PARASITOLOGICAL AND MOLECULAR STUDIES ON *TRYPANOSOMA EVANSI* OF CAMELS IN EGYPT

SOUZAN G. GHATTAS[1] and NASHWA M. HELMY[2]

1. *Parasitology Department, Animal Health Research Institute, ARC, Dokki, Giza*
2. *Biotechnology Department, Animal Health Research Institute, ARC, Dokki, Giza*

(Manuscript received 26 January 2016)

Abstract

This study was conducted on a total of 187 one humped camels (*Camelus dromedarius*). Blood samples were collected from 40 camels suspected for *Trypanosoma* infection from farms at Giza Pyramids area. In addition, 147 samples were collected from apparently healthy camels (97 and 50 from El-Bassatin and El-Warraq abattoirs respectively) for screening of *Trypanosoma* species infection. All samples were examined parasitologically by Giemsa stained blood smear and haematocrit centrifugation technique, serologically by card agglutination test (CATT) for detection of anti-trypanosomal antibodies and polymerase chain reaction (PCR) for DNA amplification with aspecific primers for detection of trypanozoan parasites. Out of 187 camels, 14 camels were positive by parasitological methods with a percentage of 7.49%, 21 positive samples were detected by anti-*Trypanosoma* antibodies using CATT with a percentage of 11.23%. Fourteen out of the positive blood samples by parasitological and serological techniques were used for PCR amplification. Thirteen out of 14 positive blood samples were PCR-positive and one was negative by using specific primers for *T. evansi* minicircle EVA1 and EVA2. The results of examined camels after using the polymerase chain reaction (PCR) for detection of DNA of trypanosomes in infected camels, showed 138 bp PCR product for the specific detection of *Trypanosoma evansi*. It was concluded that the use of PCR, beside parasitological and serological methods, is recommended for exact diagnosis in survey and control programmes of *Trypanosoma evansi*.

Keywords: *Trypanosoma*- Camels - Stained Blood smear- Buffy coat- CATT - PCR

INTRODUCTION

Trypanosomosis is a disease caused by obligated flagellate blood parasite; that infect members of every vertebrate class. Surra is an animal disease occurring in Africa, Asia and Latin America, caused by *Trypanosoma evansi*. The parasite is transmitted by biting flies such as Tabanidae and Stomoxys species (Meiloud *et al*., 2011). Trypanosomosis caused by *Trypanosoma evansi* (*T. evansi*) is an important livestock disease in Egypt causing significant losses in camels. Surra disease manifests

itself usually as a chronic infection characterized by weight loss, anemia, infertility, emaciation and abortion (Luckins, 1988). The disease occurs both in chronic and acute form (Gutierrez *et al*., 2006). The chronic form of the disease is most common and is likely to be associated with secondary infection due to immune-suppression (Njiru *et al*., 2004). Clinical signs and pathological lesions caused by *T. evansi* in camels are unreliable for definitive diagnosis (Chaudhary and Igbal, 2000). *T. evansi* is usually detected by the microscopical examination of infected blood (wet blood film, stained blood smears and buffy coat examination), mouse inoculation and immunological methods. However, microscopical observation requires skilled technicians and has poor sensitivity. Mouse inoculation is impractical for a large-scale epidemiological study. The immunological methods yield false negatives and positives due to antigenic variation of *T. evansi* and it cannot differentiate between infected and treated cases (Gonzales *et al*., 2007). The conventional parasitological methods lack sensitivity and the serological techniques which detect antibodies or antigens lack specificity or sensitivity, respectively. Therefore, a molecular technique, especially polymerase chain reaction (PCR) has been developed in order to overcome the problems faced with conventional and serological techniques. In addition, it was reported that PCR is a reliable method for diagnosis and epidemiological studies (Gutierrez *et al*., 2006).

The present study aimed to use the parasitological (Giemsa stained blood smears and haematocrit centrifugation), serological (CATT) and molecular methods (PCR) for detection and confirmation of *Trypanosoma evansi* in camels.

