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MOLECULAR DIAGNOSIS OF RICKETTSIAE INFECTING CAMELS AND IXODID TICKS IN EGYPT

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ABSTRACT

Rickettsioses including their pathogens, vectors, and hosts have an epidemiological importance and zoonotic importance. The objective of the present article was to define the prevalence and genotypic properties of *Rickettsia* in camels and their ticks in Egypt. Sixty one blood samples and 99 adult ticks were taken from camel hosts from Cairo, Giza and Sinai, during a period extending from 2013 to 2014. Based on the morphological identification of both male and female tick specimens, 91.9 % of the collected ticks were *Hyalomma dromedarii*. The prevalence of *Rickettsia* in camels using Gimenez staining technique and PCR was 0 and 41 %, respectively. The rickettsiae infection in ticks recorded 10.1 and 1.01 %, by Gimenez stain and PCR, respectively. Further, the phylogenetic analysis was conducted based on the sequences of *OmpA* and *glt*Agenes and three intergenic spacers (*mppA, dksA* and *rpmE*) of *Rickettsia* species. The phylogenetic analyses revealed a novel strain of *Rickettsia africae* in *Hyalomma marginatum* that was collected from camel in Sinai province. In addition, the phylogenetic analysis based on Clustal omega suggested that *Rickettsia* sequences which detected in camels were *R. africae*. Moreover, the highest Rickettsial infection rate was recorded in age groups of 17 to 19 years (80.0 %), Abady camel breeds (56.8 %) and ticks-infested camels (42.8 %). Concerning hematological changes, macrocytic anemia and leucopenia were recorded in camels with rickettsioses. The molecular characterization of *Rickettsia* detected in camels and their tick vectors will help in a better understanding of the epidemiological approach of rickettsioses in Egypt.

Keywords: Camels, *Hyalomma* species, rickettsioses, Multi-genes typing

INTRODUCTION

Rickettsioses are considered emerging and re-emerging zoonotic vector-borne diseases (**Parola and Raoult, 2001; Dantas-Torres *et al*., 2012; Kernif *et al*., 2012b; Parola *et al*., 2013**). Rickettsioses in general have high morbidity and low mortality except some *Rickettsia* spp. such as *Rickettsia rickettsia*, which showed high mortality in both dogs and human (**Raoult and Roux, 1997; Parola *et al*., 2005; 2013**).

The order Rickettsiales are simply known obligatory intracellular gram negative bacilli, cocci or thread-like bacteria that retained basic fuchsin when stained by Gimenez stain (**Gimenez, 1964; Fournier and Raoult, 2007; Kang *et al*., 2014**). The taxonomy of Rickettsiae has undergone extensive reorganization **(Raoult and Roux, 1997; Hechemy *et al*., 2003**). The order Rickettsiales includes Anaplasmataceae and Rickettsiaceae families. The 16S rRNA, *gltA*, *ompA*, *ompB*, and *sac 4* genes were suggested for rickettsial taxonomy (**Dumler *et al*., 2001; Fournier *et al*., 2003**). The rickettsiae are divided into four groups; Spotted Fever (SFG), Typhus (TG), *R. belli* and *R. candensis* group (**Fournier and Raoult, 2007; Merhej and Raoult, 2011**).

Ticks are considered secondary to mosquitoes in their ability to transmit diseases (**Hillyard, 1996**). They are the main vectors and reservoirs of *Rickettsia* spp.; especially SFG Rickettsiae that were transmitted transstadially through the developmental stages and transovarial (**Raoult and Roux, 1997; Anderson and Magnorelli, 2008; Socolovschi *et al*., 2009b**). Ixodid ticks (hard ticks) transmit the microorganisms to vertebrates through tick bites via their salivary secretions, or through feces and blood transfusion (**Socolovschi *et al*., 2009b**).

