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Prevalence of *Trypanosoma evansi* in Dromedary Camels (*Camelus dromedarius*) in Saudi Arabia

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نسبة انتشار طفيل التريبانوسوما إيفانزاي في الإبل في السعودية

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Agglutination, haematocrit, parasitological, seropositive
إيجابي المصل، التلصيق، الطرق الطفيلية، الهيماتوكريت

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ABSTRACT

The present study investigates the prevalence of *Trypanosoma evansi* in dromedary camels in Al Ahsa, Saudi Arabia, using both parasitological and serological methods. Six hundred camels were examined in this study at three different locations in the Al Ahsa area, namely the University Veterinary Clinic, Hufof Veterinary Clinic, and Hufof Slaughterhouse. These camels were grouped according to their age into three groups: from one to two years, from two to nine years, and over nine 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 diarrhoea, and 5.2% showing loss of weight, while the rest of the surveyed camels (79.2%) were apparently healthy. Parasitological examination, including the wet mount technique (WMT), stained blood smears (SBS), and the haematocrit centrifugation technique (HCT), of blood samples obtained from the 600 surveyed camels failed to reveal any trypanosomes in these animals. The 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 from two to nine years, followed by those aged over nine years, while the least seropositive cases were those aged one to two 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.

الملخص

الدراسة الحالية تبحث نسبة انتشار طفيل التريبانوسوما إيفانزاي في الإبل في منطقة الأحساء شرق المملكة العربية السعودية باستخدام الاختبارات الطفيلية وأحد الاختبارات المناعية. تم فحص عدد 600 رأس من الإبل 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 وفقا لأعمارها، أقل من عام إلى عامين، أكثر من عامين إلى تسعة أعوام، وأكثر من تسعة أعوام من العمر. وقد تمت أيضا 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 أية إصابة بهذه المثقبيات. فحص عينات المصل التي جمعت من هذه الإبل عن طريق اختبار CATT أظهر وجود أجسام مضادة لطفيل التريبانوسوما إيفانزاي في مصل 12.17 % من الحيوانات التي شملها الاستقصاء. معظم هذه الإبل كان من الفئة العمرية أكثر من عامين إلى تسعة أعوام، تلتها المجموعة الأكثر من 9 سنوات، في حين أن المجموعة التي أظهرت أقل وجود للأجسام المضادة كانت الفئة العمرية الأقل من سنة إلى سنتين. أظهرت الدراسة أيضا أن إناث الإبل أكثر عرضة للإصابة مقارنة بالذكور.

1. Introduction

Blood parasites are a major constraint on 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; the principal affected hosts are equines and dromedaries (Desquesnes et al., 2013). Unlike African trypanosomes, the parasite is transmitted mechanically by haematophagous 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 the Eastern, Jazan, Northern Borders, Riyadh, and Tabouk Regions, with a prevalence rate ranging from 5–40%. The parasite was also detected in the Al Qassim area (El-Metenawy, 1998; Omer et al., 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 the 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.

2. Materials and Methods

2.1. Study Area:

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

2.2. Animals:

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

2.3. Collection of Blood Samples:

Five-ml blood samples were collected by jugular vein puncture from

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camels into plain vacutainer tubes (WHO, 2010). These samples were used in the wet mount technique (WMT), stained blood smears (SBS), haematocrit centrifugation technique (HCT), and for preparation of serum samples.

2.4. Parasitological Techniques:

2.4.1. 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 ×40 magnification) for detection of trypanosome motility.

2.4.2. Stained Blood Smears (SBS)

Both thin and thick blood smears were prepared according to Cruickshank et al. (1975). The smears were air-dried and stained with a Leishman stain for three minutes. The slides were then flooded with distilled water, left to stand for one minute, then washed and air-dried. Then the slides were examined microscopically using a 100× objective lens for parasite identification.