MATERIALS AND METHODS

Animals:

The present study was conducted on a total of 187 one humped camels (*Camelus dromedarius*)**.** Forty camels were suspected for *Trypanosoma* infection from Giza farms in Pyramids area. One hundred and forty seven apparently healthy camels from El-Bassatin and El-Warraq abattoirs (97 and 50 respectively) all over one year were included in this study. These animals were subjected to careful clinical and laboratory investigations for *Trypanosoma* spp. infection.

Sampling:

Two blood samples were collected from each camel by jugular vein puncture, one in a tube containing disodium salt of EDTA and the second in a tube without anticoagulant for subsequent serum collection. One part from blood samples collected on EDTA were used for parasitological examination using Giemsa stain blood smear and haematocrit centrifugation technique. The other part is preserved at -20°C for

DNA extraction of trypanosomes for PCR technique. Blood samples without anticoagulant were centrifuged at 3000 rpm for 10 minutes. Clear serum was collected and stored at -20°C until used for detection of *Trypanosoma* antibodies using serological test (card agglutination test).

Parasitological examination:

Giemsa stained blood smears:

Two thin blood smears were prepared, fixed by methanol and stained with Giemsa stain. The stained smears were examined under oil immersion lens of microscope at a total magnification of X 1000 for the presence of *Trypanosoma* species. The parasites were identified according to the characters described by Soulsby (1982).

Haematocrit centrifugation technique:

The capillary tubes were filled with blood samples and sealed at one end using plasticin, then centrifuged at 3000 rpm for 10 minutes. Buffy coat in the tubes were examined for the presence of trypanosomes using a microscope with oil immersion objective (*Coles,1986*).

Serological test:

Card agglutination test (CATT):

The method was described by Bajyana and Hammers (1988). Serum samples were tested with CATT/*T. evansi* ® following the instructions of the manufacturer (laboratory of serology, institute of tropical medicine, Antwerp, Belgium). Briefly one drop of camel serum diluted up to 1:5 in CATT-buffer, was pipetted onto a plastic coated test card and then added with one drop of CATT reagent.The reaction mixture was spread out using a clean stirring rod and allowed to react on the card with help of manual rotation for 5 minutes. Blue granular agglutinations indicate a positive reaction visible to the naked eye.

Samples for PCR:

Extraction of DNA from blood samples:

The positive blood samples by using microscopical and serological examinations were used for PCR amplification. DNA of blood samples were extracted by using thermo scientific kits (Gene JET Geno mic DNA Purification Kit #K0721, #K0722). Oligonucleotide primers for conventional PCR, set of oligonucleotide primers were synthesized and designed specific for *T. evansi* mini circle EVA1 and EVA2 (*Njiru et al., 2004*).

Table 1. Oligonucleotide primer sequences specific for *Trypanosoma evansi.*

Primer	Sequences	Amplified product
T. evansi EVA1	5- ACATATCAACAACGACAAAG -3	138 bp
T. evansi EVA1	5- CCCTAGTATCTCCAATGAAT -3	

DNA amplification in conventional PCR:

DNA amplification was done in 25 µl reaction volume containing 12.5 µl of 2X Taq PCR mixes (Tiangen, Cat No. KT201) containing loading dkye, 10 PM of each oligonucleotide primers, 5 µl of DNA template and fill up to 25 µl with DNAse and RNAse free water. The optimized cycle program for PCR were as follow: initial denaturation at 94ºC for 5 min; 30 cycles of 30 sec. at 94ºC, 30 sec. at 60ºC and 30 sec. at 72ºC; and final extension step at 72ºC for 5 min (Njiru, *et al.*,2004).