In Egypt, *Hyalomma* species are the most dominant ticks on camels, especially *H. dromedarii*, *H. marginatum*, *H. excavatum*, and *H. impeltatum* (**Abdel-Shafy, 2000; El-Kammah *et al*., 2001; Abdel-Shafy *et al*., 2012**).

Tick-borne rickettsioses have been diagnosed serologically in animals and human (**Botros *et al*., 1989; Soliman *et al*., 1989; Corwin *et al*., 1992; 1993; Reynolds, 2004**). In previous studies, SFG were detected in *Rhipicephalus sanguineus* and *Hyalomma* spp. from Sinai (**Lange *et al*., 1992; Loftis *et al*., 2006ab**). Socolovschi and his colleagues detected *R. sibirica mongolitimonae* in a traveler returned from Egypt to France (**Socolovschi *et al*., 2010**). Moreover, *R. africae* was recorded for the first time in *Hyalomma* spp. in Egypt by **Abdel-Shafy *et al*. (2012)**. In addition, *R. aeschlimannii* alsohas been reported in *Hyalomma* spp. by **Loftis *et al*. (2006ab)** and **Abdel-Shafy *et al*. (2012)**.

The diagnosis of rickettsioses is still considered a challengedue to the non-specific clinical signs, laboratory abnormalities and/or subclinical infection (**Gasser *et al*., 2001; Parola *et al*., 2005; 2013**). Molecular techniques were applied targeting accurate and rapid detection and identification of *Rickettsia* spp. PCR followed by sequencing improved the sensitivity of diagnosis and specificity of taxonomy (**Parola *et al*., 2013; Guillemi *et al*., 2015**). Primers amplifying the *OmpA* and *gltA* genes were less conserved genes, so it had a higher discriminating power between genomes of SPG *Rickettsia* spp. (**Roux *et al*., 1997; Fournier *et al*., 1998**). Moreover, intergenic spacers (*mppA, dksA* and *rpmE*) were more variable than genes and conserved spacers (**Fournier *et al*., 2004**). Therefore, they had highly variable sequences.

Few previous studies aimed to detect rickettsioses in camel ticks. However, to the best of our knowledge, this is the first study to detect camels' rickettsioses in Egypt. Camels have been used in meat and milk production, security purposes in desert and border areas, also in racing as a traditional sport. Therefore, the objectives of this study were the determination of the prevalence of tick-borne rickettsioses in camels and their ixodid tick vectors at different provinces in Egypt, in addition the molecular characterization of novel genotypes of *Rickettsia* compared to the previously published genotypes. The genotypic relationship between these *Rickettsia* species and previously recorded worldwide is targeted by *OmpA*, *gltA*, *mppA*, *dksA* and *rpmE* sequences alignment with GenBank related records.

MATERIALS AND METHODS

Sampling sites and collections (Animals and Ticks)

Sixty one camels were examined for the presence of ticks at Cairo, Giza and Sinai from Mar. 2013 - Oct. 2014. AnEDTA-whole blood (5 ml each) was collected from jugular vein of each animal. The blood samples were used for hematological studies, preparing blood smears for Gimenez staining techniques (**Gimenez, 1964**). An amount of blood per animal was stored at -20 °C for molecular studies. Other blood samples were collected without anticoagulants for serum separation. Sera were used for further biochemical parameters investigations. The animals were checked for tick infestations through their whole body (**Abdullah *et al*., 2016b**). Ninety nine adult ticks were collected from animals. Ticks were removed from animals by forceps and put into plastic tubes. The tubes were transferred to the lab for further processing.

Ticks Identification

Ticks were identified according to the taxonomic keys of **Hoogstraal and Kaiser (1958)**, and **Estrada-Peña *et al.* (2004)**. Further, hemolymph staining technique was performed for all collected ticks according to **Gimenez (1964)**. Then, the ticks were stored at -20 °C until DNA was extracted for molecular studies.

