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Evaluation of *T. evansi* Using Different Diagnostic Techniques with Experimentally Infected (Dromedary) Camels

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Abstract

Trypanosoma evansi is a parasitic protozoan that allows surra disease to spread across subtropical and tropical regions of the universe. The objective of the present study was to evaluate the diagnostic techniques using experimentally infected (dromedary) Camels. Thirty-five apparently healthy adult camels of both sexes were used in this experiment. The camels were administered intravenously with 0.5 ml of blood infected with *T. evansi* via the lateral abdominal vein. Blood samples were obtained from the camels on days 0, 4, 8, 12, 16, 20, 24, and 28 after infection to evaluate the detection rate of the various diagnostic tests. The receiver operating characteristic curve (ROC-Curve) was used for the evaluation of the sensitivity of the diagnostic techniques; BCT=50.0%, CATT/*T. evansi*=71.84%, MI=61.63%, PCR=81.43%, TBS=68.37% and WBF=68.37% respectively. On day 4, no *T. evansi* were detected for the following techniques; WBF, BCT, TBS, and MI. While the detection rate of the CATT/*T. evansi* and PCR techniques were 70% and 100% respectively. The detection rate of WBF, TBS, BCT, MI, CATT/*T. evansi* and PCR on day 24 of the experimental analysis were not statistically different. The prevalence rate of WBF was significantly lower (p>0.01) compared with PCR (WBF=60%, CI=44%, 74%; PCR=91%, CI=78%, 97%). The association between the different diagnostic techniques and detection of *T. evansi* infection in experimentally infected Camels was strong and significant (Chi-squared=13.720, degree of freedom (df)=5, p=0.0175). In conclusion, PCR has the highest detection rate and is the most sensitive technique. The association between the different diagnostic techniques and detection of *T. evansi* infection in experimentally infected Camels was strong and significant.

Keywords

Camels • Diagnostic techniques • Prevalence • *T. evansi*

Introduction

Trypanosoma evansi is a protozoan organism of that causes surra disease all through the tropical and subtropical regions of the world, as well as for Asia, Africa and Latin America [1,2]. Livestock trypanosomes triggered by *T. evansi*, a member of the Trypanozoon subgenus, it has a major socio-economic effect and limits the performance of animal proteins worldwide [3-5]. It has been the most widely distributed pathogenic animal *T. evansi* affecting domesticable animals in Africa, Asia, Central and South America [5,6]. In Africa, it is primarily a protozoan disease of camels, prevalent in the Sahel zone, above the Tsetse belt, where it is disseminated mechanically by haematophagous insects such as stomoxys and tabanids [7]. The role of transmission can even be highlighted by the fact that there are serious forms of the disease in horses and camels, while cattle and buffalo are deemed to be impactful sources of the disease [8]. Its economic impact is often underestimated and losses may be due to mortality, reduced animal production, lower productivity resistance, poor carcass quality, weight loss, decreased milk yield, low reproductive output and therapy costs [9]. Classical clinical symptoms of surra entail recurrent fever, anaemia, mostly owing to haemolysis, and erythrophagocytosis [10].

Specific canonical parasitological approaches for the evaluation of trypanosomosis such as microscopic analysis of blood or lymph node aspirates were used. However, these are not very sensitive, but a range of techniques are used in order to enhance the sensitivity by enrichment of sample, inoculation of mouse and DNA methods. In addition to *T. evansi*, specific detection by microscopy, molecular tools are very useful for species specific diagnosis [11]. Numerous sorts of genetic and molecular approaches have been constantly revised to increase the accuracy of the diagnosis and classification of Trypanozoon species. In particular, the evaluation and comprehensive review of genomes have been strongly linked to *T. evansi* recognition and can give rise to a verifiable gene hypothesis that may be accountable for variations in dysfunctions amongst parasitic variants or subspecies [12]. Several methods have been developed for the detection of *T. evansi* infection, but among these, Polymerase Chain Reaction (PCR) is identified to be the most sensitive and specific, and it has a wider range of application [13,14]. Reliable epidemiological research (such as PCR-based methods) is a precondition for designing effective control programs against trypanosomosis. PCR allows detections of specific trypanosomal DNA through the use of species-specific primers [15]. PCR is likely attributed to the parasitic and tests of antigen detection owing to its responsiveness in assessing the pre-patent and protracted process of infection [16].

