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Original article

Epidemiological study on tropical theileriosis (*Theileria annulata* infection) in the Egyptian Oases with special reference to the molecular characterization of *Theileria* spp

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ABSTRACT

Theileria annulata infection is a tick-borne disease known as Egyptian fever since 1947. It is a destructive obstacle for the livestock production in the Egyptian Oases (EL-Wady EL-Geded Province). The present study was conducted on 1068 cattle, ranged from below one year to more than eight years old; belonged to different farms and villages in EL-Wady EL-Geded Province. The infection was confirmed by blood smears, Tams-1 target based polymerase chain reaction (Tams-1 PCR), 18Ss rRNA polymerase chain reaction and semi nested-polymerase chain reaction (nPCR) followed by DNA sequencing and phylogenetic analyses, in addition to tick identification. Molecular techniques confirmed the infection in 63.6% (679/1068) of the examined animals while Giemsa-stained blood smears confirmed it in 36.8% (393/1062). Male and female animals showed molecular confirmed infection rates of 64.5 and 62.7%, respectively. Animals less than one year old were more infected (83.33%, 400/480) followed by animals less than three years (57.31%, 149/260) and animals less than five years (42.45%, 90/212), respectively. On the other hand, animal of five years old or above were less infected and the infection rate in this group was estimated to be 34.48% (40/116). Two tick species were identified during the present study: *Hyalomma anatolicum* and *Rhipicephalus annulatus*. *Theileria annulata* was the only *Theileria* species found in the Egyptian oases in respect to phylogenetic analysis of the obtained sequences.

1. Introduction

The Egyptian oases (EL-Wady EL-Geded Province) is an area located in the middle and southern part of the western desert plateau, it is one of the frontier Provinces in the Arab Republic of Egypt. It is considered as a promising area for agricultural expansion in Egypt. It occupies about 44% of the total area of the Arab Republic of Egypt (440.098 km^2). This region is characterized by dry climate and rare rain but with important underground water resources (Ministry of Agriculture & Land Reclamation, 2009). Agricultural expansion, meat and milk production contribute to the economic development in this region that will lead to re-deployment of the human population reducing then its density in and around the Nile Valley and its Delta (Ministry of Agriculture & Land Reclamation, 2009). Unfortunately, tropical theileriosis is still one of the main obstacles for cattle herd industry development in this region (AL-Hosary, 2013). *Theileria annulata* infection causes severe losses in the livestock production (Michael et al., 1989). Compared to native and cross-bred cattle and buffaloes, lethality is higher in exotic cattle breeds (Mahmmod et al., 2011; Mohamed et al., 2012; Nasir et al., 2000).The present study was performed to evaluate the epidemiological status of tropical theileriosis and determine the occurrence of new species of *Theileria* in this locality as previously mentioned in other localities in both Upper and Middle Egypt (Fig. 1) (AL-Hosary et al., 2015b).

2. Materials and methods

2.1. Study areas and samples collection

A total number of 1068 cattle (550 females and 518 males) from different localities in EL-Wady EL-Geded Province (Egyptian Oases) were sampled from January 2015 till January 2016. EL-Wady EL-Geded is located in southwestern Egypt at the plateau of the Egyptian western desert; it is limited by the Sahara Desert in the South, River Nile in the East, Northern Sudan in the South and Southeastern Libya in the West (Fig. 1). This area is characterized by dry and hot weather and the year