DNA extraction

The DNA was extracted from blood samples using GF-1 Tissue Blood Combi DNA Extraction Kit (SNF, Vivantis, Malaysia) according to the manufacturer's instructions. The DNA of adult ticks was extracted after dividing the tick body into quarters. The DNA was extracted by high salt concentration protocol (**Zilberman *et al*., 2006**). The purity and concentration of DNA were measured by nanodrop 2000c (Thermo Scientific) and stored at -20°C.

Primers Design

The primers of *OmpA* and *gltA* genes were designed according to **Fournier *et al.* (1998)**, **Roux *et al.* (1997)** and **Mediannikov *et al*. (2004)** (Table 1). *Rickettsia* positive sample further characterized using primers targeting intergenic spacers (*mppA*, *dksA* and *rpmE*) (Fournier et al. 2004) (Table 1).

Table 1 Primers utilized in amplification and sequencing of genes

DNA Marker	5'- Primers Sequences-3'	Amplified Fragments	References
OmpA gene 190.70-F 190.701-R	 5'-ATGGCGAATATTTCTCCAAAA-3' 5'-GTTCCGTTAATGGCAGCATCT-3'	590-634 bp	Fournier et al. (1998)
gltA gene CS2d-F CSEnd-R	 5'-ATGACCAATGAAAATAATAAT-3' 5'-CTTATACTCTCTATGTACA-3'	852-1265 bp	Roux et al. (1997) Mediannikov et al. (2004)
Intergenic spacers:			Fournier et al. (2004)
mppA-purC-F mppA-purC-R	5'-GCAATTATCGGTCCGAATG-3' 5'-TTTCATTTATTTGTCTCAAAATTCA-3'	155-197 bp	
dksA-xerC-F dksA-xerC-R	5'-TCCCATAGGTAATTTAGGTGTTTC-3' 5'-TACTACCGCATATCCAATTAAAAA-3'	164-292 bp	
rmpE-tRNA-F rmpE-tRNA-R	5'-TCAGGTTATGAGCCTGACGA-3' 5'-TTCCGGAAATGTAGTAAATCAATC-3'	297-417 bp	

PCR amplification of target sequences

The PCR amplifications were performed in a PTC-100™ thermal cycler according the protocol described by **Abdel-Shafy *et al.* (2012)** and **Abdullah *et al*. (2016a)**. PCR products were electrophoresed in 1.5 % agarose gels. The gels were stained with ethidium bromide. A 100 bp ladder was used with each gel. The Gel photos were analyzed by Lab Image software (BioRad).

Sequencing of PCR products

The PCR products were purified by ExoSAP-IT PCR Product Cleanup Kit according to manufacturer's recommendation. Sequencing reactions were performed in an MJ Research PTC-225 Peltier Thermal Cycler using an ABI PRISM®BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme; Applied Biosystems), following the protocols supplied by the manufacturer. The sequencing was performed in Macrogen Center, Seoul, South Korea. Each sequencing reaction was repeated at least three times.

Data submission in GenBank

The sequences of *OmpA*, *gltA* and intergenic spacers were aligned, assembled and corrected using ChromasPro 1.49 beta (Technelysium Pty. Ltd., Tewantin, QLD, Australia), then the corrected *Rickettsia* sequences were submitted in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) to record each sequence with accession number.

Phylogenetic relationship and Multigene typing

Amplified sequences of each fragment were aligned using Blastn program of NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) for sequence homology searches against *Rickettsia* spp. GenBank database. Multiple sequences alignments for evolutionary relationships between new Egyptian records and other reference isolates were inferred using the ClustalW 1.8® program (**Dessen *et al.*, 1990**) after modification of sequences length by BioEdit sequence alignment editor (v. 7.0.9.0). In addition, the percent of the identity matrix were constructed by using Clustal omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Using the MEGA4 software, two phylogenetic trees were constructed with the neighbor joining method (NJ) (**Saitou and Nei, 1987; Tamura *et al*., 2007**), and the unweighted pair group method with arithmetic mean (UPGMA) (**Dawyndt *et al*., 2006**). The evolutionary distances were calculated by the maximum composite likelihood method (**Tamura *et al*., 2004**). Percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (**Felsenstein, 1985, 2004**).