Early detection may play a role for the control of *T. evansi*, and amidst the adoption of a number of screening procedures, the diagnosis of trypanosomosis remains difficult and the widely used tests have significant drawbacks, and diagnosis basically relies on traditional Giemsa staining of thin and thick blood smears. Tests of Serology that are used for antibodies detection against pathogens, faces reproducibility drawbacks owing to antigenic heterogeneity and large proportions of false negative and false positive outcomes [17-19]. To resolve the sensitivity and specificity limitations enforced by other diagnostic approaches, the molecular identification of *T. evansi* Deoxyribonucleic Acid (DNA) is favored, and some molecular markers have been widely constructed to detect, differentiate, and study trypanosome species such as Internal Transcribed Spacer-1 (ITS-1) and Rhode Trypanozoon Antigen Type 1.2 VSG gene (RoTat-1.2 VSG) [20,21].

Therefore, the present study was designed to determine the diagnostic sensitivity of the current parasitological techniques for the detection of *T. evansi* in experimentally infected camels using (Thin Blood Smear (TBS), Wet Blood Film (WBF), Buffy-coat Technique (BCT), Mouse Inoculation (MI), antibody detection test (CATT/*T. evansi*) and PCR. Furthermore, to compare all these techniques, ROC-curve was employed to identify the most sensitive technique amongst the different methods of identifying *T. evansi*.

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Materials and Methods

Source of trypanosomes

The *Trypanosoma evansi* used in the present study was isolated from natural infections of surra in camels at the University of Maiduguri, Faculty of Veterinary Medicine, Department of Veterinary Parasitology and Entomology. It was defined in the negative Blood Inhibition and the Infectivity Test (BIIT) and for the reason of phenotype, stabilized by six passages in Wistar rats, and sent to the Nigeria Institute for Trypanosomiasis and Onchocerciasis Research (NITOR) Vom, Nigeria where it's been validated as *T. evansi* (Code: T.e.mguri 2018) by a series of parasitological and molecular characterization of the isolate. The dose of the inoculum, 1.0 x 10^3 tryps/ ml was inoculated intraperitoneally into Wistar rats to enlarge the inoculums, when parasitaemia has produced nearly 5.0 x 10^3 tryps/ ml of blood, the infected blood was collected from the rats by cardiac puncture and successively diluted with phosphate buffered glucose saline (PBSG, pH 7.2) until 1.0 x 10^3 trypanosomes/0.5 ml was attained. Each camel was subsequently inoculated by means of the abdominal vein laterally with 0.5 ml of the infected blood.

Experimental animals

Thirty-five apparently healthy adult camels of both sexes were being used for this investigation and all the camels were evaluated for intestinal, blood, and external arthropod parasites and permitted to accustom to the environment for 60 days before the research began. They were housed in concrete floors and fly-proof stalls and fed wheat bran, groundnut husks, 5 kg of dry fodder daily per camel, watermelons, and sliced cucumber, whereas drinking water was given ad-libitum during the research.

Experimental infection

Peripheral blood samples were collected from the dromedary camels on days 0, 4, 8, 12, 16, 20, 24 and 28 post-infection. Two blood samples were collected from each animal, one with an anticoagulant (EDTA) for conventional parasitological diagnosis (thin blood smear, wet blood film, Buffy coat technique and mouse inoculation) and PCR. The second sample was placed in a plain vacutainer tube for serum collection.

