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PREVALENCE OF TICK-VECTORS OF *THEILERIA ANNULATA* INFESTING THE ONE-HUMPED CAMELS IN GIZA, EGYPT

By

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Abstract

Theileria are obligate intracellular protozoan parasites transmitted by hard ticks that cause severe and mild infections in their vertebrate hosts. The objective of the present study was to identify the possible tick vector of *Theileria* spp. and to determine the prevalence of camel theileriosis in Birqash market, Giza, Egypt. These camels are previously imported from Sudan and Somalia. A total of 298 nomadic one - humped camels in the study area were selected by simple random sampling technique during the period from December 2014 to November 2015. A total of 1540 ticks were collected, four ixodid tick species; *Hyalomma dromedarii, Amblyomma lepidum, Amblyomma variegatum, and Rhipicephalus pulchellus* were found on camels. It was observed that *H. dromedarii* recorded the highest prevalence (69%), followed by *A. lepidum* (8%), *A. variegatum* (3%) and *R. pulchellus* (2%). Using light microscope *Hyalomma dromedarii* was the most tick carrier of *Theileria* spp. which recorded 1.3% (20/1540) *Theileria* infection. The highly prevalence rates for all ticks were monitored during the summer season. The molecular detection of *Theileria annulata* in Geimsa-stain positive *H. dromedarii* ticks was performed by the PCR using primer set N516/N517 derived from the gene encoding the *30 kDa* major merozoite surface antigen. This primer set amplified *T. annulata* in *H. dromedarii* at 750 bp. Thus the presence of *H. dromedarii* on camels in the field have dangerous inclusive for animal health and to some extent humans. To reduce these dangerous effects, strategic control methods could be used in the control of ticks.

Keywords: *Amblyomma, Hyalomma, Rhipicephalus,* Ixodidae, Prevelance, *Theileria; 30KDa*

Introduction

Ticks are the most important vectors that infest animals and transmit a wide range of pathogens. Ticks are considered as vertebrate's vectors and constitute the largest tick genera with about 235 different species described worldwide (Norbert. 2013). The ticks, as obligate blood-sucking ectoparasites, attack a broad range of vertebrates, including humans. They are viewed as second just to mosquitoes as vectors of pathogens for domestic and wild animals (de la Fuente *et al*, 2007). They transmit medical and veterinary pathogens including viruses, bacteria, and protozoans, all of which cause damage to livestock production. The one-humped camel, *Camelus dromedaries* is an important multipurpose animal. It has been used for transportation and produces milk, wool and meat in arid and semi-arid areas of the world (Kamani, 2008). Also, camels are hard animals and can tolerate the harsh conditions of arid regions because of their unique adaptive physiological characteristics. (Swelum *et al*, 2014).

Theileriosis is one of the most common tick-borne diseases, which have been studied and described in a wide range of livestock such as cattle, sheep, and goats. Few studeid theileriosis infected camels (Nassar, 1992; Abd El-Baky, 2001; El Kammah *et al*, 2001; El-Fayoumy *et al*, 2005; Youssef *et al*, 2015). *Theileria* spp. is tick-trans-mitted, intracellular protozoan parasites infecting leukocytes and erythrocytes of a wide range of animals (Shaw *et al*, 1991; Bishop *et al*, 2004). The organisms have been described in all livestock species and can

cause significant economic losses to farmers. They are transmitted by a variety species of the ixodid tick *Hyalomma* (Florin-Christensen, and Schnittger, 2009).

In Egypt, the most common tick species infesting camels in Sinai Peninsula are *H. dromedarri* and *H. impeltatum*, they transmitted the infective stage of *Thelieria* via their guts, salivary glands and hemolymph (El-Kady, 1998).

Detection of these blood parasites is highly helpful in early diagnosis and treatment. Classical, microscopy using Giemsa-stained hemolymph from ticks has proven to be the first method for detecting *Theileria* organism in the ticks, but not in all tick-vectors, where *Theileria* was low (Hamed *et al,* 2011). Application of polymerase chain reaction (PCR) proved valuable in diagnosing *Theileria* in ticks (Zhang *et al*, 2014).

The present study was designed to achieve the two objectives: 1) Updated knowledge about the ixodid ticks infesting camels from Giza Birqash Camel Market (Souq al-Gamal). 2) Determination of tick(s) naturally infested with *Theileria* spp. to identify the possible tick vector and to determine the prevalence of camels' theileriosis in Birqash Market, using Giemsa-stained tick hemolymph smears, and PCR assay.

