Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 4, 597-601

PCR: A sensitive diagnostic tool for *Trypanosoma evansi* in camels in Egypt

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ARTICLE INFO

Recieved: 06 January 2024

Accepted: 28 February 2024

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Keywords:

Camels
PCR
Stained blood smears
Trypanosoma evansi

ABSTRACT

Camel Trypanosomiasis, or Surra, or El Debab as better known, caused by *Trypanosoma evansi* constitutes an economically important disease that affects the health and production of camels. Two-hundred and ninety-five samples from camels of different ages and sexes were collected from five geographic locations in Egypt (Behera, Cairo, South Sinai, Matrouh, Halayeb and Shalateen). Giemsa-stained smears that were prepared from blood samples were examined microscopically, while PCR coupled with DNA sequencing was applied for molecular detection and phylogenetic analysis. Microscopic and molecular findings revealed a prevalence of 0.34% and 50.51% in the examined camels through stained blood smears and PCR techniques, respectively. *T. evansi* is enzootic in Egypt, and the PCR technique could preferably be applied in surveillance studies as a more sensitive detection method.

Introduction

Camels are known for their importance as a source of meat and milk, despite the scanty food resources they utilize in the arid areas of the world (Joshua *et al*., 2008). Thousands of camels are regularly transported into Egypt from Sudan, Somalia and Libya for breeding and slaughtering purposes (Barghash *et al*., 2016). Egypt has more camels being slaughtered for meat than ever before, especially after the global increase in protein prices (Abou El-Naga and Barghash, 2016). Camels are susceptible to many parasitic diseases, with surra disease standing out as the leading cause of high morbidity and mortality (Enwezor and Sackey, 2005). The causative agent, *Trypanosoma evansi* (*T. evansi*), represents one of the most pathogenic protozoans which affects camels and a wide variety of hosts. It belongs to the subgenus Trypanozoon, family Trypanosomatidae (Taylor *et al*., 2016). As unicellular, hemoflagellate, blood-sucking flies of the genus Tabanus and Stomoxys are the main mechanically transmitting vectors of *T. evansi* in affected countries in Africa and Asia, meanwhile, vampire bats were incriminated in biological transmission in South America (Aregawi *et al*., 2019). As a result of the loss of the maxicircle kDNA, *T. evansi* is assumed to have evolved from T. brucei which makes it unable to undergo cyclic development in tsetse flies and widen its geographical distribution and host range (Lun and Desser, 1995). Surra disease is manifested by fever, weight loss, anemia, decreased appetite, body condition and productivity (Desquesnes *et al*., 2013). Compared to horses, camels seem to have a more chronic form of the disease (Biswas *et al*., 2001). Studies on the prevalence of *T. evansi* have shown that *T. evansi* is regarded as an enzootic protozoan parasite in Egyptian camels (Abdel-Rady, 2006; Amer *et al*., 2011; Elhaig *et al*., 2013). Detection of *T. evansi* relies mainly on parasitological examination of blood films which has the disadvantage of low sensitivity especially in chronic cases and asymptomatic carriers (Desquesnes *et al*., 2022). On the contrary, molecular diagnosis of *T. evansi* revealed high sensitivity (Omanwar *et al*., 1999; Holland *et al*., 2004; Sengupta *et al*., 2010). PCR assay employing TBR1/2 primers has the highest sensitivity while maintaining a good specificity in epidemiological studies and could detect as low as a single trypanosome cell /ml of rat blood (Pruvot *et al*., 2010). The current study aimed to estimate the occurrence of *T. evansi* in camels in different localities of Egypt using both stained blood smears examination and TBR1/2-PCR based technique. DNA sequencing and analysis were set to infer the phylogenetic relationship between different isolates of *T. evansi*.

Materials and methods

Study design and area

The study was designed as a cross-sectional survey aiming to assess the prevalence of *T. evansi* in camels in different localities in Egypt and to determine the genetic relatedness between them. Therefore, five localities where camels were reared or slaughtered were selected including Behera, Cairo, South Sinai, Matrouh, Halayeb and Shalateen.

