Beni-Suef University Journal of Basic and Applied Sciences xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Beni-Suef University
Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/bjbas

Seasonal fluctuation of trypanosomiasis in camels in North-West Egypt and effect of age, sex, location, health status and vector abundance on the prevalence

Hassan M. Sobhy [a], Safaa M. Barghash [b], Tahani S. Behour [c], Esam A. Razin [b,*]

[a] Institute African Researches and Studies, Cairo University, Egypt
[b] Animal Health Department, Desert Research Center, Cairo, Egypt
[c] Animal Reproduction Research Institute, Egypt

ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form 23 December 2016
Accepted 7 January 2017
Available online xxxx

Keywords:
Trypanosoma evansi
Camels
RoTat1.2
Egypt

ABSTRACT

Follow-up surveys of the blood parasites of the livestock and their possible vectors are recommended to assess their distribution and infection rates in different areas in Egypt and to plan control measures against their vectors. By this study, the authors have completed a comprehensive survey of the extent of *Trypanosoma evansi* infection in North-West Egypt from November 2014 to October 2015, using blood film examination and RoTat1.2 PCR-based assay. The present study was carried out in the frame of PROCAMED project, supported by the European Union (ENPI-Joint operational Programme of the Mediterranean Basin-IEVP-CT). Historical data of our previous and the present survey have shown that trypanosomiasis in this particular area is constant and not subject to change over time. In addition, the present study has clarified that *T. evansi* is endemic in this area due to the interaction of a wide range of factors and features of the host which determine patterns of trypanosomiasis, and its distribution coincides with the distribution of vectors.

1. Introduction

Trypanosomes are unicellular flagellar protozoa belonging to phylum Sarcomastigophora, the order of Kinetoplastidae, family of Trypanosomatidae and the genus of trypanosome, under the *Salivaria* group. The subgenus *Trypanozoon* includes the pathogenic species *Trypanosoma evansi, T. brucei* and *T. equiperdum* (FAO, 2000). *T. evansi* thought to have evolved from *T. brucei* by adaptation to mechanical transmission enabling it to spread beyond the tsetse belt in Africa (Hoare, 1972). It can infect a variety of hosts and causes a species-specific pathology. Trypanosomiasis in camels (Surra) occurs both in chronic and acute forms (Payne et al., 1990), but chronic cases are most common and may present an association with secondary infections due to immunosuppressant caused by *T. evansi* infection, and this complicates clinical diagnosis (Luckins, 1992).

Surra is diagnosed by demonstrating the parasite in blood after infection. Parasitological diagnosis is mainly carried out by the direct microscopic examination of wet or stained blood films. It has a poor sensitivity due to parasitaemia is intermittent (Yadvendra et al., 1998). With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed. PCR is reported to be more sensitive than conventional parasitological techniques in a number of hosts and has the advantage that it can identify parasites at the subspecies level (Barghash et al., 2014; Elhaig et al., 2013; Gutierrez et al., 2004). For molecular analysis, various target sequences such as kinetoplast DNA, ribosomal DNA, internal transcribed spacer region and VSG genes are reliable targets for the detection of *T. evansi* (Sengupta et al., 2010). The various variable glycogen (VSG) RoTat 1.2 is more appropriate and can be performed easily. It is able to detect 1 trypanosome/ml of blood or as low as 1 pg of *Trypanosoma* DNA in the presence of host DNA (Claes et al., 2004).

However, trypanosomiasis in a certain area mainly remains the outcome of the interaction between the parasite; the animal host and the vector (de La Rocque et al., 2001). Two previous studies (Barghash et al., 2014; El-Naga and Barghash, 2016) have been conducted to elucidate the complex and varying role of each of these

* Corresponding author at: Department of Animal Health, Animal Production and Poultry Division, Desert Research Center, Egypt.
E-mail addresses: hassansobhy20@yahoo.com (H.M. Sobhy), Barghash_7@yahoo.com (S.M. Barghash), tahanybehour21@yahoo.com (T.S. Behour), Esam_razien@live.com (E.A. Razin).

http://dx.doi.org/10.1016/j.bjbas.2017.01.003
2314-8535/

Please cite this article in press as: Sobhy, H.M., et al. Seasonal fluctuation of trypanosomiasis in camels in North-West Egypt and effect of age, sex, location, health status and vector abundance on the prevalence. Beni-Suef Univ. J. Basic Appl. Sci. (2017), http://dx.doi.org/10.1016/j.bjbas.2017.01.003

factors in the northern west coast of Egypt. The present study was the third to discuss and follow up *T. evansi* infection in camels in this area making use of a combination of parasitological (thin blood film) and molecular methods.