Materials and Methods

Study area: All ticks were collected from camel market at latitudes 30°08'57.1"N and longitudes 30°59'42.8"E, Birqash village, Giza Governorate, Egypt. Temperature and relative humidity were monitored monthly in the studied area from December 2014 to November 2015.

Ticks collection: The camels were sampled for ticks on head, shoulder, belly, and perineal regions using curved forceps. The samples were transported alive to Laboratory Animal Acarines Research, Faculty of Agriculture, Cairo University. They were incubated at 28±1℃ & 75±5% relative humidity. Locality (Global Positioning System G.P.S.), host, date, climatic condition (temperature and relative humidity), and infestation sites were recorded. The identification of ticks was confirmed in the laboratory using standard keys (Hoogstraal, 1956; Walker *et al*, 2003).

Tick hemolymph smear preparation: Hemolymph was collected individually for examination with 100x magnifications using lightmicroscope for any stages of tick species and then stored at -20^{o}C (Burgdorfer, 1970).

DNA Extraction: From every month, 25% tick samples were divided into 5 ticks in one pooled sample. The frozen ticks were cut into small pieces using a disposable scalpel in 1.5 µL Eppendorf tubes under a sterile laminar flow hood, Molecular Biology Laboratory, Department of Zoology, Faculty of Science, Menoufia University. DNA was extracted from the ticks using the PureLink® Genomic DNA Kits (Invitrogen, USA). Each sample was covered in the tissue lysis buffer included in the kit (between 180µL and 540µL depending on tick size) and treated with proteinase K (20µL/180µL of tissue lysis buffer) and then incubated at 56℃ for 48 hr. Subsequent steps were carried out according to the manufacturer's instructions (Invitrogen, USA).

PCR: PCR amplification was performed in a final reaction volume 2X (50µL) containing 25µL 2X master mix solution (*i*-Taq™, *iNt*RON, Korea), 0.2 uM (2µL) of each primer, 4 µL template DNA, 2.5µL Bovine serum albumin, & 14.5µL injection H_2O. The designated primers were obtained from Macrogen, Korea. The oligonucleotide sequences of the primers used for detected *Theileria annulata* were forward strand primer30-kDa N516 gene (5′- GTA ACC TTT AAA AAC GT-3′) and reverse strand primer 30-kDa N517 gene (5′- GTT ACG AAC ATG GGT TT-3′) (D'Oliveira *et al*, 1995) under an initial denaturation at 94°C for 10 min followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and at 72°C for 1 min, followed by final extension at 72°C for 10 min. Amplification reactions were done in

a PCR thermal cycler Biometra T-personal/Germany S/N 1003507 and the corresponding amplicons were checked on 1% agarose-gel using TAE buffer stained with ethidium bromide, examined under UV transilluminator, and photographed using the Digital Camera.

Results

Four ixodid tick species detected on one humped camels were: *Hyalomma dromedarii, Amblyomma lepidum, Amblyomma variegatum* and *Rhipicephalus pulchellus.* In few 2 or 3 mixed infestation were on same camel. *H. dromedarii* was the commonest 69%, followed by *A. lepidum* 8%, *A. variegatum*3% and *R. pulchellus* 2%. Details were given in tables (1, 2 & 3) and figures.

Table 1: Prevalence of ticks on one-humped camels in Birqash village from December 2014 to November. 2015.

Month of collection	No. of examined camels	*H. dromedarii*		*A. lepidum*		*A. variegatum*		*R. pulchellus*	
		Infested No.	Infested (%)	Infested No.	Infested (%)	Infested No.	Infested (%)	Infested No.	Infested (%)
Dec 2014	25	14	56	1	4	-	-	-	-
Jan 2015	25	12	48	-	-	-	-	-	-
Feb 2015	24	8	33	-	-	-	-	-	-
Mar 2015	25	15	60	2	8	1	4	-	-
Apr 2015	25	16	64	2	8	1	4	-	-
May 2015	25	21	84	4	16	1	4	-	-
Jun 2015	25	23	92	-	-	-	-	-	-
Jul 2015	25	23	92	4	16	3	12	3	12
Aug 2015	25	21	84	5	20	-	-	4	16
Sep 2015	26	19	73	-		3	12	-	-
Oct 2015	24	19	79	4	17	-	-	-	-
Nov 2015	24	15	63	3	13	-	-	-	-
Total	298	206	69	25	8	9	3	7	2