Collection of samples

A total of 295 samples were acquired at random from camels that appeared healthy, of various ages and both sexes from Behera, Cairo, South Sinai, Matrouh, Halayeb, and Shalateen from Spring to late Summer (March to September), 2019. Data concerning the number of samples collected from individual localities is outlined in Table 2. Blood was

ISSN: 2090-6277/2090-6269/

collected either from the jugular vein of live animals or during slaughtering into sterile vacutainer tubes containing EDTA as an anticoagulant. Blood samples were transferred to the laboratory of the Parasitology Department, Faculty of Veterinary Medicine, Alexandria University in a cooling box. Blood samples were then processed accordingly for microscopic examination and molecular diagnosis.

Microscopic examination

Thin smears were prepared from blood samples, air-dried, and then fixed in methanol for 2 min. Smears were stained with Giemsa stain for 45 min, rinsed with water and allowed to air-dry. Fixed stained smears were examined under 40x of the light optical binocular microscope (Optika, Italy), where about 20 microscopic fields were examined per individual slide (Taylor *et al*., 2016).

Molecular diagnosis

DNA extraction

DNA was extracted from the whole blood samples by applying the GeneJet DNA Purification Mini Kit (Thermo Scientific, Lithuania) according to the default manufacturer's instructions. Two hundred microliters of DNA product were eluted, and the integrity of the eluted total genomic DNA was assessed by agarose gel electrophoresis. DNA samples were kept at -20°C until used. Control positive was kindly provided as a DNA sample by Dr. Helen Price, School of Life Sciences, Keele University, UK.

Polymerase Chain Reaction (PCR)

Primers specific to amplify a 164 bp sequence, and that targeting the minichromosomal satellite DNA in the subgenus trypanozoon, was generated and designated TBR 1/2. Sense and antisense sequences of TBR 1/2 are shown in Table 1. The ultimate primer concentration utilized was 10 pmol /µl. Using the PCR amplification technique, 295 samples in total were examined, essentially following the PCR protocol of Henidy *et al*. (2019). With a total reaction volume of 25 µl, the PCR reaction contained 12.5 µl Dream Taq Green master mix (Thermo Scientific), 1.5 µl DNA template, 1 µl of each primer (10 pmol /µl), and 9 µl nuclease-free water was performed. A total thermal cycling of 35 cycles were performed after an initial denaturation step of 95°C for 3 min. Each thermal cycle comprised of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 30 seconds. The final extension step at 72°C for 7 min was performed. PCR was performed in a 3Prime thermal cycler (TECHNE, UK).

Gel electrophoresis

Integrities of the purified whole genomic DNA and the PCR products were assessed by electrophoresing 10 µl of samples in 1% agarose gel. Electrophoresis was performed at 100 V for 1 hr, gels were stained with ethidium bromide (0.5 µg/ml), and 100 bp DNA ladder as a concentration of 105 ng/µgl (Jena Bioscience) was included as a size marker. Gels were visualized by the gel documentation system UVP PhotoDoc-itTM Imaging System (analytikjena, USA).

Sequencing reaction

Using an Applied Biosystems 3130 automated DNA Sequencer, the sequences of forward and reverse direction of the purified PCR product were obtained (ABI, 3130, USA). Sequencing reactions were performed using the same PCR primers, and by applying the ready reaction Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer/Applied Biosystems, Foster City, CA- Cat. No. 4336817). Sequencing reactions were done according to the instructions of the manufacturer. Sequence outputs were then applied in the deep-mining BLAST® (Basic Local Alignment Search Tool) analysis (Altschul *et al*., 1990), to establish the molecular identities of individual sequences with their GenBank accessions. Identities between individual sequences were aligned in a diagonal analysis to establish the homology relationship between different *T. evansi* isolates.

Phylogenetic analysis

Sequences that were retrieved from the Genbank were assessed for homologies by aligning in the CLUSTAL Omega multiple sequence alignment program of EBI tools (https://www.ebi.ac.uk/Tools/msa/clustalo/). Sequences of minisatellite repetitive TBR of average lengths of 130-135 nucleotides were fed into the DNA portal of the Clustal Omega tool. Default settings were upheld throughout the analysis. The seed tree file generated from the alignment was then applied to the phylogenetic analysis.

MEGA version 11 was used to execute the phylogenetic and molecular evolutionary studies (Tamura *et al*., 2021). The phylogenetic tree was built using the Maximum Likelihood (ML) algorithm embedded in the MEGA11 software. Default settings were upheld throughout the analysis.

Statistical analysis

The collected prevalence data of examined camels using thin blood smears and PCR technique were subjected to the chi-square analysis using SAS, 2004 software, and P values <0.05 were considered significant.