Procedures for diagnosis of *Trypanosoma evansi*

Conventional parasitological methods: Wet Blood Film: Ten microliters of peripheral blood was placed on a clean glass slide, mixed gently with 1 ml of phosphate buffered saline, covered with a coverslip and examined at a magnification of x 100 for 15-20 mins [22].

Thin blood smear: At one end of a clean microscope slide a small drop of blood was placed and a thin film was drawn. It was fixed in methyl alcohol for 1 minute air dried. The timing used was 25 min for the smears to be stained with Giemsa (one drop of Giemsa+1 ml PBS, pH 7.2), and this was followed by rinsing the slides in tap water and drying. The thin smears of Giemsa stain were examined at a magnification of x 100 with immersion oil [22].

Mouse inoculation: Two Wistar rats were intra-peritoneally inoculated with 0.2-0.3 ml of camel blood. Three days post infection, blood samples were collected from the tail vein and blood films were prepared and examined by light microscopy for detection of trypanosomes.

Buffy-coat technique: Microhaematocrit centrifuge tubes were filled with blood containing anticoagulant and sealed with plasticine and centrifuged in microhaematocrit centrifuge at 1500 g for 5 minutes. A smear was prepared by scratching and breaking the capillary tube 1 mm below the surface of the Buffy-coat and one drop of the Buffy-coat was expelled onto a microscope slide, smeared and covered with a coverslip and examined at a magnification of x 100 for 15-20 mins [23].

Serological technique

Sera have been screened for antibodies to *T. evansi* using the CATT/*T. evansi* test (Institute of Tropical Medicine, Antwerp, Belgium). An antigen roughly measuring 45 µl was conveyed to the test card and combined with 25 µl of the test sera diluted at 1⁄4 with PBS pH 7.2 as instructed by the manufacturer. The card was stirred for 5 min and the reaction was tested in a clear light with a positive reaction verified by the monitoring of agglutinations (blue agglutinates) [24].

Molecular technique (polymerase chain reaction)

Genomic DNA from whole blood specimens was collected using an obtainable commercially DNeasy blood and tissue kit (Qiagen, Inc. Germany) as instructed by the manufacturer. The specimens were evaluated using the primer set RoTat 1.2 forward and reverse targeting 205bp fragments of the predominant surface glycoprotein of *T. evansi* [25]. The PCR amplification reaction was accomplished in a total reaction volume of 25 µl enclosing 50 ng of template DNA and 12.5 µl of commercially obtainable PCR Mastermix (Qiagen, Germany). The primer was utilised at a concentration of 10 pmol/ µl, and the PCR amplifications were performed in a Biometrathermocycler.

Cycling circumstances were as follows: initial denaturation at 94/3 mins (1 cycle), accompanied by 94/min (denaturation), 57˚C/1 min (annealing), and polymerization at 72˚C/1 min (40 cycles), final extension at 72˚C/5 min. Amplified products were electrophoresed through 1.5% agarose containing ethidium bromide and the voltage was set at 60 V for the electrophoretic mobility to visualize the amplified DNA and contrasted to a standard DNA100 bp (Qiagen, USA).

Statistical analysis

Receiver Operating Characteristic Curves (ROCC) is widely used to represent the relationship/trade-off between clinical sensitivity and specificity for each potential cut-off for a test or a series of tests in a graphical format. On the y-axis, sensitivity was plotted, while 1-specificity was plotted on the x-axis. It's determined by comparing the proportion of true positives (sensitivity) to the proportion of false positives (specificity or 1-specificity) based on the total number of real negatives. On both axes, they have values between 0 and 1. The capacity of the ROC curve as a diagnostic test to depict correctly certain diagnostic techniques based on the sensitivity and specificity is provided by the region to the left and above the diagonal line known as the line of no discrimination. On the other hand, the region under a ROC curve is used to assess the effectiveness of tests since it is a measure of the effectiveness of a test in general, with a larger area indicating a more sensitive test (Figure 1). A diagnostic test's sensitivity is defined as its ability to correctly depict true positives, while specificity is defined as its ability to correctly depict false positives. The data obtained were analyzed using receiver operating characteristic curve (ROC-Curve) for sensitivity and Chi-Square test at 95% confidence interval with JMP Version 11 (SAS, Inc. NC, USA).

