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Surveillance of Camel Trypanosomosis in Al-Jouf region, Saudi Arabia

Mohamed El Wathig[1] and Bernard Faye[2,3*]

[1]Federal Ministry of Animal Resources and Fisheries, Khartoum, Sudan
[2]Camel and Range Research Center, P.O.Box 322, Al-Jouf, Saudi Arabia
[3] CIRAD-ES, UMR SELMET, TA C/dir B, Campus International de Baillarguet, 34398 Montpellier, France

[*]Corresponding Author: bernard.faye@cirad.fr

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Abstract

Three hundred and ten blood samples from clinically healthy and suspected camels (male and female) of four breeds from different location of Al-jouf (Saudi Arabia), were examined to detect prevalence rate of *T. evansi* infection by CATT test. An overall prevalence was determined as 43.8%. The variability of trypanosomosis was highly significant according to the factor moving, clinical signs and animal status regarding its age, sex and lactation status. Prevalence rate increased significantly in May and a second peak was observed in September.

Keywords: Camel, Trypanosoma evansi, CATT, Risk factor

Introduction

Trypanosomosis is a large group of disease caused by obligated flagellate blood parasite that infect members of every vertebrate class (Hoare, 1972). Camel trypanosomosis, also known as Surra, is one of the main disease of camels and is caused by *Trypanosoma evansi*, causing morbidity of up to 30% and mortality of around 3% (Ngerenwa *et al*., 1993; Egbe-Nwiyi and Chaudry 1994 and Pacholek *et al*., 2001). The disease is occurring in Africa, Asia, Central and South America, Canary Island and recently in France (Gutierrez *et al*. 2000; Luckins and Dwinger. 2004, Desquesnes *et al.*, 2008 and Tamarit *et al.*, 2010). The parasite is transmitted by biting flies such as Tabanidae and Stomoxys species, as well as by Vampire bats (Hoare, 1972).

The disease is still serious problem in camel husbandry that causes considerable economic losses in many camel farms of the world (Olaho-Mukani *et al*., 1996; Enwezor and Sacky, 2005 and Derakhshanfar *et al.*, 2010), causing decrease in milk and meat yield, premature births and abortions in pregnant females and medical cost (Boid *et al.*,1985 and Reid 2002).

The disease occurs both in chronic and acute form (Gutierrez *et al*., 2000). The chronic form of the disease is most common and is likely to be associated with secondary infection due to immunodepression (Njiru *et al.,* 2004). Clinical signs and pathological lesions caused by *T. evansi* in camels are unreliable for definitive diagnosis (Chaudhary and Igbal, 2000). Diagnosis of trypanosomosis depends on detection of the parasite in the blood or tissue fluids of infected animals. Unfortunately, parasitological techniques cannot always detect ongoing infection, as the level of parasitaemia is often low and fluctuating (Zweygarth *et al*., 1984). 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; it is a quick, simple and easy to perform and sensitive (Diall *et al*., 1994 and Pathak *et al*., 1997). Molecular markers are required to detect accurately *T. evansi* infections in the early stages. In Saudi Arabia, the disease is widely present, but few publications are available on the observed prevalence. Therefore, the aim of this study was to detect infected camels in northern part of Saudi Arabia by using conventional methods and CATT, then to evaluate the correlations between the disease prevalence and some risk factors.

Materials and Methods

Study area

This study was conducted in Al-Jouf province, which located in the north-western part of Saudi Arabia between 42.37 E and 32.29 N (Fig.1). The

Fig. 1: Study area of *T. evansi* surveillance

average annual rainfall was about 2 to 10 ml, and the vegetation mainly composed of desert grasses e.g. *Plantago amplexicali, Calligonum comosum* and *Salsala vilosa* .

Sampling procedures

According to the availability of the camel herds as well as willingness of the owner, non-probability sampling method (convenience sampling) was employed in the study site as described by Thrusfield (1996). In this study, achieved from February 2011 until October 2011, as a whole, 310 camels from 25 farms in different locations in the area were sampled. In each farm, both clinically healthy and suspected camels with trypanosomosis were

sampled and data regarding their breed, lactation status, clinical signs, movement and season were collected.