Results

Prevalence of T. evansi based on microscopic examination of blood smears

The total prevalence of *T. evansi* in the 295 examined camels was 0.43% by microscopic examination of blood smears, which corresponded to only one positive sample out of 295 examined animals. This single *T. evansi*-positive camel was in Matrouh, which accounted for only 3.45% out of 29 camels from Matrouh (Table 2). The revealed parasite was leaf-like and mono-flagellate protozoan. There was no statistical difference in the prevalence between different localities (P= 0.0562) through the examination of blood films.

Prevalence of T. evansi based on molecular diagnosis

PCR technique targeting Minichromosomal satellite DNA revealed 50.5% (149 samples) of positive camels for *T. evansi* (Table 2). Based on the specific primer, the PCR product was at 164 bp (Fig. 1). The PCR product was of multiple bands due to the tandem repeat nature of the parasite. The PCR revealed a prevalence of 60, 51.16, 56.67, 37.93 and 22.86% of *T. evansi* in Behera, Cairo, South Sinai, Matrouh, Halayeb and Shalateen,

Table 1. The sequences of TBR 1/2 primers

Primer name	Primer sequence	Amplicon length	Reference
TBR-1 Forward	5′-GAATATTAAACAATGCGCAG - 3′	164 bp	Herrera *et al*. (2005)
TBR-2 Reverse	5′-CCATTTATTAGCTTTGTTGC-3′		

respectively, which is significantly different (P= 0.0018).

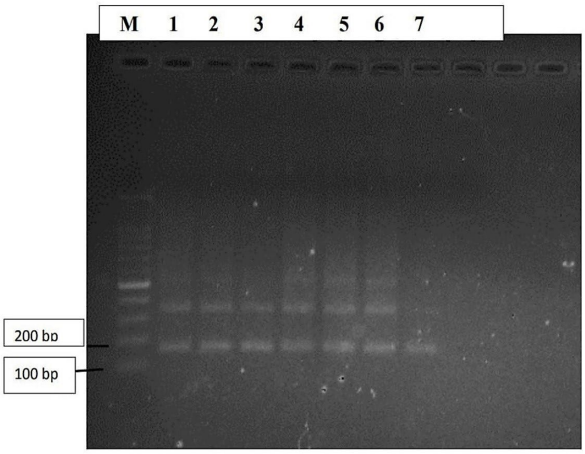

Fig. 1. Ethidium bromide-stained agarose gel of PCR for *T. evansi* showing bands at 164 bp (M= 100 bp DNA ladder, lane 1, 2, 3, 4, 5 and 6=positive samples, Lane 7= control positive. Dimers due to the repetitive nature of the target gene.

Phylogenetic analysis

Six sequences corresponded to different isolates of *T. evansi* from Kom Hamada, Behera (two sequences), Sinai, Cairo, Matrouh, and Halaib and Shalateen, showed considerable homologies to each other, while also were highly homologous to eight *T. evansi* isolates from the neighboring Sudan. The maximum homologies (identities) of 100% were detected between Cairo isolates and those originating from Gedaref 2&3, Ad Damer 2, Sidoon, and Port Sudan (Table 3). Similarly, three Sudanese isolates showed 100% identities to Halaib and Shalateen isolates, which are Ad Damer 2, Sidoon, and Port Sudan. The lowest homologies were recorded between Kom Hamada and Halaib/Shalateen sequences (87.31%), while >98% identities were generally recorded between Egyptian and Sudanese isolates (Table 3).

The considerable homologies between isolates from Egypt and Sudan were reflected in the evolutionary analysis as demonstrated by the multiple sequence alignment (Fig. 2) and the ML phylogenetic tree (Fig. 3). For the multiple sequence alignment, identity > 64% was recorded between all sixteen sequences, with most of nucleotide's substitutions were recorded at the 5′ proximities of minisatellite TBR sequences (Fig. 2). Only two insertions/deletions were identified at positions 70-71 of majority of sequences. The evolutionary relationship between various *T. evansi* isolates was measured in an ML tree, where lengths of branches were proportionally corresponding to evolutionary distances between *T. evansi* isolates. As shown in Fig. 2, and except for the outgroup branch (*T. brucei rhodesiense*), two main branches were detected with the first branch including Kom hamada two sequences, Matrouh, Kassala, and Gedaref 3. The second branch was further subdivided into two branches,