Results

Table 1 showed the detection rate of *T. evansi* infection in camels using the different techniques. On day 4 and 8 of the experimental period, WBF, BCT, TBS and MI tests did not detect *T. evansi*. However, CATT/*T. evansi* showed a detection rate of 70% on day four and PCR detection rate was 100 %, while on day eight CATT/ *T. evansi* and PCR tests detection rate were 100%. On day 12 of the study, the detection rate of WBF, BCT, TBS and MI was 80%, conversely, the detection rate of CATT/*T. evansi* and PCR techniques were 100%. On day 16 of the study, the detection rate of WBF and TBS were 80% while CATT/*T. evansi*, BCT, MI, and PCR were 100%. On day 20 of the experimental study, the detection rate of WBF, TBS and MI was 80% while that of the CATT/*T. evansi* and PCR were 100%. On day 20 the detection rate of BCT, CATT/*T. evansi* and PCR were 100% while WBF, TBS and MI were 80%. On day 24 of the experimental study, the detection rate of WBF, TBS, BCT, MI, CATT/*T. evansi* and PCR produce similar results for the entire tests with 100%. On day 28 of the experimental study, the detection rate of WBF, TBS, BCT, TBS, and MI were 80% respectively whereas CATT/*T. evansi* and PCR have a detection rate of 100%.

Table 1. Detection rate of different diagnostic techniques in the detection of *T. evansi* in experimentally infected camels.

DAYS	WBF (%)	BCT (%)	TBS (%)	MI (%)	CATT/*T. evansi* (%)	PCR (%)
4	0	0	0	0	70	100
8	0	0	0	0	100	100
12	80	80	80	80	100	100
16	80	100	80	100	100	100
20	80	100	80	80	100	100
24	100	100	100	100	100	100
28	80	80	80	80	100	100

WBF: Wet Blood Film; BCT: Buffy Coat Technique; TBS: Thin Blood Smear; MI: Mouse Inoculation; CATT: Card Agglutination Test for *Trypanosoma evansi/T. evansi*; PCR: Polymerase Chain Reaction

Table 2 showed the prevalence of *T. evansi* using different diagnostic techniques with experimentally infected camels. The number of positive tests for WBF was 21 (60%), BCT and MI were 23 (66%), TBS was 22 (63%), CATT/*T. evansi* was 29 (83%) and PCR was 32 (91%).

Table 3 showed the association between *T. evansi* infection and the different diagnostic techniques in experimentally infected (dromedary) Camels, the association was strong and significant (Chi-squared=13.720, degree of freedom (df)=5, p=0.0175).

Figure 1 showed the sensitivity of different diagnostic techniques in the detection of *T. evansi* in experimentally infected Camels using the ROC-curve. The sensitivities of the following techniques were; BCT=50.0%, CATT/*T. evansi*=71.84%, MI=61.63%, PCR=81.43%, TBS=68.37% and WBF=68.37% respectively.

Table 2. Prevalence of different diagnostic techniques using experimentally infected camels with *T. evansi.*

Tests	No. of positive tests	% Positive tests	95% Confidence interval
WBF	21	60	44,74
BCT	23	66	49,79
TBS	22	63	46,77
MI	23	66	49,79
CATT/*T. evansi*	29	83	67,92
PCR	32	91	78,97

Table 3. Association between different diagnostic techniques used in the Identification of *T. evansi* infection with experimentally infected camels.