Electrophoresis of PCR product:

After amplification 5 µl of the reaction product was mixed with 1 µl of 6X gel loading dye and subjected to electrophoresis on 1.5% agarose gel at 100V for 30 min. Gel were stained with ethidium bromide and photographed on UV transilluminator. Samples were considered positive for *T. evansi* by using EVA1 and EVA2 primers when a single band of DNA at 138 bp were evident in the ethidium bromide stained gels, compared with the molecular size marker (50 bp DNA ladder).

RESULTS

Clinical signs:

Some of the general clinical signs were recorded on camels suspected to be infected with *Trypanosoma* species such as emaciation, weight loss, intermittent fever, anemia, lacrimation, corneal opacity and diarrhea**.**

Parasitological results:

Table (2) showed that microscopic examinations revealed that out of 187 camels, 14 (7.49%) were found to be infected with *Trypanosoma* species. Three cases (7.50%) were positive by Giemsa stained blood smears and 5 (12.50%) by haematochrite centrifugation from the 40 suspected camels. In case of apparently healthy camels from El-Bassatein abattoir, there were 2 (2.06%) positive cases by both Giemsa and haematochrite methods, while there was only one positive sample (2%) from El-Waraque abattoir by the same two methods. Microscopic examination of stained blood smears revealed that *T. evansi* were monomorphic thin trypomastigote parasite with length range from 15-30 µm, long free flagellum and thin posterior extremity with subterminal small kinetoplast (Fig. 1).

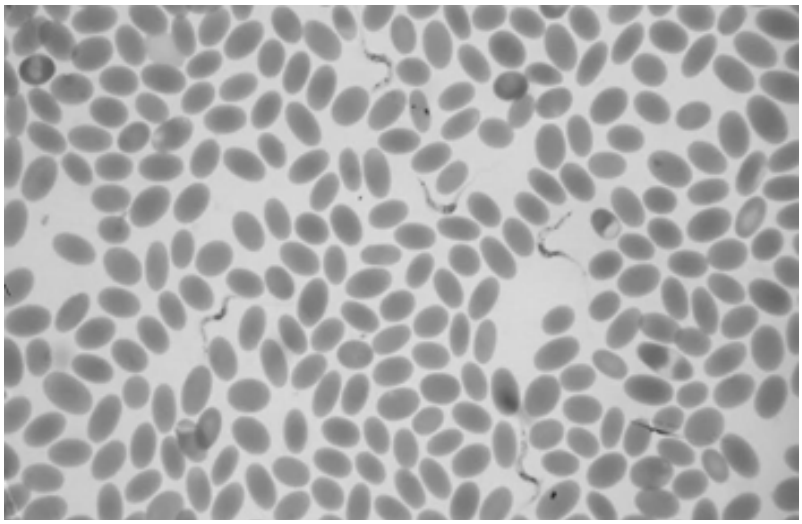

Fig. 1. *Trypanosoma* spp. in thin Giemsa stained blood film from infected camel (X100).

Serological result (CATT):

Table (3) showed that serological examinations by CATT revealed that out of 187 camels, 21 (11.23%) were positive for *Trypanosoma* spp. Eleven samples from the 40 suspected camels were positive for *Trypanosoma* infections with a percentage of 27.50 %. Six and 4 samples from the apparently healthy camels from El-Bassatein and El-Warraque abattoirs were positive for *Trypanosoma* spp. infection with a percentage of 6.19 % and 8.0% respectively.

Table 2. Parasitological results for detection of *Trypanosoma* infection in camels.

Parasitological tests	Suspected infected camels		Apparently healthy camels				Total No. of +ve samples	
	Giza farm (n= 40)		El-Bassatein abattoir (n= 97)		EL-Warraque abattoir (n= 50)			
	+v	%	+v	%	+v	%	+ve	%
Giemsa stained blood smears	3	7.50	2	2.06	1	2.0	6/187	3.21
Haematochrite centrifugation	5	12.50	2	2.06	1	2.0	8/187	4.28
Total	8	20	4	4.12	2	4.0	14/187	7.49

Table 3. Serological results (CATT) for detection of *Trypanosoma* infection in camels.