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MF142295.1_New Halfa 2   ---ACATTTAACCATACTTTTATGTGTGCATATTAATTACTAGTGTGCACCTTTAAATACAATTGTTTACAAT-TAAT 75
MF142296.1_Gedaref 1     ---CCCAATTTACATACTTTTATGTGTGCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 75
EG_Sinai                 ----CTTATACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAGTTAAT 74
EG_Matrouh               -----CTAACCCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAGTTAAT 73
EG_Kom Hamada S1         ---AATCACCCATACTTTTATGTAGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 75
EG_Kom Hamada S2         -----AAAACAAATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACGT-TAAT 73
MF142299.1_Gedaref 3     CCCCTTTAAACATAACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 78
MF142292.1_ Ad Damer 1   ----GGACTTACATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 74
EG_Cairo                 ------CTTACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 72
MF142290.1_Port Sudan    ----ACCTTACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 74
MF142297.1_Gedaref 2     ---CCATTTACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 75
MF142291.1_Sidoon        ---CCATTTACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 75
MF142294.1_New Halfa 1   ---CCATTTTCCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 75
MF142293.1_Ad Damer 2    --CCAATTTACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 76
EG_Halaib&Shalateen      -----CTTTACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 73
MF142298.1_Kassala       --CCACTTAACCATACTTTTATGTGTGCCATATTAATTACAAGTGTGCAACATTAAATACAAGTGTGTAACAT-TAAT 76
                                        **         ************ ******** * ********** *** **     ****

MF142295.1_New Halfa 2   TTGCAGGTTTGCACCGCTGTTCTTTATGGTTTAAGGTGGGCAACAAACCTAATAATGGGA     134
MF142296.1_Gedaref 1     TTGCAAGTTTGCACCCCCTGTCCTTTAGGGTTTAAGGTGGGCAACAAACCTAATAAATGGA     134
EG_Sinai                 TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     134
EG_Matrouh               TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     133
EG_Kom Hamada S1         TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     134
EG_Kom Hamada S2         TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     132
MF142299.1_Gedaref 3     TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     137
MF142292.1_ Ad Damer 1   TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     133
EG_Cairo                 TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     131
MF142290.1_Port Sudan    TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     133
MF142297.1_Gedaref 2     TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     134
MF142291.1_Sidoon        TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     134
MF142294.1_New Halfa 1   TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     134
MF142293.1_Ad Damer 2    TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     135
EG_Halaib&Shalateen      TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     132
MF142298.1_Kassala       TTGCAAGTTTGCAACAATGTTCTTTAGTGTTTAATGGGTGCAACAAAGCTAATAAATGGA     135
                         ***** ******* *  *** *****  ****** * * ******** *******  ***
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Fig. 2. Multiple sequence alignment of the minisatellite TBR repetitive sequences of *T. evansi* isolates. Sequences were extracted from GenBank and uploaded into the DNA alignment portal of the Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/). Default settings were upheld throughout the analysis. Identical nucleotides were denoted by (*) while gaps represent non-homologous nucleotides. Numbers on the left indicate the total lengths of minisatellite sequences.

Table 2. Total prevalence of *T. evansi* in examined camels using parasitological and molecular techniques.

Site of sample collection	Number examined	Thin blood smear		PCR		Chi-square value and P-Value
		Positive	%	Positive	%	
Behera	115	0	0	69	60	195.81*** P<.0001
Cairo	86	0	0	44	51.16	
South Sinai	30	0	0	17	56. 67	
Matrouh	29	1	3.45	11	37. 93	
Halayeb and Shalateen	35	0	0	8	22. 86	
Total	295	1	0.34	149	50.51	
Chi-square value and P-Value		9.20 NS (P= 0.0562)		17.16 * (P= 0.0018)		

one including four isolates (Cairo, Halaib & Shalateen, Port Sudan, Al Damer 1) and the rest of seven isolates, with the except of Sinai, are all Sudanese (Gedaref 1&2, Siddon, New Halfa 1&2, Al Damer 2). Overall, the ML phylogenetic tree failed to demarcate Egypt from Sudanese isolates, indicating very close evolutionary relatedness.