Tests	Negative tests	Positive tests	Total
WBF	14	21	35
BCT	12	23	35
TBS	13	22	35
MI	12	23	35
CATT/*T. evansi*	6	29	35
PCR	3	32	35
Total	60	150	210
Chi-squared	13.72		
Df	5		
P-value	0.0175		

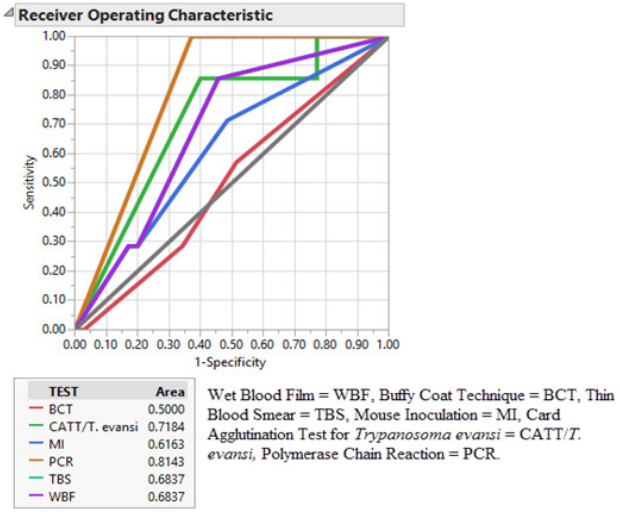

Figure 1. ROC-Curve presenting the sensitivity of different diagnostic techniques in the detection of *T. evansi* in experimentally infected(dromedary) camels.

Discussion

The present study aimed at comparing the detection rate of PCR using RoTat 1.2 primers set specific for *Trypanosoma evansi* with traditional parasitological methods (WBF, BCF, TBS and MI) and serological method (CATT/*T. evansi*) in camels experimentally infected with *T. evansi*. The detection rate of WBF, BCF, TBS and MI were much lower than that of CATT/*T. evansi* and PCR techniques in the early days, especially on days 4 and 8 of the diagnosis, which isn't unforeseen in view of the high detection rate of molecular and serological tests.

The present study showed that the diagnostic detection rate of WBF to *T. evansi* in experimentally infected (dromedary) camels was 100% on 24 day while, 80% detection rate was recorded at day 8, 12. 16, and 28 with an overall prevalence rate of 60% (21/35). The result of the current finding was higher than the report of Singh et al., who detected *T. evansi* in 4.14% of the camels examined with concurrent detection rate of 24.32% [26]. However, the detection rate of WBF was high during day 24 of the diagnosis. Moreover, Pathak et al., detected *T. evansi* in 45.37% (49/108) in naturally infected camels that was much higher than the result obtained in the present study using WBF. The variation in detection rate between the present study and that of Pathak et al., could not be unconnected to the much higher sample size used in their study. Furthermore, Jaiswal et al., reported that microscopic examination is not always sufficient to reveal the epidemiology and magnitude of surra [27,28].

The present result showed variable diagnostic detection rate for BCT with 0% on day 4 and 8. While, on day 12 and 28, 80% of detection rate was recorded, whereas, a 100% detection rate was accounted for on day 16 and 24 with an overall prevalence of 66% (23/35). The result observed in the present study was higher than the finding of Ali et al., who revealed 3.67% of detection rate [29]. The variation in detection rate might be due to the ability of parasitological tests which depends on the number and virulence of the inoculated trypanosomes.

Mouse Inoculation (MI) divulged a diagnostic detection rate of 0%, 0%, 80%, 100%, 80%, 100% and 80% on days 4, 8, 12. 16, 24 and 28 respectively, while the overall prevalence of *T. evansi* was 66% (23/35) as indicated for BCT, and MI has been reported to have a high detection rate of 20-50% trypanosomes per milliliters, which is considered the most efficient parasitological test for the diagnosis of scanty trypanosomes [30,31].