Collection of blood and serum samples

Two blood samples were collected from the jugular vein of each animal: one in a tube containing anticoagulant, ethylene diamine tetraacetic acid (EDTA) and the second one without anticoagulant. These blood samples were kept in ice and were processed on the day of collection. The blood samples with EDTA were used for parasitological diagnosis and the others were processed for serum collection. Blood samples were centrifuged at 5000 rpm for 15 minutes to separate serum for CATT.

Parasitological techniques

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

2- Hematocrit centrifugation technique (HCT) was achieved according to the method described by Woo (1970). The capillary tubes were filled with blood samples and sealed at one end using plasticin, then centrifuged at 10.000 rpm for 10 minutes at room temperature; the buffy coat of the tubes was examined for the presence of trypanosomes using a microscope with oil immersion objective.

Serological test

Serum samples were tested with Card Agglutination Test (CATT/*T. evansi*) following 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 the help of manual rotation for 5 minutes. Blue granular agglutinations indicate a positive reaction visible to the naked eye.

Statistical analysis

Each sample was described by the date of sampling, the location, the age status (young male, adult male, lactating female, non-lactating female) and the breed of the animal, the moving status (urban moving, desert moving, urban in-door and desert in-door), the clinical signs at sampling time (healthy, emaciation, general clinical signs) and treatment (not treated, treated with antibiotics, treated with antiparasitic, treated with anti-tryps) and finally the CATT results (Positive or negative). The aims of the statistical procedure were:

- To quantify the positive CATT test prevalence according to the different factors mentioned above

- To identify the relationships between the CATT results and the different parameters. For this, a two-steps analysis was achieved: (i) Analysis of cross-tables (CATT test x each explaining factors) by Chi-square test, (ii) Multivariate analysis using Multiple Correspondence analysis (MCA) to investigate the correlations between CATT test results and explaining factors. An automatic hierarchical classification (AHC) allowed getting type of animals, and cross tables types x CATT test results were analyzed by Chi-square test.

The analyses were performed with software XL stat (© Addinsoft).

Results

Variability of T. evansi prevalence rate in camels

Based on CATT results, the prevalence of *T. evansi* infection in camels was found to be as 43.8%. However, according to the factors taken in account, the prevalence rate could vary from 11.1% to 79% (Table 1).

The variability in trypanosomosis is highly significant according to the factor "moving" ($P<0.00001$), clinical signs ($P<0.0001$) and animal status regarding

Table 1: Trypanosomosis prevalence detected by CATT test according to different factors in Al-Jouf region (Saudi Arabia)

p	CATT Prevalence %		Factors
0.088	46%	1-hamra	Breed
	40%	2-malha	
	30%	3-safra	
	49%	4-wadha	
0.000	60.2%	1-Urban-moving	Moving
	27.6%	2-Desert-moving	
	37.8%	3-Urban-indoor	
	66.6%	4-Desert-indoor	
0.061	40%	1-No treatment	Pre-treatment
	50%	2-tret-with antibiotics	
	59%	3-treat-with antiparasitic	
	71.4%	4-treat-with anti-tryps	
0.0001	38.2%	1-No clinical signs	Clinical signs
	79.3%	2-Emaciation	
	73.3%	3-Genral clinical signs	
0.0001	11.1%	1-young male	Age/Sex-lactation
	48.0%	2-Adulte male	
	34.9%	3-Lactated female	
	70.8%	4-Non lactated female	

it age, sex and lactation status (P<0.0001).

Multivariate analysis

For taking in account all factors of variation in order to assess the relationships between them, an automatic classification was performed allowing the identification of 5 types of animals. Yet, these types were described according to the dendrogram showing a convenient partition in 5 classes (Fig. 2) and the cross-table allowing the composition of the classes (Table 2). The classification into 5 types explained 56.6% of the variance.

Regarding the description of each class, it appeared that the highest prevalence rate (P<0.001) was observed in class C gathering the following factors: camel *hamra* breed, non lactated female, urban moving, presenting emaciation and no treatment (Table 2).

Seasonal variation

By considering all the samples, *T. evansi* prevalence rate increased significantly in May. A second peak was observed in September (Fig.3).