Hematological and Biochemical profiles

Haematological parameters including total erythrocytic count (RBCs), total leucocytic count (WBCs), differential leucocytic count (DLC), haemoglobin (Hb) and packed cell volume (PCV) were done as described by **Schalm *et al.* (1986)**. All biochemical prameters were determined spectrophotometerically. Serum total protein (Biuret method) was determined according to **Gornal *et al.* (1949)**. Serum albumin was determined according to **Doumas *et al.* (1971)**. Serum globulin was determined by subtraction of serum albumin from serum total protein according to **Doumas *et al.* (1971)**. A/G ratio was estimated by dividing the albumin content on globulin value. Serum AST and ALT were determined according to **Reitman and Frankel (1957)**. Serum ALP was determined according to **Belfield and Goldberg (1971)**. Serum Urea was determined according to **Fawcett and Soctt (1960)**. Serum Creatinine was determined according to **Schirmeister (1964)**.

Statistical analysis

Statistical analysis for hematological and biochemical parameters was performed using Student's *t* test (SPSS 14.0 for Windows Evaluation Version). Probability values (P-value) < 0.05 were considered of statistical significant and < 0.001 were considered of high statistical significant.

RESULTS

During sampling, the main clinical signs were observed in the 61 tested camels ranged between apparently healthy (n = 49) to number of camels with fever (n = 9), anorexia (n = 6), Lethargy (n = 5), anemia (n = 8), enlargement of superficial lymph nodes (n = 2), and emaciation (n = 2). In addition, the five tick species found in Cairo, Giza, and Sinai were identified into *Hyalomma* species. Where, the camel tick *H. dromedarii* was the most dominant that recorded 91.9 % (Table 2). *H. marginatum*, *H. impeltatum*, *H. excavatum* and *H. rufipes* recorded low infestation (Table 2).

Table 2 The prevalence of *Rickettsia* spp. in camels and their tick by Gimenez stain and PCR

Camels and Ticks species	No.	Prevalence of Tick species (%)	No. of positives with *Rickettsia* spp.			
			Gimenez staining technique		PCR using *OmpA*&*gltA* genes	
			No.	%	No.	%
Camels	61	_	0	0	25	41
*Hayalomma*spp.	99	100	10/99	10.1	1/99	1.01
H. dromedarii	91/99	91.9	8/91	8.79	0	0
H. marginatum	5/99	5.05	1/5	20	1/5	20
H. excavatum	1/99	1.01	0	0	0	0
H. impeltatum	1/99	1.01	1/1	100	0	0
H. rufipes	1/99	1.01	0	0	0	0

Prevalence of tick-borne rickettsioses in camels and their tick vectors

The prevalence of *Rickettsia*spp. in camels and their tick species using Gimenez staining technique was 0 % and 10.1 %, respectively (Table 2). Moreover, all blood camels' specimens and their ticks were screened by PCR amplifying fragments of *OmpA* and *gltA* genes with the products sizes 500 to 600 bp and 1000 to 1200 bp, respectively (Fig.1). For more discrimination, the *Rickettsia* positive samples were further screened by intergenic spacers amplification (*mppA*, *dksA* and *rpmE*), where the obtained fragment sizes were 146, 229 and 344 bp, respectively. The total of 25/61 camel samples (4 *OmpA* and 23 *gltA*) and 1/99 ticks' samples (*H. marginatum* from Sinai province) were confirmed positive. Therefore, the prevalence of rickettsioses using PCR was 41.0 % in camels and 1.01 % in their tick species (Table 2).