There was a distinct monthly prevalence of each species in the studied area. *H. dromedarii* was observed to be more prevalence from May to October. *A. lepidum* infested lower number of camels than *H. dromedarii*, with peak prevalence in August. But, two peak periods were reported for *A. variegatum,* one in July and second in September. *R. pulchellus* was recorded in July and August. Also, there was a marked increase in the number of *H. dromedarii* on camels examined between June and October, which declined to a minimum number in February. The relative numbers of the total tick of the four species recorded were *H. dromedarii,* 92.3%; *A. lepidum,* 5.2%; *A. variegatum,* 1.8% and *R. pulchellus,* 0.6%.

Table 2: Abundance of ixodid ticks on camels in Birqash village from December 2014 to November 2015.

Month of collection	No. of camels examined	*H. dromedarii*		*A. lepidum*		*A. variegatum*		*R. pulchellus*		Tick number	Ticks (%)
		No. of ticks (♀ + ♂)		No. of ticks (♀ + ♂)		No. of ticks (♀ + ♂)		No. of ticks (♀ + ♂)			
Dec 2014	25	75	24	0	1	-	-	-	-	100	6.4
Jan 2015	25	50	11	-	-	-	-	-	-	61	3.9
Feb 2015	24	30	14	-	-	-	-	-	-	44	2.8
Mar 2015	25	60	13	0	3	-	-	-	-	76	4.9
Apr 2015	25	64	5	2	0	-	-	-	-	71	4.6
May 2015	25	75	37	3	6	0	3	-	-	124	8
Jun 2015	25	127	50	-	-	-	-	-	-	177	11.4
Jul 2015	25	130	32	2	23	0	22	0	3	212	13.7
Aug 2015	25	177	42	2	32	-	-	-	-	253	16.4
Sep 2015	26	168	10	-	-	0	3	0	7	188	12.2
Oct 2015	24	141	10	1	3	-	-	-	-	155	10
Nov 2015	24	65	12	2	0	-	-	-	-	79	5.1
Total	298	1162	260	12	68	-	28	-	10	1540	

Number of *A. lepidum* and *A. variegatum* was relatively low but increased in July and September. *R. pulchellus* appeared in July and September. Proportion between ticks'

number and climatic conditions increased in camels at high temperatures (Fig. 1).

Th. annulata infected camels showed no abnormal clinical picture. Hemolymph smears examination of 20 female *H. dromedarii* (1.3%) was positive for *Th. annulata*, while smears of *A. lepidum*, *A. variegatum* and *R. pulchellus* were negative.

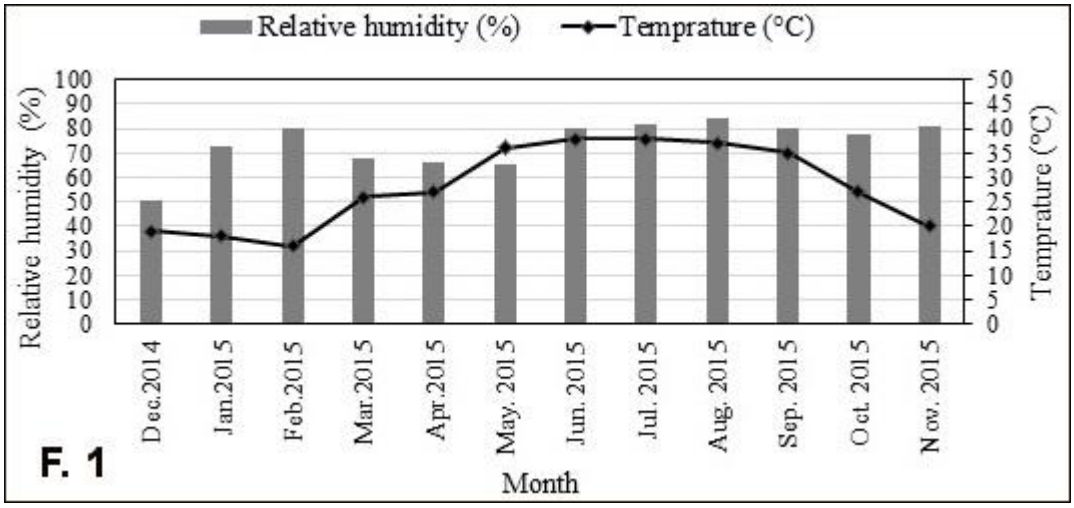

Fig. 1: Ambient temperature and relative humidity recorded in study area from Dec. 2014 to Nov. 2015

