Prevalence of *Trypanosoma evansi* in Dromedary Camels
(*Camelus dromedarius*) in Al-Ahsa Area of Eastern Saudi Arabia

Maitham Abdullah Yusuf Al-Salameen(1), El Awad Mohammed El Hassan(2), Mohamed Abd Elmonem Salem(3)(4), Omar Abdullateef Al-Jabr(2), and Fadil Mohammed Housawi(3)

(1) Ministry of Environment, Water and Agriculture, Saudi Arabia
(2) Department of Microbiology and Parasitology, College of Veterinary Medicine
King Faisal University, Al- Ahsa, Saudi Arabia
(3) Department of Clinical Studies, College of Veterinary Medicine, King Faisal University
Al- Ahsa, Saudi Arabia.
(4) Department of Medicine and Infectious Diseases, Faculty of Veterinary Medicine, Cairo University.

Received 10 July 2018 - Accepted 12 May 2019

https://doi.org/10.37575/b/med/205

ABSTRACT

The present study investigates the prevalence of *Trypanosoma evansi* in dromedary camels in Al-Ahsa area of Eastern Saudi Arabia using both parasitological and serological methods. Six hundred camels were examined in this study at three different locations in Al-Ahsa area namely University Veterinary Clinic, Hufof Veterinary Clinic, and Hufof Slaughterhouse. These camels were grouped according to their age into three groups, under 1 to 2 years, over 2 to 9 years and over 9 years old. The association of animal age and sex with *Trypanosoma evansi* infection was also investigated.

Clinical examination of the surveyed camels showed loss of appetite in 2.3% of these animals, 13.3% of the camels suffering from diarrhea, 5.2% showed loss of weight while the rest of the surveyed camels (79.2%) were apparently healthy. Parasitological examination including Wet Mount Technique (WMT), Stained Blood Smears (SBS), and Hematocrit Centrifugation Technique (HCT) of blood samples obtained from the 600 surveyed camels failed to reveal any trypanosomes in these animals. Card Agglutination Test for Trypanosomes (CATT), however, was able to detect the presence of anti-*T. evansi* antibodies in the serum of 12.17% of the surveyed animals. Most of the seropositive camels were in the age group over 2 to 9 years, followed by those aging over 9 years, while the least seropositive cases were those aging under 1-2 years. The majority of the seropositive camels showed moderate agglutination level. The sex of camels showed high seropositivity to *T. evansi* in females compared to male camels.

Key Words: Agglutination, Hematocrit, Parasitological, Seropositive.

INTRODUCTION

Blood parasites are major constraint to camel health and production. These parasites are mainly protozoa in addition to some filarial worms. Infection with these parasites represents one of the major health hazards to camels. The predominant blood parasite reported in camels in Saudi Arabia is *Trypanosoma evansi*. This parasite induces a syndrome most commonly called Surra in the majority of economically important livestock as well as wild animals however; the principal affected hosts are equines and dromedaries (Desquesnes et al., 2013). Unlike African trypanosomes, the parasite transmitted mechanically by the hematophagous flies such as Tabanus and Stomoxys, hence it is widely distributed in all countries with hot and warm climates (Eyob and Matios, 2013). Infection with this parasite is one of the most debilitating diseases in camels. The disease was first reported in Saudi Arabia in 1984 (Diab et al., 1984). In a survey study carried out by Al-Khalifa et al. (2009) on the prevalence of blood parasites of camels in Saudi Arabia, *Trypanosoma evansi* was detected in five regions of the country including Eastern, Jazan, Northern frontiers, Riyadh, and Tabouk regions with a prevalence rate ranging from 5-40%. The parasite was also detected in Al Qassim area (Omer et al., 1998; El-Metenawy, 1998) and in the Central region (Kasim, 1984).

Although there are certain reports on *T. evansi* in camels in the Eastern region of the Kingdom in general, information regarding
the susceptibility of camels and prevalence of this parasite in Al-Ahsa area in particular is lacking. Therefore, the aims of this study were to examine and determine the prevalence of *Trypanosoma evansi* in dromedary camels, in Al-Ahsa province of Saudi Arabia and to investigate the effect of camel sex and age on susceptibility to infection.

**MATERIALS AND METHODS**

**Study area:**
This study was conducted in Al-Ahsa area of Eastern Province of Saudi Arabia. The area is hot and humid in summer, cold with some rainfall in winter.