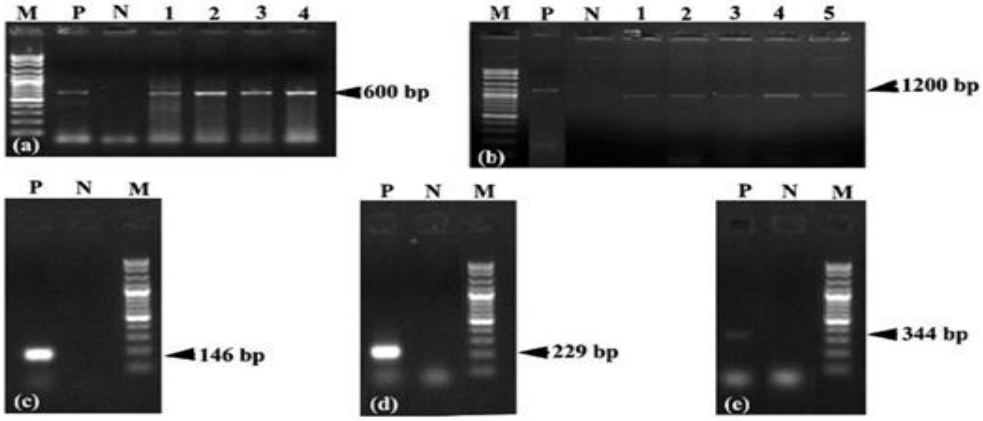

Fig 1 Molecular identification of *Rickettsia* spp. by PCR products detected in camels and *Hyalomma* species in 1.5 % agarose gels stained with ethidium bromide. In all figures, lane M: 100 bp DNA ladder and lane N: Control negative. (a) Lane P presents600 bp amplicon of *OmpA Rickettsia* positive tick sample and lanes 1 to4 present 600 bp amplicon of *OmpA Rickettsia* positive samples of camels. (b) Lane P presents1200 bp amplicon of *gltA Rickettsia* positive tick sample and lanes 1 to 5 present 1000 bp amplicon of *OmpA Rickettsia* positive samples of camels. (c) Lane P presents 146 bp amplicon of *mppA Rickettsia* positive tick sample. (d) Lane P presents 229 bp amplicon of *dksA Rickettsia* positive tick sample. (e) Lane P presents 344 bp amplicon of *rpmE Rickettsia* positive tick sample.

Sequences Analyses and Genbank Accession Numbers

The obtained Egyptian *Rickettsia* sequences of *OmpA* and *gltA* genes and intergenic spacers (*mppA*, *dksA* and *rpmE*) from *H. marginatum* tick were submitted in GenBank and registered with accession numbers KX819299, KX819298, KX819297, KX819295 and KX819296, respectively. The identities of obtained *Rickettsia* sequences were ranged from 97-100 % in comparison to *Rickettsia* strains recorded in Genbank (Table 3 and 4). The present results revealed that the Egyptian new isolates were similar to *R. africae* (KX819299, KX819298, KX819297, KX819295 and KX819296) and closely matching to the reference counterparts previously recorded in Saini-Egypt (HQ335132.1, HQ335126.1, HQ335143.1, HQ335138.1 and HQ335144.1), respectively (Table 3).Moreover, the partial sequence of *OmpA* gene of *Rickettsia* amplified from camel (no. 61) showed 48.68 % similarity with *R. africae* accession no. U83436.2 (Table 3).

Table 3 GenBank accession numbers of Egyptian Rickettsial amplified from camels and their tick vectors.

