

## Epidemiological studies (parasitological, serological and molecular techniques) of *Trypanosoma evansi* infection in camels (*Camelus dromedarius*) in Egypt

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### Abstract

Trypanosomosis in camel caused by *Trypanosoma evansi* is still a serious problem in camel husbandry causes considerable economic losses in many camel-rearing regions of the world. In the present study 193 camels clinically suspected for surra were examined parasitologically by Giemsa stained blood smear (GSBS) and haematocrit centrifugation technique, serologically for detection of anti-trypanosomal antibodies by card agglutination test for trypanosomes (CATT), and for DNA amplification, by Polymerase chain reaction (PCR), with primers yielding a 177 bp PCR product for the specific detection of *Trypanozoon* parasites. Out of 193, eight camels were positive by GSBS (4.1%) while 12 were positive with haematocrit centrifugation technique (6.2%). Detection of anti-trypanosomal antibodies with CATT yielded 84 positive samples (43.5%). Using PCR 110 out of 193 were positive (56.9 %). PCR technique is accurate, more sensitive and specific method for diagnosis of trypanosome infected camels than parasitological techniques; it overcomes the problem of specificity and can detect low parasitemic camels in chronic cases. The PCR proved to be the best test used for detection of camel trypanosomosis in Egypt.

**Keywords:** Camels, Trypanosomosis, Stained Blood smear, Haematocrit centrifugation technique (HCT), Card agglutination test (CATT), Polymerase chain reaction (PCR)

### Introduction

In Egypt, camels take a principal position. Besides being a mean of meat and milk production, they are used for transportation of crops and do other farm works; also, their hair, wool and hides are used. According to the last Official Egyptian Veterinary reports 267,000 camels live in Egypt, belonging to the one humped species *Camelus dromedarius* (Abdel-Rady, 1997). Trypanosomosis caused by *Trypanosoma evansi* is an important livestock disease in Egypt causing significant losses in camels, the disease, Surra, manifests itself usually as a chronic infection characterized by weight loss, anemia, infertility and abortion (Luckins, 1988; Lohr, et al., 1986). The maintenance of good health and productivity in herds is of vital importance to nomadic herders. The most important protozoan parasitic disease is camel trypanosomosis (Surra) which causes severe disease throughout Africa. Trypanosomosis in camels causes considerable economic losses due to a decrease in milk and meat, premature births and abortions (Boid et al., 1985). Affected camels show fever, anorexia, marked generalized edema and deteriorate rapidly and die; the chronic form of *T. evansi* infection is characterized by progressive loss of body weight,

intermittent high fever, marked generalized muscular atrophy, pale mucous membranes and occasionally abdominal edema. Affected camels also may exhibit a characteristic sweet odour due to an increase of urinary ketones (Schwartz and Dioli, 1992). Trypanosomosis in camels occurs both in chronic and acute forms (Wilson et al., 1983). The chronic form is most common and may present an association with secondary infections due to immunosuppressant caused by *T. evansi* infection, which complicates clinical diagnosis. Clinical signs recorded as emaciation, intermittent fever, anemia, lacrymation, corneal opacity, diarrhea and edema of the dependent parts (Chaudhary and Iqbal, 2000) is insufficient knowledge for diagnosis, while detection of parasites in the blood is difficult because parasitaemia is intermittent (Mahmound and Gray, 1980). Consequently, there is a need for alternative more sensitive diagnostic techniques.

Control of camel trypanosomosis depends mainly on the use of curative and prophylactic drugs. Treatment recommendations and strategies for chemotherapeutic control depend on information of trypanosomosis risk and the prevalence of trypanocidal drug resistance in the area. Sensitive

diagnostic techniques are required to detect the parasite and the efficacy of trypanocidal drug treatment. Parasitological methods used in the diagnosis of *T. evansi* in camels are considered easy, rapid and economic. However, they are not sufficient to detect all trypanosome infected animals, especially in case of low parasitaemia and also in the chronic form of the disease. The serological test such as the card agglutination test [CATT] is used for the detection of antibodies circulating in the serum of infected camels (Bajyana-Songa and Hamers, 1988), the test could be used under both laboratory and field conditions (Magnus *et al.*, 2002). It is a quick, simple and easy to perform and sensitive (Diall *et al.*, 1994; Pathak *et al.*, 1997). These serological tests are considered sensitive. However, serological techniques are not always distinguishing current from past infection due to the prolonged persistence of antibodies in the blood of treated animals (Luckins *et al.*, 1978). Particularly in these cases of treatment success evaluation, DNA based techniques, as polymerase chain reaction (PCR) are useful; DNA of *Trypanosoma evansi* parasites in the blood of animals should have disappeared, while antibodies do persist. These DNA tests are considered sensitive and specific. With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed but not yet completely validated (Wuyts *et al.*, 1995; Clausen *et al.*, 1998).