**Animals:**
Six hundreds dromedary camels (*Camelus dromedarius*) from three locations in the study area namely, University Veterinary Hospital, Hufof Veterinary Clinic, and Hufof Slaughterhouse were included in this study. They were grouped according to their age into three categories, under 1 to 2 years, over 2 to 9 years, and over 9 years old. The camels were grossly examined for clinical symptoms before sampling. Blood sampling was employed in this study as described by WHO (2010).

**Collection of blood samples:**
Five-ml blood samples were collected by jugular vein puncture from camels into plain vacutainer tubes (WHO, 2010). These samples were used in wet mount technique (WMT), stained blood smears (SBS), hematocrit centrifugation technique (HCT) and for preparation of serum samples.

**Parasitological techniques:**

**Wet Mount Technique (WMT):** (Higgins, 1983)
A drop of blood was placed onto a clean microscopic slide, covered with a coverslip and examined microscopically (20 microscopic field at x40 magnification) for detection of trypanosomes motility.

**Stained Blood Smears (SBS):**
Both thin and thick blood smears were prepared according to Cruickshank *et al.* (1975). The slides were air dried and stained with Leishman stain for 3 minutes. The slides were then flooded with distilled water, left to stand for 1 minute, then washed and air-dried. Then the slides were, examined microscopically using 100x objective lens for parasites identification.

**Hematocrit Centrifugation Technique (HCT):** (Woo, 1970)
Heparinized capillary tubes were filled with non-coagulated blood and sealed at one end using plasticine. The tubes were then centrifuged at 3000 rpm for 5 minutes using a hematocrit centrifuge and the buffy coat area was examined microscopically for the presence of trypanosomes.

**Serological Techniques:**

**Card Agglutination Test for Trypanosomes (CATT):** (Bajyana Songa and Hamers, 1988)
A CATT *Trypanosoma evansi* kit produced by Institute of Tropical Medicine (Prince Leopold) Antwerpen, Belgium was used to screen camel sera collected in this study. The test utilizes a CATT – antigen of a freeze dried suspension of purified, fixed and stained bloodstream form trypanosomes expressing a predominant variable antigen type of *Trypanosoma evansi* (Ro Tat 1.2). The test is conducted on a plastified card according to Manufacturer instructions. A two-fold dilution ranging from 1:4 to 1:64 of each serum sample was prepared in CATT buffer (Phosphate buffered saline, pH 7.2). Twenty-five µl of each dilution were applied in a test area on the card. Known positive and negative controls were also included in the test. Then 45 µl of well homogenized CATT antigen is added to each serum sample and mixed. Then the mixture was spread out to a nearly 1mm from the boundary of test area. The card was then rotated on a flat bed orbital rotator for 5 minutes at 70rpm, then read for agglutination using a naked eye.
RESULTS

Clinical examination:
Fourteen of the examined camels suffered from loss of appetite, 80 camels suffered from diarrhea, and 31 showed loss of weight while the rest of the examined camels were apparently healthy.

Parasitological examination:
Blood samples collected from the 600 surveyed camels, examined by wet mount technique (WMT), hematocrit centrifugation technique (HCT), and stained smears revealed the absence of any trypanosomes in these animals as shown in table 1.

<table>
<thead>
<tr>
<th>Location</th>
<th>Total No. of animals</th>
<th>Wet mount</th>
<th>Hematocrit technique</th>
<th>Stained smears</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hufof Vet. clinic</td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>KFU Vet. clinic</td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>0</td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>200</td>
<td>0</td>
<td>200</td>
<td>0</td>
</tr>
</tbody>
</table>

Serological examination:
Card Agglutination Test for Trypanosomes (CATT):
Seventy-three Trypanosoma evansi seropositive cases were detected out of the 600 surveyed camels using CATT test, representing 12.17%. As shown in table (2) and figure (1) the highest number of seropositive cases was reported from the university veterinary clinic where 31 out of 200 camels were seropositive representing 15.5%. This is followed by camels examined at the slaughterhouse, where 25 seropositive cases were reported, representing 12.5%. The least number of seropositive cases was reported from animals examined at Hufof veterinary clinic where only 17 seropositive cases were detected, representing 8.5%.