The diagnostic detection rate of CATT/*T. evansi* in the present study was 70% on day 4 and 100% on day 8, 12. 16, 24 and 28 respectively, while the overall prevalence of *T. evansi* was 83% (29/35) which agrees with Davison et al., Pathak et al., and Ngaira et al., who reported a higher detection rate using serological methods compared to parasitological methods [18,27,32]. However, this result was different from the reports of Dia et al., and Verloo et al., who reported a lower detection rate using serology, and this may be attributed to low antibody titers which correlates with the explanation of Verloo et al., who proved that specific antibodies appeared 6-32 days post infection [33-35].

The study also showed the high diagnostic detection rate of PCR in camels experimentally infected with *T. evansi*, which was as high as 100% on day 4, 8, 12. 16, 24 and 28 respectively, while the overall prevalence of *T. evansi* was 91% (32/35) and *T. evansi* specific PCR products were detected 4 days post infection, which was contrary to the results obtained by de Almeida et al., who detected *T. brucei* by PCR one week after infection [36]. PCR signifies a sustainable contrivance for a broad epidemiological study, and is often used to document on the factual prevalence of trypanosome infections and allows the execution of stratagems for controlling *T. evansi*. During the current study, there was a significant association between the different diagnostic tests used and *T. evansi* infection in the experimentally infected Camels. These findings reflect the study of El-Metanawey et al., where they found significant relationships between CATT/*T. evansi* and MI and also between PCR with MI [37]. After the experiment the camels were treated, and each camel was treated with a single 4 grammes intravenous dose of Naganol and those with positive response were treated after 3 months for *T. evansi* to disappear from the blood of the dromedary camels.

Using the ROC-curve to interpret the sensitivity of the different diagnostic techniques, the present study divulged variable sensitivities. This could be due to the ability of the molecular, serological and parasitological tests which depends on the number and virulence of the inoculated trypanosomes. The result of the present study showed a sensitivity of 71.8% for CATT/*T. evansi* which was dissimilar with the sensitivity of the CATT *T. evansi* evaluated under the TG-ROC analysis, with 97.5% as documented by Camoin et al., [38].

Conclusion

In conclusion, PCR has the highest detection rate and is the most sensitive technique compared to the other contrivances studied in the present study and the association between the different diagnostic techniques and detection of *T. evansi* infection in experimentally infected Camels was strong and significant.

Authorship

The concept was developed and planned by Falmata Kyari, Albert Wulari Mbaya and Abdullahi Abubakar Biu; the data were gathered by Falmata Kyari. Falmata Kyari wrote the paper, and Lawan Adamu reviewed the manuscript, evaluating and interpreting the data statistically.

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Ethical Statement

Collection of blood samples and handling of animals was authorized by the University of Maiduguri, Faculty of Veterinary Medicine.

Conflict of Interest

No conflicting interest to declare.

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References

1. Luckins, AG. "*Trypanosoma evansi* in Asia." *Parasitol Today*. 4(1988): 137-142.
2. Lun, ZR, Fang Y, Wang CJ and Brun, R. "Trypanosomiasis of Domestic Animals in China." *Parasitol Today*. 9(1993): 41-45.
3. Luckins, AG and Dwinger RH. "Non-tsetse Transmitted Animal Trypanosomiasis." In: Maudlin I Holmes PH, Miles MA. The trypanosomiases. Oxfordshire: CABI Publishing. (2004): 269-281.
4. Desquesnes, M. "Livestock Trypanosomes and their Vectors in Latin America." CIRAD-EMVT Publication, France (2004).
5. Desquesnes, Marc, Holzmuller Philippe, Lai De-Hua and Dargantes Alan, et al. "*Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Toxonomy, Morphology, Hosts and Pathogenic Effects." *Biomed Res Int*. 2013(2013): 1-20.