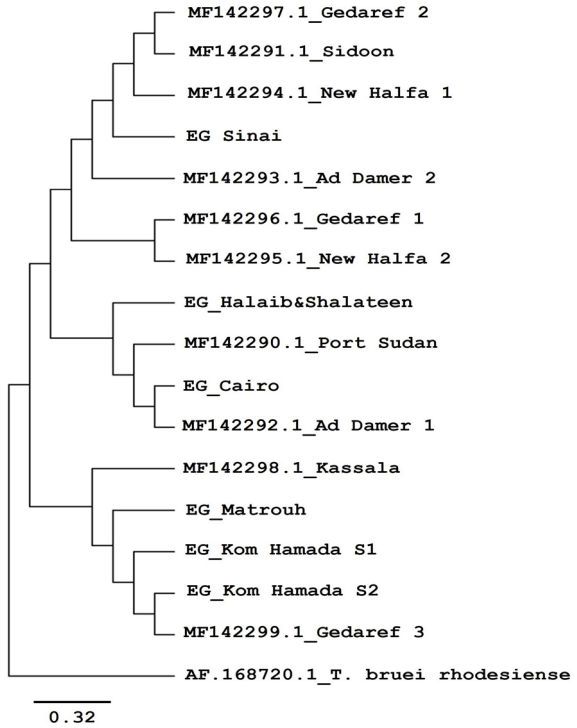

Fig. 3. Maximum Likelihood phylogenetic tree of *T. evansi* isolates. Multiple sequence alignment file was fed into the ML construct portal of MEGA 11 software (Tamura *et al*., 2021). Lengths of tree branches indicate evolutionary distances, while the scale bar = 0.32 length unit. The Minisatellite TBR sequence of T. brucei rhodesiense was included as an outgroup to confirm the authentic relatedness of the tree evolutionary branching.

Discussion

Trypanosomiasis (Surra) is known for its negative effect on the camel's industry. It is endemic in camels in Egypt with a high prevalence due to the high abundance of vector flies. A relevant trypanosomiasis control program required firstly a standardized method of diagnosis, notably with the observed limited sensitivity of microscopic examination to diagnose chronic cases of trypanosomiasis (Barghash *et al*., 2014).

Interestingly, camels imported from Sudan were a significant source of *T. evansi* in Egypt. As reported by Zaitoun *et al*. (2017), the prevalence of *T. evansi* in Abu-Simbel Veterinary Quarantine at the South border of Egypt was 43.3% among the 396 examined camels during the quarantine period (48 and 20% among clinically suspected and apparently healthy camels, respectively) using TBR1/2 PCR assay. Moreover, blood film microscopic analysis showed a prevalence of 12.17 and 0%, respectively.

A cross-sectional study was performed to assess the prevalence of *T. evansi* in Egypt in five localities, Behera, Cairo, South Sinai, Matrouh, Halayeb and Shalateen, applying the microscopic examination and the PCR molecular assay.

As expected, the examination of blood films was not sensitive enough during the investigation of trypanosomiasis and epidemiological studies.

Examination of blood films, in Matrouh, revealed only one positive sample (3.45%) for *T. evansi*. Whereas a higher prevalence of 20.24, 20.9 and 20.6% of *T. evansi* in camels was reported by Abou El-Naga and Barghash (2016); Barghash *et al*. (2014) and Sobhy *et al*. (2017) using Giemsa-stained blood smears in the Matrouh governorate.

Blood smear examination in other localities showed negative results for *T. evansi*. Rates of 1.66 and 1.4% were recorded by Khedr (2016) and Hegazy (2017) in Behera through microscopic examination. While Abo-Aziza *et al* (2017) reported a 13% infection rate in camels in Giza via microscopic examination of blood films. Also, 4.76 and 2.9% of the examined camels were reported to be positive by stained blood smears in Iran and Sudan by Ahmadi-hamedani *et al*. (2014); and Bala *et al*. (2018), respectively.

The total prevalence of *T. evansi* in the 295 camels through exam

Table 3. Diagonal analysis of homology-based identities between different isolates of *T. evansi* as identified by the NCBI BLAST analysis.