This study aimed at carrying out an epidemiological study for the detection and identification of *T. evansi* infections in camels clinically suspected of surra, in Upper Egypt by different diagnosis techniques, and to compare these techniques: Giemsa stained blood smear (GSBS), Hematocrit Centrifuge Technique (HCT) (Woo 1970), serological technique (CATT *T. evansi* ®) and DNA based polymerase chain reaction (PCR).

#### Materials and Methods

**Animals:** In this study, realized from August 2004 until February 2005, a total number 193 suspected clinical camels (*Camelus dromedarius*) were examined clinically, as body temperature, mucous membrane, muscles of thigh and hump. These animals had localities in Egypt in Aswan and Sohag Governorates. The main complaints of the camel owners were loss of appetite, decrease of productivity, and reduce of the body weight.

**Collection of blood and serum samples:** Two blood samples were collected from each suspected clinical camels through jugular vein. Whole blood samples were collected by jugular venipuncture into 10 ml EDTA-coated vacutainer tubes (Terumo, Eschborn, Germany). One part of these samples were subjected to

parasitological examination using, GSBS and HCT, but other part is preserved at -20°C for DNA extraction of trypanosomes for PCR. While blood samples were collected without EDTA in a dry, clean and sterile centrifuge tubes for serum preparation, also these samples were preserved at -20°C until used for detection of trypanosome antibodies using CATT.

Parasitological techniques:

**A. Giemsa stained blood smears (GSBS):** A drop of blood is placed on one end of a clean 94 microscope slide and a smear is drawn out. The blood smears were air-dried, fixed in methyl alcohol for 2 minutes and allowed to dry, then stained by Giemsa stain 10%. Trypanosomes were detected by microscopic examination GSBS at magnification 100x by help of oil immersion lens.

**B. Haematocrit centrifugation technique (HCT):** The method was described by Woo (1970). The capillary tubes were filled with blood sample and sealed at one end using plasticin, then centrifuged at 10,000 g for 10 minutes; the Buffy coat of the tubes were examined for the presence of trypanosomes using a microscope with oil immersion objective.

**Serological test, Card agglutination test (CATT):** 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.

#### **Molecular technique, Polymerase chain reaction (PCR):**

**DNA extraction:** DNA was prepared from camel blood as described by Higuchi (1989). Two hundred and fifty microlitres of EDTA blood was mixed with 250  $\mu$ l lysis buffer (0.32 M Sucrose, 0.01 M Tris, 5 mM MgCl<sub>2</sub>, 1% Triton X-100, pH 7.5). The mixture was centrifuged at 13,000 g for 25 sec. The supernatant was removed and the pellet washed with 500  $\mu$ l lysis buffer. The centrifugation and washing were repeated twice. The final pellet was resuspended in 250  $\mu$ l 1 x PCR buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1 % Triton X-100). 1.5  $\mu$ l of proteinase-K (10 mg/ml) was added and vortexed. The samples were incubated at 56 °C for 1 hour and at 95°C for 10 min. (to inactivate the proteinase-K) and stored at -20°C until use.

**DNA amplification:** PCR were carried out in 25  $\mu$ l reaction volumes containing 10 mM Tris-HCl (pH 8.3),

50 mM KCl, 2 mM MgCl<sub>2</sub>, 250 μM each of the four dNTP's (dATP, dCTP, dGTP, dTTP), 1 unit of Ampli Taq Gold® polymerase (Applied Biosystems, GmbH, Darmstadt, Germany), 5 μl template and 2 μM of each of the oligonucleotide primers. The primer sequences used were as follows (Artama et al., 1992):

Nuclid Repeat Primer 1- (NRP1): 5'-CGAATGAATATTAACAATGCGCAGT-3'

Nuclid Repeat Primer 2- (NRP2): 5'-AGAACCATTATTAGCTTTGTTGC-3'

The reaction mixture was overlaid with one drop of mineral oil and centrifuged at 10,000 g for 15 seconds. The tubes were transferred immediately into the thermocycler (TRIO-Thermo block, Biometra®, Germany).

**Cycling parameters:** PCR was performed by incubating the samples at three temperatures corresponding to three steps (denaturation, annealing and extension) in a cycle of amplification. programmed to perform a denaturation step at 95°C for 10 min (to activate the Ampli Taq Gold® DNA Polymerase), followed by 35 cycles consisting of 30 sec at 94°C, 30 sec at 55 °C and 30 sec at 72°C. The last extension step was 10 min longer. The samples were stored at 4°C until use in the next step.

