PARASITOLOGICAL AND MOLECULAR STUDIES ON TRYPANOSOMA EVANSI OF CAMELS IN EGYPT

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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 obliged 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 Bajyna 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 Genomic 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*.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences</th>
<th>Amplified product</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>T. evansi</em></td>
<td>EVA1 5'-ACATATCAACAAACGACAAG-3'</td>
<td>138 bp</td>
</tr>
<tr>
<td><em>T. evansi</em></td>
<td>EVA2 5'-CCCTAGTATCTCCAATGAAT-3'</td>
<td></td>
</tr>
</tbody>
</table>

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 dye, 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 94°C for 5 min; 30 cycles of 30 sec. at 94°C, 30 sec. at 60°C and 30 sec. at 72°C; and final extension step at 72°C 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 haematocrit 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 haematocrit 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.

<table>
<thead>
<tr>
<th>Parasitological tests</th>
<th>Suspected infected camels</th>
<th>Apparently healthy camels</th>
<th>Total No. of +ve samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Giza farm (n= 40)</td>
<td>EL-Bassatein abattoir (n= 97)</td>
<td>EL-Warraque abattoir (n= 50)</td>
</tr>
<tr>
<td>Giemsa stained blood smears</td>
<td>+v</td>
<td>%</td>
<td>+v</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.50</td>
<td>2</td>
</tr>
<tr>
<td>Haematochrite centrifugation</td>
<td>5</td>
<td>12.50</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>20</td>
<td>4</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Serological test</th>
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</tr>
<tr>
<td>CATT</td>
<td>+v</td>
<td>%</td>
<td>+v</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>27.50</td>
<td>6</td>
</tr>
</tbody>
</table>
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).

![Ethidium bromide stained agarose gel](image)

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-
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-Bassatin and El-
Warraq 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%, 47.0% 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


دراسات طفيلية و جزئية على التريبانوسوما إيفانزاي في الجمال بمصر

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2. قسم البيوتكنولوجيا - معهد بحوث صحة الحيوان - مركز البحوث الزراعية - الديق - الجيزة

أجرت هذه الدراسة على عدد 187 من الجمال منهم 40 جمل بمزرعة خاصة بمحافظة الجيزة في منطقة الأهرامات متوقع إصابتها بالتريبانوسوما لظهور بعض الأعراض المشابهة وعدد 147 من الجمال السليمة ظاهرا تم تجميعها من مجزري البساتين (76) والوريق (50) وذلك لاستبان نسبة الإصابة وتأكيد نوع التريبانوسوما الذي يصيب الجمال في مصر في مقارنة بين الاختيارات المختلفة. تم فحص جميع العينات بطرق طفيلي مختلفة (صبغة الجيما والهيماتوكريبت) وطرق سيرولوجية (الإلكات) وتفاعل البلازما المتسلسل، أظهر الفحص الطفيلي بطريقة صبغة الجيما والهيماتوكريبت أن نسبة الإصابة 7.50 % و 12.50 % من أصل 40 من الجمال التي تظهر عليهم الأعراض المشابهة على التوالي، بينما كانت نسبة الإصابة 20.0 % وكلا الطريقيتين للجمال السليمة ظاهرا من مجزري البساتين والوراق على التوالي. تم أخذ جميع العينات الإيجابية بواسطة الفحص الميكروسكوبى والسيروولوجى وعددهم 14 عينة لفحصهم باستخدام تفاعل البلمرة المتسلسل لتأكيد التشخيص وتحديد نوع التريبانوسوما وذلك باستخدام بادئ خاص للتربيانوسوما إيفانزاي عند 138 قاعدة زوجية وقد تبين أن جميع العينات إيجابية لهذا النوع من التريبانوسوما إيفانزاي ماعدا عينة واحدة كانت سلبية. تستنتج من ذلك أنه من الأفضل استخدام أكثر من طريقة للوصول إلى التشخيص الدقيق.