2.4.3. Haematocrit Centrifugation Technique (HCT) (Woo, 1970)

Heparinised capillary tubes were filled with non-coagulated blood and sealed at one end using plasticine. The tubes were then centrifuged at 3,000 rpm for five minutes using a haematocrit centrifuge, and the buffy coat was examined microscopically for the presence of trypanosomes.

2.5. Serological Techniques:

2.5.1. Card Agglutination Test for Trypanosomes (CATT) (Bajyana Songa and Hamers, 1988)

A CATT *Trypanosoma evansi* kit produced by the Prince Leopold Institute of Tropical Medicine (Antwerp, Belgium) was used to screen the camel sera collected in this study. The test utilises 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* (RoTat 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 homogenised CATT antigen were added to each serum sample and mixed. Then the mixture was spread out to nearly 1mm from the boundary of the test area. The card was then rotated on a flatbed orbital rotator for five minutes at 70rpm, then read for agglutination using the naked eye.

3. Results

3.1. Clinical Examination:

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

3.2. Parasitological Examination:

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

Table 1: Parasitological examination of the surveyed camels

Location	Total no. of animals	Wet mount		Haematocrit technique		Stained smears	
		+	−	+	−	+	−
Hufof Vet. Clinic	200	0	200	0	200	0	200
KFU Vet. Clinic	200	0	200	0	200	0	200
Slaughterhouse	200	0	200	0	200	0	200

3.3. Serological Examination:

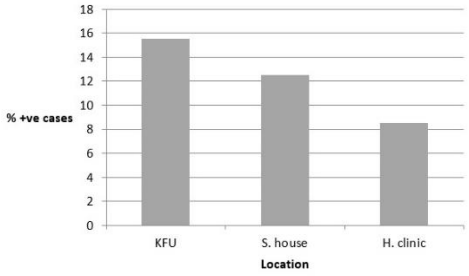
3.3.1. Card Agglutination Test for Trypanosomes (CATT)

Seventy-three *Trypanosoma evansi* seropositive cases were detected out of the 600 surveyed camels using the 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 (15.5%) were seropositive. 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 2: Seroprevalence of *Trypanosoma evansi* in camels in the Al Ahsa area of Saudi Arabia

District	No. of animals	CATT positive	CATT negative	Percentage +ve
Hufof Vet. Clinic	200	17	183	8.5%
KFU Vet. Clinic	200	31	169	15.5%
Slaughterhouse	200	25	175	12.5%
Total	600	73	527	12.17%

Figure 1: Seroprevalence of *T. evansi* in camels in the Al Ahsa area



3.3.2. Agglutinating level of anti-*T. evansi* antibodies

As shown in Table 3, 18 of the 73 seropositive camels showed very strong agglutination, 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-*T. evansi* antibodies in the three surveyed localities. At Hufof Veterinary Clinic, 5 out of 17 seropositive camels were found to produce a very strong level, three a strong level, and nine cases a moderate level of agglutination (Figure 2). At the KFU Veterinary Clinic, 3 out of 31 seropositive camels showed a very strong level, one a strong level, and 27 a moderate level of agglutination (Figure 3). At the slaughterhouse, 10 of the 25 seropositive cases showed a very strong level, seven a strong level, and eight a moderate level of agglutination (Figure 4).

Table 3: Agglutination level of anti-*T. evansi* antibodies in camels in the Al Ahsa area

District	No. of animals	Total +ve	+	++	+++	−ve
Hufof Vet. Clinic	200	17	9	3	5	183
KFU Vet. Clinic	200	31	27	1	3	169
Slaughterhouse	200	25	8	7	10	175
Total	600	73	44	11	18	527

$\chi^2 = 18.266$ DF = 4 P = 0.0011

Key:

+ Positive (moderate agglutination).

++ Positive (strong agglutination).

+++ Very strong agglutination.

χ^2 = Chi-squared test.

DF = Degrees of freedom.

P = Probability.