<table>
<thead>
<tr>
<th>District</th>
<th>No. of animals</th>
<th>CATT positive</th>
<th>CATT negative</th>
<th>Percentage +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hufof Vet. clinic</td>
<td>200</td>
<td>17</td>
<td>183</td>
<td>8.5%</td>
</tr>
<tr>
<td>KFU Vet. clinic</td>
<td>200</td>
<td>31</td>
<td>169</td>
<td>15.5%</td>
</tr>
<tr>
<td>Slaughterhouse</td>
<td>200</td>
<td>25</td>
<td>175</td>
<td>12.5%</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>73</td>
<td>527</td>
<td>12.17%</td>
</tr>
</tbody>
</table>

Figure 1: Seroprevalence of T. evansi in camels in Al-Ahsa area of Saudi Arabia
Agglutinating level of anti-\textit{T. evansi} antibodies:

As shown in Table (3), 18 out of the 73 seropositive camels showed very strong agglutination, while 11 showed strong agglutination and the majority of the seropositive camels showed moderate agglutination. Figures 2, 3, and 4 illustrate the agglutinating level of anti-\textit{T. evansi} antibodies in the three surveyed localities. At Huof veterinary clinic 5 out of 17 seropositive camels were found to produce a very strong level, three with strong level and nine cases with moderate level of agglutination (Figure 2). At the KFU veterinary clinic 3 out of 31 seropositive camels showed very strong level, one with a strong level while 27 showed moderate level of agglutination (Figure 3). At the slaughterhouse 10 out of the 25 seropositive cases showed very strong level, seven with strong level while eight with moderate level of agglutination (Figure 4).

<table>
<thead>
<tr>
<th>District</th>
<th>No. of animals</th>
<th>Total +ve</th>
<th>+</th>
<th>++</th>
<th>+++</th>
<th>ve-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huof Vet. Clinic</td>
<td>200</td>
<td>17</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>183</td>
</tr>
<tr>
<td>KFU Vet. Clinic</td>
<td>200</td>
<td>31</td>
<td>27</td>
<td>1</td>
<td>3</td>
<td>169</td>
</tr>
<tr>
<td>Slaughter house</td>
<td>200</td>
<td>25</td>
<td>8</td>
<td>7</td>
<td>10</td>
<td>175</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>600</strong></td>
<td><strong>73</strong></td>
<td><strong>44</strong></td>
<td><strong>11</strong></td>
<td><strong>18</strong></td>
<td><strong>527</strong></td>
</tr>
</tbody>
</table>

\(^\chi^2 = 18.266\)  \ DF = 4  \ P = 0.0011

Key:
+ Positive (moderate agglutination).
++ Positive (strong agglutination).
+++ very strong agglutination.
\(\chi^2 = \) Chi Squares test.
DF = Degrees of freedom.
P = Probability.

Figure 2: Agglutination level at Huof Veterinary Clinic
Age susceptibility to *Trypanosoma evansi* infection:
As shown in Table 4, the highest seroprevalence rate was detected in animals aging over 2 to 9 years where 35 seropositive cases out of 73 were reported in this age group. This is followed by those aging over 9 years, where 30 seropositive cases were detected. The least susceptibility was detected in animals aging under 1 to 2 years where only eight seropositive cases were reported in this age group.

Table 4: Effect of age on seroprevalence of *Trypanosoma evansi*

<table>
<thead>
<tr>
<th>District</th>
<th>No. of animals</th>
<th>-ve at all ages</th>
<th>+ve at under 1 to 2 years</th>
<th>+ve at over 2 to 9 years</th>
<th>+ve at above 9 years</th>
<th>-ve at all ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hufof Vet. clinic</td>
<td>200</td>
<td>183</td>
<td>3</td>
<td>9</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vet. Clinic (KFU)</td>
<td>200</td>
<td>169</td>
<td>4</td>
<td>20</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Slaughter house</td>
<td>200</td>
<td>175</td>
<td>1</td>
<td>6</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>600</td>
<td>527</td>
<td>8</td>
<td>35</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

$\chi^2 = 15.72\quad DF = 4\quad P = 0.0034$
Figures 5, 6, and 7 show the effect of age on seroprevalence of *T. evansi* at the three investigated areas. The highest prevalence rate was detected in camels aging over 2 to 9 years at both Hufof vet. clinic and the University clinic. This is followed by those aging over 9 years and then those aging under 1 to 2 years (Figures 5 and 6). At the slaughterhouse, camels aging over 9 years showed the highest prevalence, followed by those aging over 2 to 9 years and then by those aging under 1 to 2 years (Figure 7).