Sequence type	Animals and Ticks		Egyptian Rickettsial isolates	GenBank No.	Similarity with recorded *Rickettsia* species in GenBank		
	Species	Sex			Identity (%)	Covering (%)	Reference strains of *Rickettsia* spp.
OmpA	*H. marginatum*	♂	*Rickettsia africae*	KX819299	100	99	HQ335132.1
gltA				KX819298	100	94	HQ335126.1
mppA				KX819297	97	99	HQ335143.1
dksA				KX819295	99	99	HQ335138.1
rpmE				KX819296	99	100	HQ335144.1
OmpA	Camel (no. 61)	♂	*Rickettsia* spp.	_	48.68	_	U83436.2

HQ335132.1 = *Rickettsia africae* strain EgyRickHmm-Qalet El-Nakhl-2 outer membrane protein A (*OmpA*) gene, partial cds.
HQ335126.1 = *Rickettsia africae* strain EgyRickHd-Qalet El-Nakhl citrate synthase (*gltA*) gene, partial cds.
HQ335143.1 = *Rickettsia africae* isolate EgyRickHmm-Qalet El-Nakhl-11 *mppA-purC* intergenic spacer, partial sequence.
HQ335138.1 = *Rickettsia africae* isolate EgyRickHimp-El-Arish-4 *dksA-xerC* intergenic spacer, partial sequence.
HQ335144.1 = *Rickettsia africae* strain EgyRickHimp-El-Arish-6 RpmE (*rpmE*) gene, partial sequence; *rpmE-trnM* intergenic spacer, complete sequence; and tRNA-Met (trnM) gene, partial sequence.
U83436.2 = *Rickettsia africae*strain ESF 2500-1 cell surface antigen *rOmpA* (scaO) gene, partial cds.

Phylogenetic Relationships and Multi-genes Typing

The percent identity matrix of Egyptian Rickettsial sequences was constructed based on Clustal omega multiple alignments (Table 4), and the phylogenetic analyses of two genes and three intergenic spacers for each *Rickettsia* amplicon using two methods UPGMA (not shown) and NJ by MEGA4 using *Rickettsia felis* as outgroup (Fig.2). The NJ phylogenetic trees indicated that *R. africae* strains of *H. marginatum* were grouped together with other *R. africae* recorded in GenBank (Table 4 and Fig.2). The *dksA* and *rpmE* were fallen in a separate clade in the NJ trees (Fig.2 d, e) indicated a novel strain of *R. africae* within *H. marginatum* picked from camel from Sinai province. In addition, the similarity percent of the Egyptian *Rickettsia* camel *OmpA* amplicons was 48.68 in comparison to *R. africae* GenBank record U83436.2 (Table 4).

Table 4 Identity matrix generated with the nucleotide sequences obtained from the different *Rickettsia* spp. Isolates from *H. marginatumOmpA*(a), *gltA*(b), *mppA*(c), *dksA*(d) and *rpmE* (e) and camel 61 *OmpA* gene (f).

a) *OmpA*

1: KF791242	100.00	98.26	98.09	98.64	98.60	98.59	97.28	97.92	98.13	98.30	98.30	98.45
2: KT345980	98.26	100.00	99.83	98.99	98.98	99.13	97.64	98.92	98.32	98.48	98.48	98.65
3: DQ097082	98.09	99.83	100.00	98.82	98.81	98.96	97.47	98.66	98.15	98.32	98.32	98.48
4: U83436.2	98.64	98.99	98.82	100.00	100.00	99.48	97.79	98.96	99.05	99.21	98.58	99.33
5: GU247115	98.60	98.98	98.81	100.00	100.00	99.48	97.84	98.91	99.00	99.17	99.17	99.32
6: GQ853063	98.59	99.13	98.96	99.48	99.48	100.00	97.57	99.17	98.79	98.96	98.96	99.13
7: EU715288	97.28	97.64	97.47	97.79	97.84	97.57	100.00	98.44	97.32	97.48	96.69	97.47
8: KX819299	97.92	98.92	98.66	98.96	98.91	99.17	98.44	100.00	100.00	99.22	99.22	98.93
9: HQ335132	98.13	98.32	98.15	99.05	99.00	98.79	97.32	100.00	100.00	99.53	98.90	99.33
10: HQ335131	98.30	98.48	98.32	99.21	99.17	98.96	97.48	99.22	99.53	100.00	99.37	99.49
11: HQ335136	98.30	98.48	98.32	98.58	99.17	98.96	96.69	99.22	98.90	99.37	100.00	99.49
12: JQ691730	98.45	98.65	98.48	99.33	99.32	99.13	97.47	98.93	99.33	99.49	99.49	100.00