Figure 2: Agglutination level at Hufof Veterinary Clinic

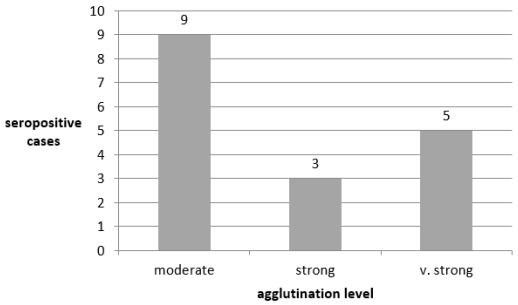


Figure 3: Agglutination level at KFU Veterinary Clinic

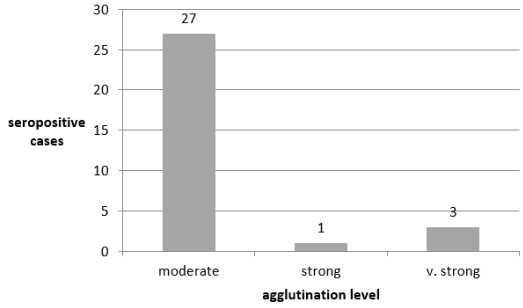
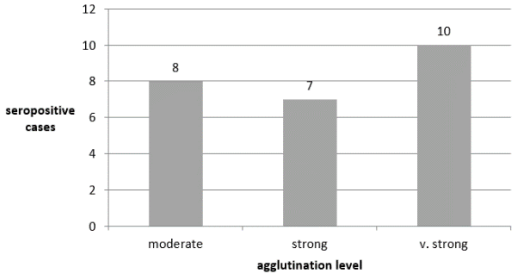


Figure 4: Agglutination level at Hufof Central Slaughterhouse



3.3.3. Age susceptibility to *Trypanosoma evansi* infection

As shown in Table 4, the highest seroprevalence rate was detected in animals aged from two to nine years: 35 seropositive cases out of 73 were reported in this age group. This is followed by those aged over nine years, where 30 seropositive cases were detected. The least susceptibility was detected in animals aged from one to two years: only eight seropositive cases were reported in this age group.

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

District	No. of animals	Age			
		+ve at 1 to 2 years	+ve at 2 to 9 years	+ve at above 9 years	-ve at all ages
Hufof Vet. Clinic	200	3	9	5	183
KFU Vet. Clinic	200	4	20	7	169
Slaughterhouse	200	1	6	18	175
Total	600	8	35	30	527

$\chi^2 = 15.72$ DF = 4 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 aged from two to nine years at both Hufof Veterinary Clinic and the University Clinic. This is followed by those aged over nine years and then those aged from one to two years (Figures 5 and 6). At the slaughterhouse, camels aged over nine years showed the highest prevalence, followed by those aged from two to nine years and then by those aged from one to two years (Figure 7).

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

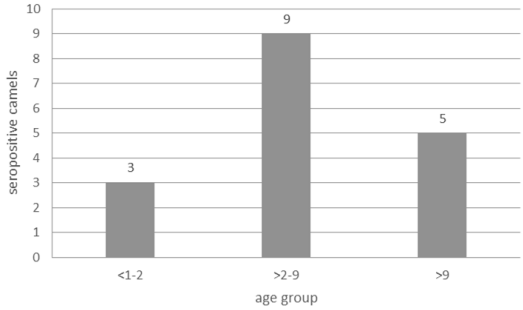


Figure 6: Effect of camel age on seroprevalence of *T. evansi* at KFU Vet. Clinic