**Gel-electrophoresis:** Visualization of the PCR-products was achieved by electrophoresis using a 2% agarose and ethidium bromide-staining.

## Results

**Clinical signs:** Camels infected with trypanosomes showed two forms, acute form of the disease characterized by loss of appetite, dullness, loss of body condition and increase of body temperature upto 40°C, in this form the parasite easy detected by microscopic examination after Giemsa stain blood smears. Whereas, the chronic form showed severe emaciation, weakness, the mucous membrane of conjunctiva become pale, atrophy in the muscles of the thigh, edematous swelling in testis, lacrymation and thin of the hump and also drop it to one side (figure 1 a&b).

**Parasitological results:** The Giemsa stain blood smears allowed detecting trypanosomes parasite in 8 out of 193 camels (4.1%), while haematocrit centrifugation technique detected 12 out of them (6.2%).

**Serological results:** CATT detected trypanosome antibodies in 84 camels out of 193 (43.5 %).

**Result of Molecular technique (PCR):** PCR detected 110 positive camels out of 193 (56.9%).

The results of examined camels after using the Polymerase chain reaction (PCR) for detection of DNA of trypanosomes in infected camels, showing a 177 bp PCR product for the specific detection of *Trypanosoma evansi*.

Parasitological methods detected only 20

camels out of 193 (10.3 %). All parasitological positive camels were also positive with CATT and PCR (100 % of sensitivity). On the other hand, CATT detected 84 out 193 (43.5 %).

## Discussion

In the present study, parasitological methods used for diagnosis of camel trypanosomosis are unsatisfactory. These results are in agreement with Godfrey and Killick-Kendrick (1962) who recorded those parasitological methods detected only 50 % of infected animals, because infection with trypanosomes in camels is usually in chronic form during which they exhibit very low parasitaemia. Moreover, Raisinghan and Lodha (1986) recorded parasitological methods used for detection of trypanosomes are not sensitive enough for diagnosis of Surra in camel. Such results were also observed by Paris, et al., (1982). The chronic form is most common and may present an association with secondary infections due to immuno-suppression caused by *T. evansi* infection, which complicates clinical diagnosis. Published clinical signs (emaciation, fever, anaemia, lacrymation, corneal opacity, diarrhoea and oedema of the dependent parts) (Chaudhary and Iqbal, 2000) are insufficient for diagnosis, while detection of parasites in the blood is difficult because parasitaemia is intermittent (Mahmound and Gray, 1980). Consequently, there is a need for alternative more sensitive diagnostic techniques.

Serological tests have been developed and evaluated for diagnosis of trypanosomosis in camels. The antibody-detecting tests such as the CATT/*T. evansi* (Bajyana-Songa and Hamers, 1988), So that, detection of infected camels by serological test (CATT) revealed 43.5% positive and all parasitological camels were positive by CATT; this result revealed a good correlation with parasitological methods. These findings are similar with those of Dially, et al., (1994) and Gutierrez, et al., (2000) who reported sensitivity of CATT test 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. Results on confirmed negative samples indicate that also the specificity of the PCR is satisfactory.

The PCR technique as used in this study was detects the chronic infection and low parasitaemia consistently the most sensitive technique. PCR also has some major advantages over the parasitological techniques. Sample processing does not have to be done within minimum time after collection but can be delayed at least 180 days after preservation at -20 °C. The PCR technique has been verified on blood samples of infected camels, confirming its higher sensitivity and specificity when compared to

parasitological techniques (Clausen, et. al., 1998).

The PCR technique is accurate, more sensitive and specific method in diagnosis of trypanosomes infected camels than other parasitological methods and overcome the problem of non specific reaction in case of serological tests; it can detect low parasitic camels in the chronic cases. The strength of PCR was shown in detection of infection in a parasitaemic camel showing clinical signs of diseases and was negative using parasitological tests. In this study, the camels shown to be PCR and HCT-positive had lower PCVs compared to uninfected camels.

The PCR test showed the best sensitivity compared with parasitological methods using GSBS or HCT. The chronic infection of the disease and low sensitivity of parasitological tests in Egypt, Moreover, CATT/*T.evansi*. Use of PCR as accurate and specific diagnostic technique, so treatment has to be carried out immediately in the field, and control programmed.

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**Table.- 1. PCR, parasitological and serological methods for detection of *T. evansi* infections in camels showing clinical signs of Surra**

Test	Positive	Negative	Total
Parasitological	20 (10.3%)	173	193
CATT	84 (43.5%)	109	193
PCR	110 (56.9%)	83	193

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