Seq/Seq Identities (%)	KH_Seq1	KH_Seq2	Sinai	Cairo	Matrouh	Halaib & Shalateen	Kassala	Gedaref 2	New Halfa 1	Ad Damer2	Sidoon	Port Sudan	Ad Damer 1	Gedaref 3
KH_Seq* 1	100	92.54	90.3	88.8	88.06	87.31	99.22	99.22	99.22	99.22	99.22	99.22	99.22	99.22
KH_Seq** 2	92.54	100	93.28	94.66	95.5	93.94	99.2	99.2	99.2	99.2	99.2	99.2	99.2	99.2
Sinai	90.3	93.28	100	95.52	97.01	96.27	98.51	99.23	99.23	99.23	99.23	99.23	99.23	99.23
Cairo	88.8	94.66	95.52	100	94.74	98.48	99.24	100	99.23	100	100	100	99.24	100
Matrouh	88.06	95.5	97.01	94.74	100	96.24	98.48	99.22	99.22	99.22	99.22	99.22	99.21	98.44
Halaib & Shalateen	87.31	93.94	96.27	98.28	96.24	100	99.24	100	99.24	100	100	100	99.23	99.22
Kassala	99.22	99.2	98.51	99.24	98.48	99.24	100	97.76	97.01	98.52	97.76	97.04	93.33	94.16
Gedaref 2	99.22	99.2	99.23	100	99.22	100	97.76	100	92.48	92.48	91.11	91.79	91.79	91.79
New Halfa 1	99.22	99.2	99.23	99.23	99.22	99.24	97.01	92.48	100	97.78	99.25	96.27	95.52	91.79
Ad Damer 2	99.22	99.2	99.23	100	99.22	100	98.52	92.48	97.78	100	98.52	97.76	94.07	93.43
Sidoon	99.22	99.2	99.23	100	99.22	100	97.76	91.11	99.25	98.52	100	97.01	94.78	92.7
Port Sudan	99.22	99.2	99.23	100	99.22	100	97.04	91.79	96.27	97.76	97.01	100	95.49	91.24
Ad Damer 1	99.21	99.2	99.23	99.24	99.21	99.23	93.33	91.79	95.52	94.07	94.78	95.49	100	89.78
Gedaref 3	98.44	99.2	99.23	100	98.44	99.22	94.16	91.79	91.79	93.43	92.7	91.24	89.78	100

*: Kom Hamada Sequence 1; **: Kom Hamada Sequence 2

ination of blood smears was 0.34% which confirms the minimal sensitivity of parasitological examination especially in chronic trypanosomiasis. That makes parasitological examination of low value in epidemiological studies. PCR is a powerful tool for diagnosis with high sensitivity that can detect low parasitemia.

Using the PCR technique, Behera recorded the highest prevalence (60%). A similar rate of infection in Behera (63%) was reported in camels by Henidy *et al*. (2019). Even though, Hegazy (2017) reported 90% infection rate which is higher than the reported prevalence. This may be attributed to variations in the time of sample collection.

While South Sinai, Cairo, Matrouh, Halayeb and Shalateen reported a prevalence of 56.67, 51.16, 37.93 and 22.86%, respectively. In Matrouh, Sobhy *et al*. (2017) detected a prevalence rate of 64.3% in 378 camels by RoTat1.2 –PCR. Also, Elhaig *et al*. (2013), in Ismailia, reported a prevalence of 10, 16 and 46% using the RoTat1.2 VSG gene, ITS-1, and TBR1 /2, respectively. This reported variation in the prevalence rates may be attributed to differences in localities and subsequently climatic conditions that affect vector abundance (Baldacchino *et al*., 2017).

PCR results indicated a higher sensitivity (P<.0001) that could detect as low as a single trypanosome parasite/10 µl blood when compared with parasitological means of diagnosis (Wuyts *et al*., 1994).

Phylogenetic analysis of the obtained sequences revealed high similarity between the different isolates which confirms their near origin. Moreover, there was a high genetic relatedness demonstrated by a high homology of the isolates (this study) with eight isolates from Sudan which constitute a main portal for camel importation to Egypt. The hundred percent homologies between current isolates and that of Gedaref 2&3, Ad Damer 2, sidoon, and Port Sudan proved the evolutionary relatedness.

Conclusion

Data from the present study indicated that *T. evansi* is enzootic in camels in Egypt. The low sensitivities of microscopic examination in detecting *T. evansi* once again have confirmed its limited application in diagnosing *T. evansi*-infected cases. PCR, on the other hand, has proved its effectiveness as a molecular detection technique, which could be used effectively in epidemiological studies.

Acknowledgments

This research was funded by the Science and Technology Development Fund (STDF) through Alexandria University, Egypt, and Keele University, UK collaboration (Newton-Fund Institutional Links Grants) with a project entitled "Investigating drug resistance and new treatments for *Trypanosoma evansi* in Egyptian camels" (code number 27730).

Conflict of interest

The authors declare that there are no conflicts of interest.

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