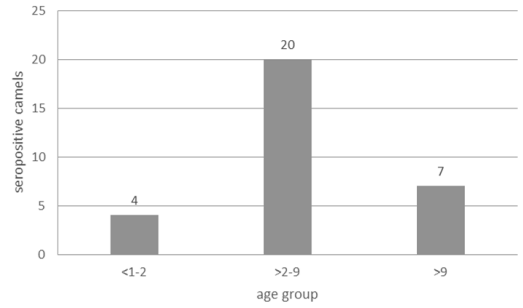
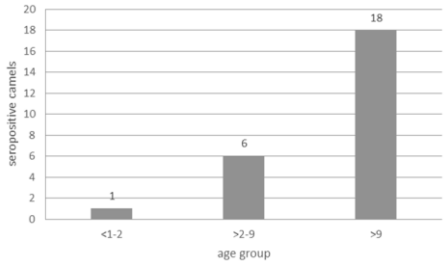


Figure 7: Effect of camel age on seroprevalence of *T. evansi* at the Slaughterhouse



3.3.4. 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

District	No. of animals	Sex			
		Male	Female	+ve males	+ve females
Hufof Vet. Clinic	200	36	164	1	16
KFU Vet. Clinic	200	43	157	4	26
Slaughterhouse	200	32	168	5	20

$\chi^2 = 5.465$ DF = 2 P = < 0.05

Figure 8: Susceptibility of camel sex to *Trypanosoma evansi* infection

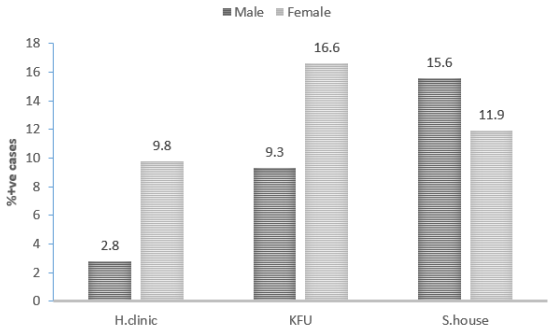
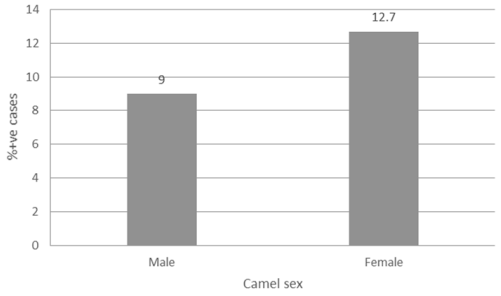


Figure 9: Overall effect of camel sex on seroprevalence of *T. evansi*



4. 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 diarrhoea, 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, they are not pathognomonic for these infections and are insufficient for their diagnosis. Recorded clinical signs of trypanosomosis include emaciation, rise in body temperature, anaemia, lacrimation, opacity of the cornea, diarrhoea, and oedema of the dependent parts (Chaudhary and Iqbal, 2000). These signs are due to a series of biochemical reactions within the body of the 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 staining of blood smears, and haematocrit technique, did not detect any blood parasite in blood samples collected from the 600 surveyed camels, possibly due to either absence or low parasitaemia. Parasitaemia usually denotes an acute phase of the disease, while during the chronic phase the number of parasites in the blood is too low to be detected by parasitological methods (Mottelib et al., 2005). *T. evansi* is known to invade tissues during the chronic phase of the disease, and the parasite is rare in 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 the diagnosis of camel trypanosomosis 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 parasitaemia.

Although parasitaemia 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 a strain from a dromedary camel during undetectable parasitaemia. The seropositivity in the present study ranged from very strong to moderate agglutination; the majority of the animals showed a moderate level, but the titre 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, the highest level was detected in animals brought to the 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 the origin of these animals. Low *T. evansi* seropositivity levels among camels was also reported in the Al-Qassim region of Saudi Arabia by El-Metenaway (1998), who reported 7% seropositivity in that region, and in the 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 the transportation work required from these animals, or due to previous infection, as the test used can also detect 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 in 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 are usually brought to slaughter at a very old age and seropositivity reported in this study for this age group was lower than that in the age group from two to nine years.

Further investigation of 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 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 in the study area for combating blood parasites of camels and the awareness among local camel producers of camel health and fitness.

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