6. Office of International Epizootics, OIE. "World Organisation for Animal Health." *Trypanosoma evansi* infection (Surra). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. (2018).
7. Hoare, CA. "The Trypanosomes of Mammals." In: A Zoological Monograph. Blackwell Scientific Publications, Oxford, UK, (1972): 749.
8. Soulsby, EJL (1982). "Helminths, Arthropods and Protozoa of Domesticated Animals." (7th edn). *Bailliel Tindall, London*, (1982): 809.
9. Partoutomo, S, Soleh M, Politedy F and Day A, et al. "A Study on the Pathogenesis of *Trypanosoma evansi* in Buffalos, Holstein-Friesian and Ongole Cattle." *Indo J Anim Vet Sci*. 1(1995): 41-48.
10. Bhatia, BB, Pathak KML and Banerjee DP. "Textbook of Veterinary Parasitology." (2nd edn), Kalyani publishers, Ludhiana, New Delhi, (2006): 304-315.
11. Office of International Epizootics (OIE).(2010). "Terrestrial Manual." 2(2010): 1-17.
12. Wei, Guojian, Bull Harold, Zhou Xia and Tabel Henry. "Intradermal Infections of Mice by Low Numbers of African Trypanosomes are controlled by Innate Resistance but Enhanced by Susceptibility to Reinfection." *Infect Dis*. 203(2011): 418-429.
13. Mugittu, KN, Silayo RS, Majiwe PA and Kimbita EK, et al. "Application of PCR and DNA Probes in the Characterization of Trypanosomes in the Blood of Cattle in Farms in Morogoro, Tanzania." *Vet Parasitol*. 94(2001): 177-189.
14. Reid, Simon A. "*Trypanosoma evansi* Control and Containment in Austrialia." *Trends Parasitol*. 18(2002): 219-223.
15. Baticados, Waren N, Fernandez Cherry Pambid and Baticados Abigail. "Molecular Detection of *Trypanosoma evansi* in cattle from Quirino Province, Philippines." *Vet Arch*. 81(2011): 635-646.
16. Tehseen, Sonia, Jahan Nusrat, Qamar Muhammad Fiaz and Desquesnes Marc, et al. "Parasitological, Serological and Molecular Survey of *Trypanosoma evansi* Infection in Dromedary Camels from Cholistan Desert, Pakistan." *Parasit Vector*. 8(2015): 415.
17. Viseshakul, Nareerat and Panyim S. "Specific DNA Probe for the Sensitive Detection of *Trypanosoma evansi*." *Southeast Asian J Trop Med Public Health*. 21(1990): 21-27.
18. Davison, HC, Thrusfield, MV, Muharsini, S and Husein A, et al. "Evaluation of Antigen Detection and Antibody Detection Tests for *Trypanosoma evansi* Infection of Buffaloes in Indonesia." *Epidemiol Infect*. 123(1999): 149-155.
19. Chansiri, Kosum, Khuchareoontaworn Sintawee and Sarataphan Nopporn. "PCR-ELISA for Diagnosis of *Trypanosoma evansi* in Animals and Vector." *Mol Cell Probes*. 16(2002): 173-177.
20. Büscher, Philippe, Gonzatti Mary Isabel, Hébert Laurent and Inoue Noboru, et al. "Equine Trypanosomosis: Enigmas and Diagnostic Challenges." *Parasit Vector*. 12(2019): 1-8.
21. Salim, Bashir, Bakheit Mohammed A, Kamau Joseph and Nakamura Ichiro, et al. "Molecular Epidemiology of Camel Trypanosomiasis Based on ITS1 rDNA and RoTat 1.2 VSG Gene in the Sudan." *Parasit Vector*. 4(2011): 31.
22. Office of International Epizootics (OIE). "World Animal Health Information Database." *Paris, France*, (2012).
23. Murray, M, Murray PK and McIntyre WI. "An Improvzed Parasitological Technique for the Diagnosis of African Trypanosomiasis." *Trans R Soc Trop Med Hyg*. 71(1977): 325-356.