Figure 5: Effect of camel age on seroprevalence of *T. evansi* at Hufof Clinic

Figure 6: Effect of camel age on seroprevalence of *T. evansi* at KFU Vet. Clinic

Figure 7: Effect of camel age on seroprevalence of *T. evansi* at Slaughterhouse
**Sex susceptibility to** T. evansi **infection:**

Table 5) and figure (8) illustrate the susceptibility of camel sexes to T. evansi infection at each locality of the study area. While figure (9) shows the overall percentage of the seropositive males and females in the three locations.

Table 5: Susceptibility of camel sex to **Trypanosoma evansi** infection

<table>
<thead>
<tr>
<th>District</th>
<th>No. of animals</th>
<th>Sex</th>
<th>+ve males</th>
<th>+ve females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hufof Vet. Clinic</td>
<td>200</td>
<td>Male</td>
<td>36</td>
<td>164</td>
</tr>
<tr>
<td>Vet. Clinic (KFU)</td>
<td>200</td>
<td>Female</td>
<td>43</td>
<td>157</td>
</tr>
<tr>
<td>Slaughter House</td>
<td>200</td>
<td>Male</td>
<td>32</td>
<td>168</td>
</tr>
</tbody>
</table>

χ² = 5.465  DF = 2  P =< 0.05

![Figure 8: Susceptibility of camel sex to Trypanosoma evansi infection](image)

![Figure 9: Overall effect of camel sex on seroprevalence of T. evansi](image)

**DISCUSSION**

In the present study clinical examination of 600 camels, showed loss of appetite in 2.3% of the surveyed camels, 13.3% of the camels were suffering from diarrhea and 5.2% from weight loss while the rest of the camels were...
apparently healthy. Although these clinical signs were reported in infection with blood parasites, yet they are not pathognomonic for these infections and are insufficient for their diagnosis. Recorded clinical signs of trypanosomosis include emaciation, rise in body temperature, anemia, lacrimation, opacity of the cornea, diarrhea and edema of the dependent parts (Chaudhary and Iqbal, 2000). These signs are due to a series of biochemical reactions within the body of infected animal as revealed by a significant increase in measured oxidative stress biomarkers (El-Bahr and El-Deeb, 2016).

Parasitological examination, including wet mount, Giemsa stained blood smears and hematocrit technique, did not detect any blood parasite in blood samples collected from the 600 surveyed camels possibly due to either absence or low parasitemia. Parasitemia usually denotes acute phase of the disease while during chronic phase the number of parasites in blood is too low to be detected by parasitological methods (Mottelib et al., 2005). T. evansi is known to invade tissues during chronic phase of the disease and the parasite is scanty or totally absent from the blood of infected animals. The failure of parasitological methods to detect any Trypanosoma evansi parasites in this study is in agreement with those of Hussain et al. (1991) and Abdel-Rady (2008), who reported unsatisfactory results of parasitological methods in diagnosis of camel trypanosomiasis due to the chronic nature of the disease in these animals. Godfrey and Killick-Kendrick (1962) stated that trypanosome infections in camels are usually chronic and the parasite exhibits very low parasitemia.

Although parasitemia was undetectable, serological examination of the surveyed camels, using CATT/T. evansi in this study showed the presence of anti-T. evansi antibodies in 12.17% of the camels. This test is sensitive compared to microscopic parasitological methods and can detect early and late antibodies to T. evansi (Verloo et al., 2000). Similarly Hilali et al. (2004) was able to detect anti-T. evansi antibodies in water buffaloes experimentally infected with strain from dromedary camel during undetectable parasitemia. The seropositivity in the present study ranged from very strong to moderate agglutination with the majority of the animals showed moderate level but the titer remains at a detectable level. There was a poor association between clinical signs and seropositivity suggesting chronic or previous infections. Although the overall level of seropositivity is moderate in the study area, yet the highest level was detected in animals brought to university veterinary clinic followed by those examined at the slaughterhouse and then animals at Hufof veterinary clinic. This variation is probably attributed to variation in origin of these animals. Low T. evansi seropositivity levels among camels was also reported in Al-Qassim region of Saudi Arabia by El-Metenaway (1998) who reported 7% seropositivity in that region and in Jazan region (18.3%) by Hussain et al. (1991). However, Abdel-Rady (2008) using CATT/T. evansi was able to detect 43.5% seropositive camels and Al-Khalifa et al. (2009) reported higher incidence of T. evansi infection among camels in five regions of this country.

