020): Prevalence of different trypanosomes in livestock in Blue Nile and West Kordofan States, Sudan. Acta Trop. 203: 105-302.

Muieed, M. A.; Chaudhry, Z. I. and Shakoori, A. R. (2010): comparative studies on the sensitivity of polymerase chain reaction (PCR) and microscopic examination for the detection of *Trypanosoma evansi* in horses. Turkish J. Vet. Anim. Sci. 34(6): 507-512.

Musinguzi, S. P.; Suganuma, K.; Davaasuren, B.; Battsetseg, B.; Battur, B. and Inoue, N. (2022): A PCR-based survey of animal trypanosomes among domestic animals herded together in the Bayan-Ulgii and Khovd provinces of Mongolia. J. Protozool. Res. 32(1-2): 1-10.

Nantulya, V. M. (1990): Trypanosomiasis in domestic animals: the problem of diagnosis. Rev. Sci. Tech. off. Int. Epiz. 9(2): 357-367.

Parreira, D. R.; Jansen, A. M.; Abreu, U. G.; Macedo, G. C.; Silva, A. R.; Mazur, C.; Andrade, G. B. and Herrera, H. M. (2016): Health and epidemiological approaches of *Trypanosoma evansi* and equine infectious anemia virus in naturally infected horses at Southern Pantanal. Acta Trop. 163: 98-102.

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

Reid, S. A. (2002): *Trypanosoma evansi* control and containment in Australasia. Trends Parasitol. 18(5): 219-224.

Setiawan, A.; Nurcahyo, W.; Priyowidodo, D.; Budiati, R. T. and Susanti, D. S. R. (2021): Genetic and parasitological identification of *Trypanosoma evansi* infecting cattle in South Sulawesia, Indonesia. Vet. World. 14(1): 113.

Sharma, R.; Choudhary, N. S.; Bagherwal, R. K.; Agrawal, V.; Mehta, H. K.; Choudhary, K. and Parashar, R. (2018): Incidence of trypanosomosis in buffaloes in Indore and Ratlam districts of Madhya Pradesh.Vet. Pract. 19(2): 222-224.

Silva, R. A. M. S.; Ban-os, A. T. M. and Herrera, H. M. (1995): Trypanosomosis outbreaks due to *Trypanosoma evansi* in the Pantanal, Brazil. A preliminary approach on risk factors. Revue Elev. Méd. Vét. Pays Trop. 48(4): 315-319.

Sloss, M. W.; Kemp, R. L. and Zajac, A. M. (1994): Veterinary Clinical Parasitology. 6th edit. Lowa State University Press/ Ames: 101-120.

Sumba, A. L.; Mihok, S. and Oyieke, F. A. (1998): Mechanical transmission of

Trypanosoma evansi and *T. congolense Stomoxys niger* and *S. taeniatus* in a laboratory mouse model. Med. Vet. Entomol. 12(4): 417-422.

Ventura, R. M.; Takeda, G. F.; Silva, R. A. M. S.; Nunes, V. I. B.; Buck, G. and Teixeira, M. M. G. (2002): Genetic relatedness among *Trypanosoma evansi* stocks by random amplification of polymorphic DNA and evaluation of a synapomorphic DNA fragment for species-specific diagnosis. Int. J. Parasitol. 32(1): 53-63.

Verma, B. B. and Gaut, O. P. (1978): Studies on buffaloes and cow calves. Indian Vet. J. 55: 648-633.

Viseshakul, N. and Panyim, S. (1990): Specific DNA probe for the sensitive detection of *Trypanosoma evansi*. Southeast Asian J. of Trop. Med. Public Health. 21(1): 21-27.

Zahoor, J.; Kashif, M.; Nasir, A.; Bakhsh, M.; Qamar, M. F.; Sikandar, A. and Rehman, A. (2022): Molecular detection and therapeutic study of *Trypanosoma brucei evansi* from naturally infected horses in Punjab, Pakistan. Pol. J .Vet. Sci. 25(3): 429-435.

Zayed, A. A.; Habeeb, S. M.; Allam, N. A. T.; Ashry, H. M. Z.; Mohamed, A. H. H; Ashour, A. A. and Taha, H. A. (2010). A critical comparative study of parasitological and serological differential diagnostic methods of *Trypanosoma evansi* infections in some farm animals in Egypt. Am.-Eurasian J. of Agric. Environ. Sci. 8(6): 633-642.

الملخص العربى

يعتبر مرض (س ُرا)، والذى يسببه طفيل مثقبيات الجمال (تريبانوسوما إيفانسى)، من الأمراض الهامه والتى تؤدى إلى خسائر إقتصاديه كبيره فى الحيوانات المصابه من الأبقار والجاموس والأغنام والماعز والخيول والكلاب.

تم إجراء الدراسه الحاليه لقياس معدل إنتشار الطفيل فى الحيوانات التى تم فحصها من الأبقار والجاموس والأغنام والماعز والخيول والكلاب فى محافظتى الاسكندريه والبحيره.

تم تجميع عدد 300 عينة دم من الحيوانات من الأبقار والجاموس والأغنام والماعز والخيول والكلاب حيث تم أخذ 50 عينه من كل فصيله من المجازر والمزارع والقطعان الصغيره فى الفتره ما بين يوليو 2021 حتى ديسمبر 2022 وتم تشخيص الإصابه بطفيل مثقبيات الجمال عن طريق عمل مسحات من الدم الخفيفه المصبوغه بصبغة الجيمسا وإختبار تفاعل إنزيم البلمره المتسلسل.

وقد اوضحت الدراسه أنه لا يوجد أى إصابات عند فحص 300 من مسحات الدم الخفيفه من الحيوانات من مختلف الأعمار والأجناس فى كلتا المحافظتين بينما تبين إصابة 104 حيوان عند فحص عدد 180 عينه بإستخدام تفاعل إنزيم البلمره المتسلسل بمعدل إصابه 57.78% حيث كان معدل الإصابه فى الحيوانات المختلفه كالآتى:46,67% فى الأبقار و 70% فى الجاموس و40% فى الأغنام و30% فى الماعز و66.67% فى الخيول و93.33% فى الكلاب.

وقد قدمت هذه الدراسه نسب الاصابه بمثقبيات الجمال (تريبانوسوما إيفانسى) فى الحيوانات المختلفه بإستخدام الفحص الميكروسكوبى وإختبار تفاعل إنزيم البلمره المتسلسل وقد تبين من النتائج ان إختبار تفاعل إنزيم البلمره المتسلسل هو الاكثر حساسيه فى تشخيص العدوى بمثقبيات إيفانسى فى الحيونات المختلفه ولذلك ينصح بإستخدامه فى الدراسات المستقبليه الخاصه بهذا الطفيل.