Theileria annulata were observed in *H. dromedarii* with highest infestation rate in summer and relatively few in autumn and spring, whereas none during winter season (Tab. 3). The 20 Giemsa-stained hemolymph smears positive were examined for the detection of nucleic acid of *Th. annulata* using one primer for *Th. annulata*. The PCR identified 20 positive samples for *Th. annulata*, bands appeared at 750 bp were consistent with the expected size for PCR products in *Theileria* sp. (Fig. 2).

Table 3: Positive *Theileria* infection in ixodid ticks using Giemsa stain in different seasons, 2014, 2015

Tick species	Tick number	Theileria positive number					
		Winter	Springer	Summer	Autumn	Total	*Theileria* (%)
H. dromedarii	1422	-	-	20	-	20	1.4
A. lepidum	80	-	-	-	-	-	-
A. variegatum	28	-	-	-	-	-	-
R. pulchellus	10	-	-	-	-	-	-
Total	1540	-	-	20	-	20	1.3

Molecular detection of *Th. annulata* in Geimsa-stain positive *H. dromedarii* was done by PCR using primer set N516/N517 derived from gene encoding *30 kDa* major merozoite surface antigen that amplified *Th. annulata* at 750 bp (Fig. 2).

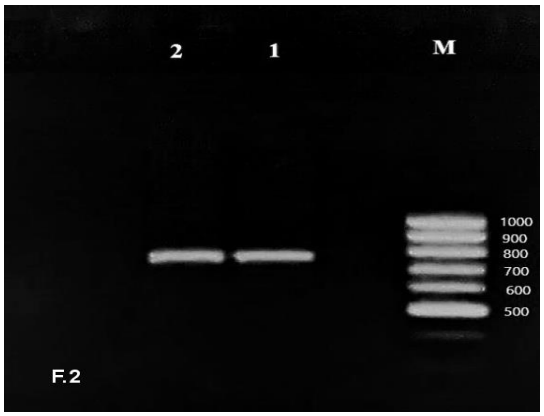

Fig. 2: PCR showing diagnostic bands for *Th. annulata* at 750 bp lane M 100 bp Ladder = DNA marker.

Discussion

Camels were selected in the present study, because of their distribution in most African countries and also due to their economic importance. They are considered the main host for many species of ixodid ticks that occurred in Africa (El Bahnasawy *et al*, 2012). Egypt is a permanent importer of camels from many African countries such as Sudan, Ethiopia, Nigeria and Somalia. Thousands of camels enter Egypt every year where these camels are carried different species of ticks where some of them probably not present in Egypt (Abdullah *et al*, 2016). Some of tick species that come to Egypt with imported camels may infest local camels or other domestic animals. The changes in weather that became more hot and wet in Egypt during the last years probably induce the imported tick species to adapt and become endemic in Egypt (Mazyad and Khalaf, 2002).

The economic damage of ixodid ticks to camels does not restrict to blood sucking but it includes the most fatal tick-borne theileriosis disease, beside other bacterial and protozoal diseases (Dautel, 1999; Dantas-Torres *et al*, 2012). Therefore, the tick fauna on the camels should be updated from time to time to avoid the problems induced from ticks and tick borne diseases in an appropriate time.

In the present study, a total of 298 camels were examined for ixodid tick infestation. Four tick species, *H. dromedarii, A. lepidum. A. variegatum*, and *R. pulchellus*were recorded on camels. The camel tick *H. dromdarii* gave highest prevalence rate (69%). This tick species is considered the commonest tick on Egyptian camels either local bred or imported ones. The three tick species gave low prevalence rate between 2 to 8%. This low prevalence rate may be due to their weak adaptation to the Egyptian environmental conditions. Meanwhile, these tick-species was not recorded before on the local camels or other domestic ones in Egypt, but, *Amblyomma* spp. was recorded on imported camels (Barghash *et al*, 2016).