(b)*gltA*

1: AY743327	100.00	98.78	98.62	98.43	98.80	98.76	98.78	98.94	98.75	98.64	98.95	98.85	98.72	99.03	99.03	99.11	98.82	99.03
2: U59729	98.78	100.00	98.86	99.03	99.11	99.03	99.03	99.20	99.13	99.06	99.35	99.29	99.19	99.43	99.43	99.51	99.51	99.43
3: U59719	98.62	98.86	100.00	99.35	99.27	98.67	98.70	98.85	98.75	98.73	99.03	98.94	98.86	99.11	99.11	99.19	99.19	99.11
4: HQ335153	98.43	99.03	99a.35	100.00	99.36	98.94	98.87	99.12	99.04	98.98	99.19	99.21	98.96	99.27	99.27	99.35	99.30	99.27
5: DQ365804	98.80	99.11	99.27	99.36	100.00	99.12	98.95	99.30	99.23	99.07	99.27	99.38	99.12	99.35	99.35	99.43	99.44	99.35
6: JN043505	98.76	99.03	98.67	98.94	99.12	100.00	99.47	99.65	99.52	99.29	99.20	99.20	99.29	99.29	99.47	99.56	99.56	99.47
7: U59733	98.78	99.03	98.70	98.87	98.95	99.47	100.00	99.82	99.23	99.07	99.19	99.21	99.19	99.43	99.43	99.51	99.51	99.43
8: HM050288	98.94	99.20	98.85	99.12	99.30	99.65	99.82	100.00	99.42	99.47	99.38	99.38	99.47	99.47	99.65	99.74	99.74	99.65
9: KX819298	98.75	99.13	98.75	99.04	99.23	99.52	99.23	99.42	100.00	100.00	99.33	99.33	99.42	99.42	99.62	99.71	99.71	99.62
10: HQ335126	98.64	99.06	98.73	98.98	99.07	99.29	99.07	99.47	100.00	100.00	99.24	99.38	99.41	99.32	99.49	99.58	99.58	99.49
11: U59730	98.95	99.35	99.03	99.19	99.27	99.20	99.19	99.38	99.33	99.24	100.00	100.00	99.35	99.59	99.59	99.68	99.68	99.59
12: HM050292	98.85	99.29	98.94	99.21	99.38	99.20	99.21	99.38	99.33	99.38	100.00	100.00	99.38	99.56	99.65	99.65	99.65	99.65
13: HM050296	98.72	99.19	98.86	98.96	99.12	99.29	99.19	99.47	99.42	99.41	99.35	99.38	100.00	99.43	99.59	99.68	99.68	99.76
14: U59728	99.03	99.43	99.11	99.27	99.35	99.29	99.43	99.47	99.42	99.32	99.59	99.56	99.43	100.00	99.68	99.76	99.76	99.68
15: U59732	99.03	99.43	99.11	99.27	99.35	99.47	99.43	99.65	99.62	99.49	99.59	99.56	99.59	99.68	100.00	99.92	99.92	99.84
16: KU310587	99.11	99.51	99.19	99.35	99.43	99.56	99.51	99.74	99.71	99.58	99.68	99.65	99.68	99.76	99.92	100.00 1100.0	100.00	99.92
17: KM288711	98.82	99.51	99.19	99.30	99.44	99.56	99.51	99.74	99.71	99.58	99.68	99.65	99.68	99.76	99.92	0	100.00	99.92
18: DQ097081	99.03	99.43	99.11	99.27	99.35	99.47	99.43	99.65	99.62	99.49	99.59	99.56	99.76	99.68	99.84	99.92	99.92	100.00