Serological test	Suspected infected camels		Apparently healthy camels				Total No. of +ve samples	
	Giza farm (n= 40)		El-Bassatein abattoir (n= 97)		EL-Warraque abattoir (n= 50)			
CATT	+v	%	+v	%	+v	%	+v	%
	11	27.50	6	6.19	4	8.0	21/187	11.23

Results of molecular technique (PCR):

The results of examined camels after using the polymerase chain reaction (PCR) for detection of DNA of trypanosomes in infected camels, showed 138 bp PCR product for the specific detection of *T. evansi*. Thirteen out of 14 positive blood samples by parasitological and serological examinations were PCR positive and one was negative by using specific primers for *T. evansi* minicircle EVA1 and EVA2 (Fig. 2).

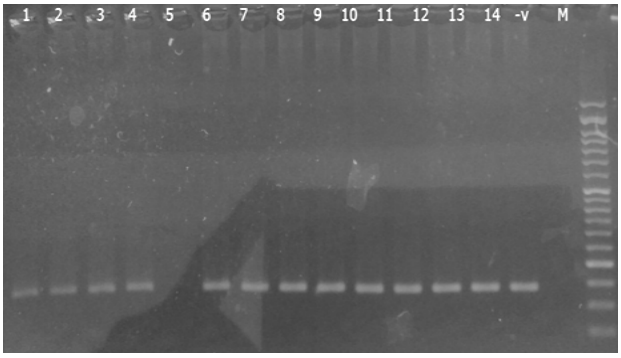

Fig. 2. Ethidium bromide stained 1.5% agarose gel electrophoresis showed PCR amplified fragment of expected size 138 bp (lane 1-14) resulted from amplification of DNA extracted from blood samples using EVA1 and EVA2 primers specific to *Trypanosoma evansi* Lane M: 50 bp DNA size marker. (Lane: 5 negative).

DISCUSSION

The clinical signs of *T. evansi* in camels that recorded in this study were in agreement with that recorded by Padmaja (2012) and Ismail *et al*., (2014). Published clinical signs (emaciation, fever, anemia, lacrimation, corneal opacity and diarrhea) are insufficient for diagnosis (Chaudhary and Iqbal, 2000) while detection of parasites in blood is difficult because parasitaemia is intermittent (Nantulya, 1990).

The present study was conducted on total of 187 one humped camels (*Camelus dromedarius*). Forty camels were suspected for *Trypanosoma* infection from Giza farms in Pyramids area. One hundred and forty seven camels from El-Bassatin and El-Warraq abattoirs (97 and 50 respectively) were apparently healthy for screening of *Trypanosoma* species infection. It was found that the prevalence of infection in the suspected infected camels and from El-Bassatein and El-Warraque abattoirs using thin blood films together with haematocrit centrifugation test were 20%, 4.12% and 4.0% respectively. Our finding are nearly similar to those recorded in Egypt by Mottelib *et al*., (2005) 5.82% and Abdel-Rady (2011) 4.1%. Such results were also observed by Derakhshanfar *et al*., (2010) who found that the prevalence of *T. evansi* in Iran was

7.5%. The chronic form is most common and may be present in association with secondary infections due to immuno-suppression caused by *T. evansi* infection, which complicates clinical diagnosis. On the other hand, Shah *et al*., (2004) in Pakistan, Chaudhary and Iqbal (2000) and El-Haig *et al*., (2013) in Egypt recorded higher incidence of 13.72%, 10.67% and 12% respectively of *T. evansi* infection in camels. Mahran (2004) in upper Egypt found high incidence (31%) in non-clinical infection of *T. evansi* among the investigated camels. Variation of incidence of *T. evansi* among camels may be attributed to the difference of locality, insect reservoir and methods of diagnosis. Raisinghan and Lodha (1986) recorded parasitological methods used for detection of trypanosomes are not sensitive enough for diagnosis of Surra in camel. *Singh et al., (2004*) studied the comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural *T. evansi* infection in camels. They found the prevalence was 4.14, 4.60%, 9.67% and 17.05% by using wet blood films, thin blood films, ELISA and PCR amplification respectively.