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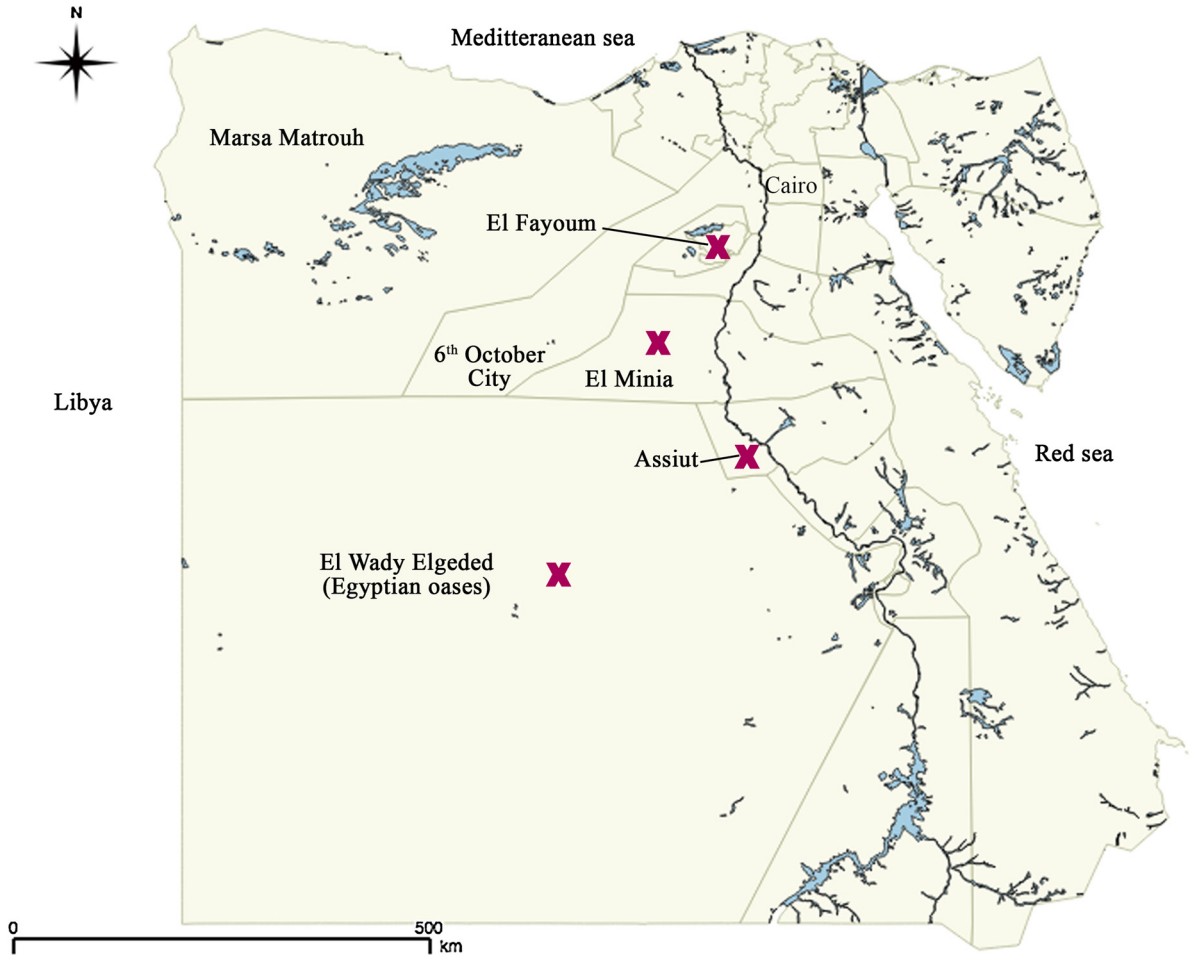

Fig. 1. Map of Egypt showing the geographical location of the Egyptian Oases.

is usually classified into two main periods include hot months starting from March until the late of September and non-hot months from October till the late of February (Domroes and El-Tantawi, 2005). The age of examined animals ranged from less than one year to more than eight years (6.28 ± 3.61 years). Two blood samples were collected from healthy (N = 868) and clinically infected animals (N = 200); one from the ear vein used for the preparation of Giemsa stained blood smears and the second collected from the jugular vein on EDTA then the samples were preserved in −20 °C until used for DNA extraction (Coles, 1986).

2.2. Clinical examination

All animals were subjected to clinical examination (Radostitis et al., 2006).

2.3. Tick identification

The collected tick samples were preserved on 70% ethanol and the adult ticks were morphologically identified according to the identification keys (Estrada-Peña et al., 2004; Hoogstraal, 1956).

2.4. Molecular diagnosis

DNA extraction was performed from whole blood using QIAamp DNA Blood kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Tams-1 primers, Tams1 F (5′-ATG CTG CAA ATG AGG AT-3′) and Tspms1R (5′-GGA CTG ATG AGA AGA CGA TGA G-3′) were used to amplify 785-bp fragment of the *Theileria annulata* 30 KDa major merozoite surface antigen gene (Kirvar et al., 2000). 18Ss rRNA primers consisting of forward primer (5′-GAC ACA GGG AGG TAG TGA CAA G-3′), Nested-forward primer (5′-GAC AAG AAA TAA CAA TAC RGG GC-3′) and reverse primer (5′-CTA AGA ATT TCA CCT CTG ACA GT-3′) were used to perform both PCR and semi-nested PCR providing specific amplicons of 460–520 bp (Gubbels et al., 1999). Positive and negative control tubes were included in each PCR run, consisting of genomic *T. annulata* DNA and molecular grade water, respectively. Positive nPCR products were purified using QIAquick PCR Purification Kit (Cat No. 28104, Qiagen, Germany) according to manufacturer's instructions. Thirteen amplicons were sequenced in both directions and subjected to BLAST similarity searches. Phylogenetic analyses were performed after aligning sequences by neighbour joining method using the Mega 6.0 program. The phylogenetic tree was constructed using Kimura two parameters distance and the nodes were tested for robustness by 1000 bootstrap replications, *Babesia bovis* (L19077) was used as an out group (Kimura, 1980). The sequence distance was tested with DNASTAR's (MegAlign) Lasergene software.

2.5. Statistical analysis

Data were compared by means of Chi Square test using SPSS Statistical Program (SPSS, Chicago, USA). The differences were considered significant at 5% threshold values.

3. Results

Clinically infected animals showed specific clinical signs of tropical theileriosis including fever (≥40 °C), lymph node enlargement