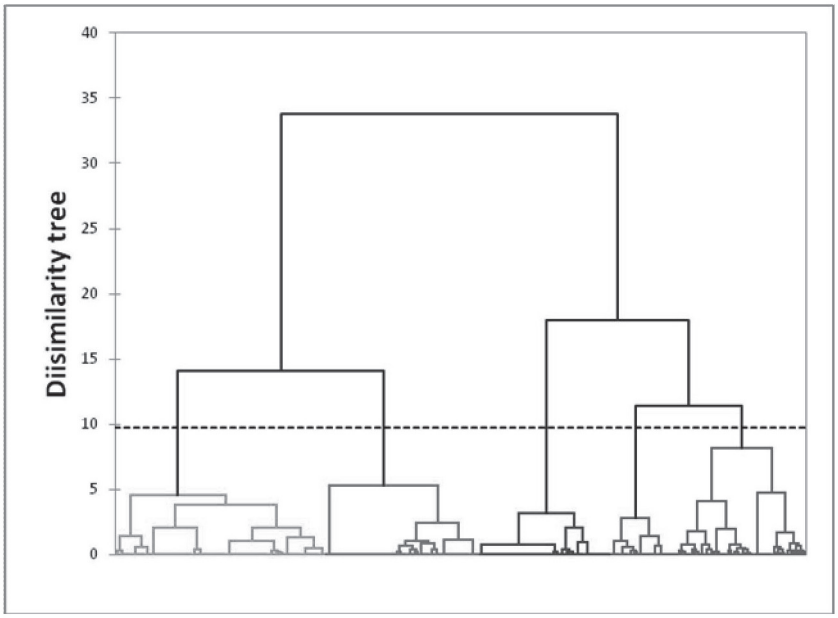

Fig. 2: Dendrogramme issued from automatic hierarchical classification representing the 5 classes of camels according to their status (breed, age, sex, lactation status, clinical signs, prevention and moving).

Table 2: Cross-table between camel classes and the different potential qualitative explaining factors

Class	Pretreatment	Clinical signs	Moving	Age/Sex-lactation	Breed	Tryps %
A	Used treatment	General	Non	Young male	Hamra	54.3
B	No treatment	Non	Non	Lactating female	Wadha	44.1
C	No treatment	Emaciation moving	Urban	Non lactating female	Hamra	66.6
D	No treatment	Healthy	Urban in door	Non	Safra	35.1
E	No treatment	Non	Urban in door	Non	Hamra	35.6

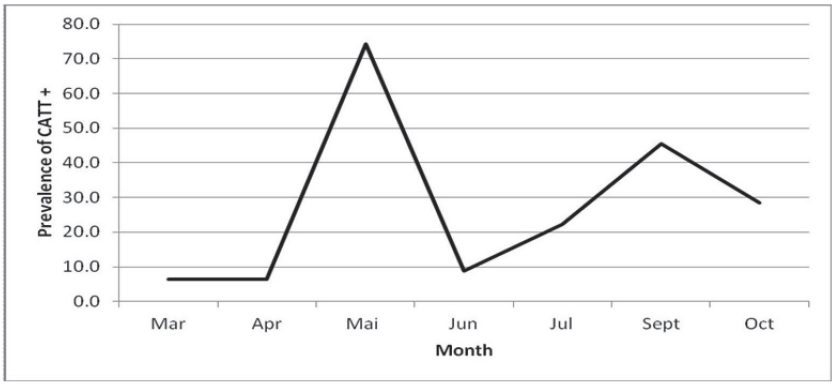

Fig. 3: Seasonal variation of the camel trypanosomosis prevalence rate (in %) in Al-Jouf, Saudi Arabia.

Discussion

The prevalence of trypanosomosis could vary according to the diagnosis test used. For example, in the survey of Delafosse and Doutoum in Chad (2004), the apparent prevalence was 5.3% using Buffy Coat Techniques and 30.5% with CATT.