CATT is a quick and easy test which can be performed under field condition. In the present study, the serological prevalence of camel trypanosomosis using CATT revealed that 11 (27.50%), 6 (6.19%) and 4 (8.0%) were positive in the suspected infected camels, El-Bassatein and El-Warraque abattoirs respectively. All positive samples by using parasitological examination were positive by CATT. Those results revealed a good correlation with parasitological methods and it was agreed with those of Gutierrez *et al*., (2006) who reported sensitivity of CATT test compared to parasitological methods varied from 86 to 100%. Pathak *et al.,* (1997) reported that CATT can be used to study the seroprevalence of *T. evansi* since it is simple, quick and field test.

The results of this study in the examined camels after using the polymerase chain reaction (PCR) for detection of DNA, showing a 138 bp PCR product for the specific detection of *T. evansi*. Thirteen out of 14 positive blood samples by microscopic examination were PCR-positive and one was negative by using specific primers for *T. evansi* minicircle EVA1 and EVA2. The one missed case in this study could either be due to degraded DNA and/or loss of DNA during extraction. PCR is a useful tool for detection and confirmation of the *Trypanosoma* spp. Clausen *et al*., (2003), Hilali *et al*., (2006), Rjeibi *et al*., (2015) and Masiga and Nyang'ao, (2001) were successfully detected *T. evansi* infection in horses, water buffalo calves, dogs and camels respectively. Singh *et al*., (2004) found the specific band in positive cases of *T. evansi* infection in camels was 277bp by using PCR amplification, while in the present work it was 138 bp. They added that the intensity of PCR bands was variable in

different test samples depending upon the level of infection in the test samples. Njiru *et al*., **(**2004**)** used 4 diagnostic tests (MI, MHCT, CATT/*T. evansi* and PCR) for diagnosis of *T. evansi* infection in 549 camels in Kenya. They found the overall prevalence of infection was 9.4%, 5.3%, 45.5% and 26.6% respectively. Abdel-Rady (2011) used PCR for *T. evansi* infection in camels with primers yielding a 177 bp PCR product for the specific detection of Trypanozoan parasites. Trypanosomosis continues to pose a great risk to camel keeping in Egypt. Amer *et al*., (2011) examined 600 camels for screening of *T. evansi* infection in Egypt. The tests used were parasitological (thin smear film and buffy coat), serological (CATT) and DNA amplification by PCR. They found that the prevalence of *T. evansi* infection in camels was detected in parasitological, serological and PCR were 11.6%, 47.o% and 62.5% respectively. They added that the PCR revealed a specific 200 bp band in positive samples. PCR technique was used for detection of minute amounts of trypanosomal DNA in infected and apparently healthy camels (OIE, 2012). Noting the chronic nature of the disease, the use of PCR beside parasitological and serological methods, is recommended for exact diagnosis in survey and control programmes.

REFERENCES

1. Abdel-Rady, A. 2011. Epidemiological studies (parasitological, serological and molecular techniques) of *Trypanosomae vansi* infection in camels (*Camelus dromedarius*) in Egypt Veterinary World, 1(11): 325-328.
2. Amer,S., O. Ryu, C. Tada, Y. Fukuda, N. Inoue and Y. Nakai. 2011. Molecular identification and phylogenetic analysis of *T. evansi* from dromedary camels (*Camelus dromedarius*) in Egypt, a pilot study. Acta Trop., 117: 39-46.
3. Bajyana, S. E. and R. Hammers. 1988. A card agglutination test (CATT) for use based on early Vat Ro Tat ½ of *T. evansi*. Annales de la Societe Belge de Medecine Tropicale, 68:233-340.
4. Chaudhary, Z.I. and M.J. Iqbal. 2000. Incidence, biochemical and hematological alterations induced by natural Trypanosomiasis in racing dromedary camels. Acta Tropica., 77: 209–213.
5. Clausen, P.H., S. Chuluun, R. Sodnomdarjaa, M. Greiner, K. Noeckler C. Staak, K.H. Zessin, E. Schein. 2003. Afield study to estimate the prevalence of *Trypanosoma equiperdum* in Mongolian horses. Vet. Parasitol., 115: 9-18.
6. Coles, E. H. 1986. Veterinary Clinical Pathology. 4th edition. W.B. Sounders Company Philadelphia.