2. Materials and methods

2.1. Study area and sample selection

The northern west coast, Egypt is the major Egyptian western entrance from Libya where camels rearing is the main activity of nomads. Camels managed under pastoral production systems, pastoralists usually take their animals to rainy western areas, which are also favorable grounds for flies. Camels were chosen purposively where they congregated for watering at different grazing points. Four areas were selected (Namely: Mersa-Matrouh, Sidi-Barrany, El-Negeila and the main desert research station for Desert Research Center in Maryout) as demonstrated in Fig.1.

2.2. Study design

A cross-sectional study was designed and adopted in this survey participating with camel owners. It was carried out from November 2014 to October 2015 in the frame of PROCAMED project, supported by the European Union (ENPI-Joint operational Programme of the Mediterranean Basin – IEVP-CT). The selection of these areas was based on the reported claims that camel trypanosomosis was prevalent in the northern west coast of Egypt, besides of Maryout was believed to be free from the disease. Each animal was examined clinically and information on different aspects of age, gender, date of sampling, vectors present, previous trypanocides treatment, history of abortions, and secondary infections was also recorded. Random samples from camels were examined for the presence of *T. evansi* and screened with thin B. film and species-specific RoTat1.2 –PCR.

2.3. Sample collection

A total of 378 randomly selected camels of different ages and both sexes were obtained from selected regions and kept under nomadic conditions. All samples were processed for parasitological examination and Deoxyribonucleic acid (DNA) extraction.

Fig. 1. Map of Egypt showing the northern west coast where blood samples were collected from camels reared in this area. Black dots pointed to the four selective sites.

2.4. Blood samples collection

Five ml of blood were collected from the jugular vein of each camel into a clean sterile Vacutainer tube containing ethylene diamine tetra acetic acid (EDTA). The whole blood samples were used for parasitological examination and for extraction of DNA as a target for PCR amplification. It was stored and preserved at −20 °C till further analysis.

2.5. Diagnosis of T. evansi by parasitological examination

Thin blood smears were prepared, air-dried, fixed in absolute methanol, stained with Giemsa stain and examined microscopically for blood parasites with light microscopy (×40 and oil immersion objectives) according to Hoare, 1972.

2.6. Diagnosis of T. evansi by polymerase chain reaction

2.6.1. Extraction of DNA from blood samples

Extraction of total genomic DNA was done using commercially available DNeasy Blood and Tissue kit (Qiagen, USA) according to the manufacturer's instructions.

2.6.2. PCR amplification and detection

PCR assay was conducted in a total volume of 25 µl composed of 12.5 µl of commercial Master Mix (Qiagen, USA), 25 ng of genomic DNA and sterile water. PCR reaction was performed in an automatic DNA thermocycler (Bio-Rad, Hercules, CA, USA) as follows: one cycle of pre-denaturation at 94 °C for 4 min., followed by 35 cycles of a denaturation step at 94 °C for 1 min., annealing step at 57 °C for 1 min. and an extension step at 94 °C for 1 min. Polymerization step with a final extension was one cycle of 72 °C for 5 min. PCR products were separated by 1.5% agarose gel electrophoresis to assess the presence of specific band indicative of *T. evansi.*

2.7. Statistical analysis

The obtained results of the survey were introduced for analysis. Samples were categorized in six ways (a) Season (b) Vector (c) Site of collection (d) Clinical manifestation (e) Age and (f) Sex. Data analysis was performed using SPSS V20.0, (IBM SPSS Statics 20, USA). Data were summarized by descriptive statistics for mean and standard deviation. Comparisons between groups were evaluated using an analysis of variance (ANOVA) test. All statistics were considered significant at $p \leqslant 0.05$.

3. Results and discussion

3.1. Surveillance of Trypanosoma evansi

This is the third study on *T. evansi* infection in camels in this area making use of a combination of parasitological and molecular tests. Since no test is 100% sensitive and 100% specific and not all tests can be applied in the field during surveys. For routine diagnostic practice in a desert, the parasitological examination is desired despite its low sensitivity. In the present study, results of the survey are shown in Tables from 1 to 5. A total of 378 camels were sampled, of which 78 (20.6%) were harbored *T. evansi* by staining B. films, whereas 243 (64.3%) were positive by PCR assay as represented in Fig. 2. Nearly, similar results were obtained by Barghash et al. (2014) and El-Naga and Barghash (2016) in the same area who found trypanosomiasis was prevalent in camels with percentages of 20.9%, 65.9% and 20.24%, 67.06% using B. film and PCR, respectively. Also, a comparable infestation (21.6%) and

Please cite this article in press as: Sobhy, H.M., et al. Seasonal fluctuation of trypanosomiasis in camels in North-West Egypt and effect of age, sex, location, health status and vector abundance on the prevalence. Beni-Suef Univ. J. Basic Appl. Sci. (2017), http://dx.doi.org/10.1016/j.bjbas.2017.01.003