In the present study, generally, adult animals showed the highest seropositivity, possibly due to stress caused by pregnancy, lactation and transportation work required from these animals or due to previous infection as the test used can detect also persisting antibodies circulating in the blood of these animals. Seropositivity and active infection with T. evansi were reported to increase with age (Dia et al., 1997; Atarhouch et al., 2003; Bhutto et al., 2010; Tadesse et al., 2012; Al-Salameen et al., 2016) and was attributed to large-scale movement of adult camels that increases their risk to infection compared to younger animals.

High seropositivity was reported in female camels compared to that in males in the current study, possibly due to stress encountered on these animals during pregnancy and lactation. Similarly, Shah et al. (2004) and Bhutto et al. (2010) reported high incidence
of trypanosomosis in female camels. On the other hand, Tadesse et al. (2012) using parasitological methods did not detect any significant difference in the prevalence of *T. evansi* infection between male and female camels in Ethiopia, this could possibly be due to differences in detection methods used in their study compared to that of the present study. Animals examined in the slaughterhouse, however, showed high seropositivity in male camels compared to that reported in females, possibly due to the fact that female camels usually brought to slaughter at very old age and seropositivity reported in this study for this age group was lower than that of the age group over 2-9 years.

Further investigation on the epidemiology and prevalence of *T. evansi* in camels all over the country using more sensitive methods such as molecular techniques is needed to verify and update information regarding this parasite in order to plan an effective control practices.

In conclusion, the moderate seroprevalence of *Trypanosoma evansi* in camels in the study area, and the failure of parasitological methods to detect any trypanosomes despite the large number of animals examined may indicate the scarceness of this parasite in the area. Results obtained in the present study possibly reflect the efficacy of the active policy adopted at the study area for combating blood parasites of camel and the awareness of local camel producers with camel health and fitness.

ACKNOWLEDGEMENT

This work is a part of a M.Sc. thesis sponsored by the Deanship of Scientific Research, King Faisal University (DSR 112016) to whom we are very grateful. The financial support made by King Abdul-Aziz City for Science and Technology (KACST, Project # AT-32-185) also greatly appreciated.

REFERENCES


Prevalence of Trypanosoma evansi in Dromedary Camels... Maitham Abdullah Yusuf Al-Salameen et al.


دراسة الحالية تبحث نسبة انتشار طفيل التريبانزوما إيفانزاي في الإبل في منطقة الأحساء شرق المملكة العربية السعودية باستخدام الدراسات الطفيلية وأحد الاختبارات المناعية. تم فحص عدد 600 إبل في هذه الدراسة في ثلاثة مواقع مختلفة في منطقة الأحساء، تشمل المستشفى البيطري بجامعة الملك فيصل، المستشفى البيطري لوزارة الزراعة بالهفوف، ومسلخ الهفوف. وقد قسمت هذه الإبل إلى ثلاث مجموعات وفقاً لأعمارها: أقل من عام إلى عامين، أكثر من عامين إلى تسعة أعوام، وأكثر من تسعة أعوام من العمر. وقد تم دراسة تأثير العمر والجنس على قابلية الحيوانات للإصابة. الاختبارات الطفيلية على عينات الدم التي تم الحصول عليها من الإبل التي شملتها الدراسة لم تكشف عن أي إصابة. فحص عينات المصل التي جمعت من هذه الإبل عن طريق اختبار CATT أظهر وجود الأجسام المضادة لطفيل التريبانزوما إيفانزاي. المجموعة التي أظهرت أقصى وجود الأجسام المضادة كانت الفئة العمرية الأقل من سنة إلى سنتين. أظهرت الدراسة أيضاً أن إناث الإبل أكثر عرضة للإصابة مقارنة بالذكور.

الكلمات المفتاحية: إيجابي المصل، التلصيق، الطرق الطفيلية، الهيماتوكريت.