7. Derakhshanfar, A., A.A. Mozaffari and Z. Mohaghegh. 2010. An outbreak of trypanosomiasis (Surra) in camels in the Southern Fars Province of Iran: clinical, hematological and pathological finding. Res. J. Parasitol.,5: 23-26.
8. El-haig, M., I.Y. Ahmed, K. Amal El-Gayar. 2013. Molecular and parasitological detection of *Trypanosoma evansi* in camels in Ismailia, Egypt. Vet. Parasitol., 198: 214-218.
9. Gonzales, J.L., E. Chacon, M. Miranda, A. Loza and L.M. Siles.2007. Bovine trypanosomosis in the Bolivian Pantanal. Veterinary Parasitology, 146: 9-16
10. Gutierrez, C., J.A Corbera, and M. Moralesand P. Büscher. 2006. Trypanosomosis in goats (Current status). Annals of the New York Academy of Sciences, 1081: 300-310
11. Hilali,M., A. Abd El-Gawad, A. Nassar and A. Abdel-Wahab. 2006. Hematological and biochemical changes in water buffalo calves (*Bubalus bubalis*) infected with *Trypanosoma evansi*. Vet. Paraasitol., 139:237-243.
12. Ismail, S.M. and M.Y. Eman. 2014. Haematological and biochemical studies in camels infested with *T. evansi* in some areas of Sini. Vet. Parasitol., 198: 8-17
13. Luckins, A.G. 1988. *Trypanosoma evansi* in Asia. Parasitol. Today 4, 137–142. Mahran, O.M. 2004. Some studiesin blood parasites in camels at Shalatin, Red Sea Governorate. Assiut. Vet. Med. J., 50 (102):172-184
14. Masiga, R.C. and J.M. Nyang'ao. 2001. Identification of trypanosome species from camel using polymerase chain reaction and procyclic transformation test. Camel Pract. Res. 8, 17–22.
15. Meiloud, G.M., I.N. Ould Bouraya, A. Samb, and A. Houmeida. 2011. Composition of Mauritanian Camel milk. Results of first study. Int. J. Agric. Bio., 13:145-147.
16. Mottelib, A.A., H.I., Hosein, I. Mourad, A.H. El-Sherifand A.S.I. Abozeid. 2005. Comparative evaluation of various diagnostic techniques for T. evansi naturally infected camels. ISAII- Warsaw- Poland 2: 205-207.
17. Nantulya, N. M. 1990. Trypanosomiasis in domestic animals: the problems of diagnosis. Rev. Sc. Tech. Off. Int. Epiz.,9: 357-367.
18. Njiru, Z.K; I Robertson, S. Okaye, C.C. Constantine, J.M. Ndung'u, R.C.A. Thompson and Reid, S.A. 2004. Detection of *Trypanosoma evansi* in camels using PCR and CATT/*T. evansi* tests in Kenya. Vet. Parasitol., 124 (3-4):187-199.
19. O.I.E. 2012. Terrestrial Manual – Version adopted by the World Assembly of Delegates of the OIE in May 2012 Chapter 2.1.17.
20. Padmaja, K. 2012. Haemato- biochemical studies of camels infested with trypanosomiasis. Vet. World., 5 (6):356-358.