24. Songa, Bayjana E and Hamers R. "A Card Agglutination Test (CATT) for Veterinary use based on an Early VAT Rotat 1-2 of *Trypanosoma evansi*." *Ann Soc Belg Med Trop*. 68(1988): 233-240.
25. Claes, Filip, Radwanska Magda, Vrakawa Toyo and Majiwa Phelix AO, et al. "Variable Surface Glycoprotein RoTat 1.2 PCR as a Specific Diagnosis Tool for the Detection of *Trypanosoma evansi* Infections." *Kinetoplastid Biol Dis*. 3(2004): 1-6.
26. Singh, Narender, Pathak KML and Kumar Rajender. "A Comparative Evaluation of Parasitological, Serological and DNA Amplification Methods for Diagnosis of Natural *Trypanosoma evansi* Infection in Camels." *Vet Parasitol*. 126(2004): 365-373.
27. Pathak, KML, Singh Yadvendra, Meirvenne NV and Kapoor M. "Evaluation of Various Diagnostic Techniques for *Trypanosoma evansi* Infections in Naturally Infected Camels." *Vet Parasitol*. 69(1997): 49-54.
28. Jaiswal, Amit Kumar, Neha Vikrant Sudan and Verma Amit Kumar. Insight into Trypanosomiasis in animals: Various Approaches for its Diagnosis, Treatment and Control: A review. Asian *J Anim Sci*. 9(2015): 172-186.
29. Ali, Nahla, Croof Hamid IMN and Abdalla Hamid S. "Molecular Diagnosis of *Trypanosoma evansi* Infection in Camelus Dromedaries from Eastern and Western Regions of the Sudan." *J Food Agri*. 23(2011): 320-329.
30. Chaudhri, SS, Gupta RP and Singh V. "Experimental Trypanosomosis in Crossbred Calves: Its Diagnosis and Chemoprophylaxis with Quinapyramine Prosalt." *Indian J Anim Sci*. 66(1996): 662-665.
31. Singh, N, Pathak KML, Kumar R and Manohar GS. "Observations on the Validity of Mouse Inoculation Test in the Surveillance of *Trypanosoma evansi* in Camel." *J Camel Practice Res*. 10(2003): 153-155.
32. Ngaira, JM, Bett B, Karanja SM and Njagi ENM. "Evaluation of antigen and antibody rapid detection tests for *Trypanosoma evansi* infection in camels in Kenya." *Vet Parasitol*. 114(2003): 131-141.
33. Dia, ML, Van Meirvenne EM, Magnus AG and Luckins, C, et al. "Evaluation of Four Diagnosis Tests: Blood Smears, CATT, IFAT and ELISA Ag in a Study of the Epidemiology of *Trypanosoma evansi*, Camel Trypanosomosis in Mouritania." *Breed Vet Med Rev Trop*. 50(1997): 29-36.
34. Verloo D, Holland W, My LN and Thanh, NG, et al. "Comparison of Serological Tests for *Trypanosoma evansi* Natural Infections in Water Buffaloes from North Vietnam." Vet Parasitol. 92(2000): 87-96.
35. Verloo, D, Magnus E and Büscher P. "General Expression of RoTat 1.2 Variable Antigen Type in *Trypanosoma evansi* Isolates from Different Origin." *Vet Parasitol*. 97(2001): 183-189.
36. De Almeida, Paulo J, Ndao Momar, Van Meirvenne Nestor and Geerts Stanny. "Diagnostic Evaluation of PCR in Goats Experimentally Infected with Trypanosoma vivax." *Acta Trop*. 66(1997): 45-50.
37. El Metanawey, TM, Nadia ME, Abdel El Aziz MM and Hassanane MS, et al. "Comparative studies on Diagnosis of *Trypanosoma evansi* in experimentally infected goats." *Global Vet*. 3(2009): 348-353.
38. Camoin, M, Kocher A, Chalermwong P and Yangtarra S, et al. "The Indirect ELISA *Trypanosoma evansi* in Equids: Optimisation and Application to a Serological Survey including Racing Horses, in Thailand." *BioMed Res Int*. (2019).

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