Hyalomma dromedarii on the camels was more prevalence from May to October, while, *A. lepidum* infested was in August. However, *A. variegatum* was in July and September, *R. pulchellus* was in July and August. Umar *et al.* (2011) in Nigeria examined 410 camels found high prevalence in *H. dromedarii* (58%) and low in *A. variegatum* (42%) and *R. pulchellus* (25%). They stated that the environmental conditions in Nigeria were more suitable for survival and development of *A. variegatum* and *R. pulchellus*, and that *H. dromedarii* was more prevalence from May to October, *A. variegatum* was more prevalnce in July and *R. pulchellus* was recorded in June and October.

In the present study, *H. dromedarii* represented the main tick species infesting the camels (92.3%) followed by *A. lepidum* (5.2%), *A. variegatum* (1.8%) and then *R. pulchellus* (0.6%). Besides, *H. dromedarii* females were found engorged on the examined camels, while females on *A. lepidum, A. variegatum*, and *R. pulchellus* were not found or rarely partially engorged. This could be due to the fact that camels are not the preferred hosts for these tick species. But, Elghali and Hassan (2009) in northern Sudan, reported *H. dromedarii* (88.9%) as the main tick species infesting camels. Also, Semere *et al*, (2014) reported that *R. pulchellus* the most abundant tick species (92.7%) on the examined camels that represented 53.7% of all collected ticks. The low prevalence of this tick in the present study might be due to the fact that *R. pulchellus* is a tick of savanna and desert climitic regions as the commonest tick species in the Rift Vally areas. Also, *H. dromedarii* was (42.7%) and constituted 12.8% of collected ticks. In the present study, *H. dromedarii* was the commonest tick species and it was reported in desert climates where camels live (Walker *et al*, 2003). On the

other hand, El-Bahnasawy *et al*. (2013) in Egypt stated that in the 21st Century, vector-borne infectious diseases was accepted as the disaster issues with considerable significant morbidity and mortality that must be considered by public health, veterinary and agricultural authorities.

In the present study, the annual prevalence of tick infestation was 69%. The prevalence of tick infestation on the dromedary camels in eastern Ethiopia and Iran was 94% and 59.25%, respectively (Taddese *et al,* 2013; Moshaverinia and Moghaddas, 2015). The difference in the rate of prevalence in three areas could be attributed to different climatic conditions and particularly differences in the sampling periods.

In the present study, *H. dromedarii* was the predominant species found in infested camels 92.3%, and this agreed with Youssef *et al,* (2015) who reported high prevalence of *H. dromedarii* in Egypt. *Hyalomma dromedarii* proved to be the principal vector of *Theileria spp.* in vertebrates (Hoogstraal, 1956). This parasite has various developmental stages, different shapes and forms inside the ticks. These forms were in the hemolymph as ring form, slender spine-like form and round form (El-Refaii *et al,* 1998; Hamed *et al,* 2011). There were no clinical pictures in all examined camels, but this disagreed with Hamed *et al.* (2011) who reported that 3/15 camels infected with *Theileria* had enlargement of superficial lymph node and fever.

In the present study, *Theileria* species developmental stages were found in 6.56% (101/1540) of tick-hemolymph. This result disagreed with Hamed *et al,* (2011) who found 9.38% (21/224) in examined tick-hemolymph. Such variations may due to different localities, examination season, camels' population density, sampling different periods and climatic conditions.

In the present study, the primer set N516/N517 derived from the gene encoding the *30 kDa* major merozoite surface antigen was used in molecular diagnosing of *T. annulata* in Geimsa positive *H. dromedarii* ticks. This gene was used in molecular studies to detect *T. annulata* in either animals (Abd Ellah and Al-Hosary, 2011; Ganguly *et al,* 2015; Youssef *et al,* 2015; Majidiani *et al,* 2016; Sudan, *et al,* 2016) or ticks (Jacquiet *et al,* 1990; D'Oliveira *et al,* 1997). All these studies used this gene in PCR screening confirmed that the camel tick *H. dromedarii* proved to be the main vector of *Th. annulata*. They found fragment 721 bp closes with the fragment 750 bp that was recorded in this study.

Conclusion

Some parasitic zoonoses are confined to certain geographic areas in Egypt, such as the deserts and the Sinai Peninsula. Animal reservoirs of parasitic zoonoses were identified, particularly rodents, stray dogs and stray cats as well as mosquitoes and ticks and other vectors, which constitute a potential risk of disease transmission.

Based on the outcome results, the camels mostly harbor *Hyalomma dromedarii*. This species is the most notorious ticks for the transmission of animal diseases. Thus, appropriate tick control measures are needed to employ and pour-on the method for acaricide application.

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