The overall prevalence of trypanosomosis in camels recorded in our study (43.8%) is higher than the finding by previous workers who reported, based on CATT diagnosis a prevalence of 33% in Sudan (Elamin *et al*., 1998), 28% in Kenya (Njiru *et al*., 2001), 30.5% in Chad (Delafosse and Doutoum, 2004) and 21% in eastern Ethiopia (Zeleke and Bekele., 2001). This pattern of prevalence could be due to the ecology of the study area and seasons of the year when the studies were

conducted which have a direct effect on the distribution of biting flies, responsible for the mechanical transmission of *T. evansi*.

Our finding stated that the younger males were less susceptible to *T. evansi* than adults. It's possible that camel calves were less exposed to infection due to fly feeding preference, or become infected but may control infection more effectively than adults. The same finding was reported by different authors (Elamin *et al*., 1998; Dia *et al*., 1997 and Delafosse and Doutoum, 2004) considering the risk of infection increasing with the age, but with acute disease form more common in young animals. This result is however reverse to the observations of Lemecha *et al*. (2008) in Ethiopia who reported a higher prevalence in young males compared to adults by using standard parasitological detection techniques. In some cases, no significant differences between age groups were reported, for example by Pacholek *et al.* (2000).

The quite highest prevalence of trypanosomosis in non lactating females compared to lactating females may be due to heavy stress and poor management, but mainly to the moving status as the lactating camels could be kept close to the farm contrary to the non–lactating camels susceptible to move far away from their farm. According to Delafosse and Doutoum (2004), the prevalence was higher in transhumant herd compared to settled herds. Large-scale movements clearly increase the risk of infection, probably due to an increase in the probability of contact between camels and parasites when traversing certain environments. The same observation was noted in Mauritania (Dia *et al*., 1997).

Highest infection rate in wadha breed could be partly linked to the color coat. Indeed, we observed a decreasing prevalence from breed with white coat (49%) to breed with dark color as Malha (40%) or Safra (30%). Indeed, there is no clear evidence that genetic difference in trypanosomosis susceptibility was reported. For example, Pathak and Khanna (1995) reported that all camels were equally susceptible to trypanosome infection regardless of breed and age. Moreover, regarding the Saudi camel breeds, a recent publication (Almathen *et al*., 2012) reported that the different phenotypes based on the coat color did not differ regarding their genotypes: in fact, the involved breeds in our study belonged to the same genotype. Elsewhere, it is widely admitted that trypanosome vectors as *Glossina* sp. have preference for some colors as blue which is used for trap, but the preference of tabanides for some colors is not clear (Mekuria and Gadissa, 2011).

Surveys in various tropical areas have shown a definite correlation between seasonal outbreaks of *Trypanosoma evansi* infection and increase in number of flies responsible for disease transmission during the rainy season (Mahmoud and Gray, 1980; Njiru *et al.*, 2002). Despite that, *T. evansi* prevalence

rate increased significantly in May and September in the current study. These months are intermediate regarding the mean temperature between spring and summer (very hot time) and between summer and autumn. They could correspond to the highest flies' activities rather than heat stress, the camel being able to resist to immunodepression due to high temperature (Abdoun *et al*., 2012). However, the efficacy of the different flies in transmitting *Trypanosoma evansi* was reported to vary in different geographic conditions and was also dependent on the interval between two successive feeds and the intensity of the fly challenge (Lukins, 1998). Moreover, congregation of camel herds around water and in pasture into close proximity facilitated efficient transmission of the parasite by the flies.

The prevalence rates observed according to clinical signs highlighted highest prevalence in emaciated animals or with general bad condition compared to animals with no clinical signs. These signs are obviously the main symptoms of trypanosomosis infection. Swai *et al.* (2011) stated that a higher infection might be due to lower body resistance due to nutritional stress or other infection and therefore rendering them more susceptible to *T. evansi* infection.

Conclusion

The study revealed that camel trypanosomosis detected by CATT test was prevalent in Al-Jouf at relatively high levels during the dry season. The finding in this study might not reflect the true situation because the CATT technique is relatively less efficient compared with PCR techniques (Nahla *et al*., 2011). Therefore, detailed studies should be carried out involving both dry and rainy seasons and the relative importance of various biting flies in transmission of the disease in different ecologies. Sensitive diagnostic methods as PCR could give a higher prevalence.

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