21. Pathak, K.M.L., N. Singh, V. Meirvenne and Kapoor, M. 1997. Evaluation of various diagnostic techniques for *Trypanosoma evansi* infections in naturally infected camels. Vet. Parasitol., 69:49-54.
22. Raisinghan, P.M. and K.R. Lodha. 1986. Prognostic values of some haematological and biochemical parameters of camels affected with Surra following the treatment with Antrycide methyl sulphat. Nagonal and Berenil. Indian Vet. J., 50:479-484.
23. Rjeibi, M.R. B.H. Taoufik, D. Zaro, T. Mahjoub, A. Rejeb, W. Dridi and Gharali 2015. First report of Surra (*T. evansi* infection) in a Tunisian dog. Parasite, 22: 3.
24. Shah, S.R., M.S. Phalan, M.A. Memon, R. Rind and W.M. Bhatti. 2004. Trypanosomes infection in camels. Pakistan Vet. J., 24 (4):209-210.
25. Singh, N., K.L.M. Pathak and R. Kumar. 2004. A comparative evaluation of parasitological, serological and DNA amplification methods for diagnosis of natural *T. evansi* infection in camels. Vet. Parasitol., 126:365-373.
26. Soulsby, E.J.L. 1982. Helminthes, arthropods, and Protozoa of domesticated animals,3rd Ed. Bailiere Tindall and Cassell Ltd., London (Uk) 65pp.

دراسات طفيلية و جزيئية على التريبانوسوما إيفانزاى فى الجمال بمصر

سوزان جرجس غطاس[١]– نشوى محمد حلمى[٢]

١. *قسم الطفيليات – معهد بحوث صحة الحيوان– مركز البحوث الزراعية–الدقى– الجيزة*
٢. *قسم البيوتكنولوجى – معهد بحوث صحة الحيوان– مركز البحوث الزراعية– الدقى– الجيزة*

أجريت هذه الدراسة على عدد ١٨٧ من الجمال منهم ٤٠ جمل بمزرعة خاصة بمحافظة الجيزة فى منطقة الأهرامات متوقع إصابتها بالتريبانوسوما لظهور بعض الأعراض المشابهة وعدد ١٤٧ من الجمال السليمة ظاهريا تم تجميعها من مجزرى البساتين (٩٧) و الوراق (٥٠) وذلك لإستبيان نسبة الإصابة وتأكيد نوع التريبانوسوما الذى يصيب الجمال فى مصر والمقارنة بين الإختبارات المختلفة. تم فحص جميع العينات بطرق طفيلية مختلفة (صبغة الجيمسا والهيماتوكريت) وطرق سيرولوجية (الكات) وتفاعل البلمرة المتسلسل. أظهر الفحص الطفيلى بطريقتى صبغة الجيمسا والهيماتوكريت أن نسبة الإصابة ٧.٥٠ % و١٢.٥٠% من أصل ٤٠ من الجمال التى تظهر عليهم الأعراض المشتبه على التوالى. بينما كانت نسبة الإصابة ٢.٠٦% و٢% بكلا الطريقتين للجمال السليمة ظاهريا المأخوذة من مجزرى البساتين والوراق على التوالى . تم أخذ جميع العينات الإيجابية بواسطة الفحص الميكرسكوبى والسيرولوجى وعددهم ١٤ عينة لفحصهم باستخدام تفاعل البلمرة المتسلسل لتأكيد التشخيص وتحديد نوع التريبانوسوما وذلك باستخدام بادئ خاص للتريبانوسوما إيفانزاى عند ١٣٨ قاعدة زوجية وقد تبين أن جميع العينات إيجابية لهذا النوع من التريبانوسوما إيفانزاى ماعدا عينة واحدة كانت سلبية. نستنتج من ذلك أنه من الأفضل إستخدام أكثر من طريقة للوصول إلى التشخيص الدقيق.