(c) *mppA*

1: KX819297	100.00	48.39	46.24	48.39	48.39	47.62	48.15	48.89	48.89	49.07
2: EF140692	48.39	100.00	96.08	97.39	97.39	97.26	97.39	98.04	98.04	97.83
3: DQ008285	46.24	96.08	100.00	98.69	97.39	97.26	97.39	98.04	98.04	97.83
4: DQ008283	48.39	97.39	98.69	100.00	98.69	98.63	98.69	99.35	99.35	99.28
5: DQ008299	48.39	97.39	97.39	98.69	100.00	98.63	98.69	99.35	99.35	99.28
6: AY345087	47.62	97.26	97.26	98.63	98.63	100.00	98.75	99.38	99.38	99.34
7: HQ335142	48.15	97.39	97.39	98.69	98.69	98.75	100.00	98.98	99.49	99.35
8: KC870931	48.89	98.04	98.04	99.35	99.35	99.38	98.98	100.00	100.00	100.00
9: HQ335141	48.89	98.04	98.04	99.35	99.35	99.38	99.49	100.00	100.00	100.00
10: HQ335143	49.07	97.83	97.83	99.28	99.28	99.34	99.35	100.00	100.00	100.00

(d)*dksA*

1: KX819295	100.00	59.88	55.45	55.84	51.11	59.15	53.33	54.55	56.57	55.56	56.00
2: AY820021	59.88	100.00	87.11	92.94	88.72	93.97	92.64	84.06	87.5	88.72	94.67
3: HQ335138	55.45	87.11	100.00	100.00	93.83	92.89	96.51	97.77	93.30	93.75	98.31
4: HQ335140	55.84	92.94	100.00	100.00	96.09	98.27	96.51	97.18	97.74	98.31	98.31
5: HQ335139	51.11	88.72	93.83	96.09	100.00	93.43	95.93	94.25	90.27	90.71	97.75
6: AY428741	59.15	93.97	92.89	98.27	93.43	100.00	98.21	89.62	92.36	92.6	100.00
7: EF215902	53.33	92.64	96.51	96.51	95.93	98.21	100.00	97.09	97.67	98.26	98.26
8: AY820036	54.55	84.06	97.77	97.18	94.25	89.62	97.09	100.00	90.42	90.83	98.88
9: AY820034	56.57	87.50	93.30	97.74	90.27	92.36	97.67	90.42	100.00	97.05	99.44
10: AY820026	55.56	88.72	93.75	98.31	90.71	92.60	98.26	90.83	97.05	100.00	100.00
11: KR492916	56.00	94.67	98.31	98.31	97.75	100	98.26	98.88	99.44	100.00	100.00

(e) *rpmE*

1: KX819296	100.00	45.45	45.45	45.00	46.10	46.53	46.53	45.45	47.16	48.50	43.56	45.82
2: DQ008250	45.45	100.00	97	97.00	97	97.00	97.00	97.5	88.00	87.00	91.48	92.23
3: DQ008248	45.45	97.00	100.00	99	99.00	99	99.00	99.50	89.00	88.00	92.61	93.26
4: KF539830	45.00	97.00	99.00	100.00	98.65	98.65	98.65	99.50	91.25	90.88	92.61	93.77
5: KC870937	46.10	97	99	98.65	100	98.82	98.81	99.5	92.28	91.94	92.61	93.77
6: HQ335144	46.53	97.00	99.00	98.65	98.82	100.00	100.00	99.50	92.42	92.08	92.61	93.77
7: HQ335145	46.53	97.00	99	98.65	98.81	100.00	100.00	99.50	92.40	92.08	92.61	93.77

8: AY836521	45.45	97.5	99.50	99.50	99.50	99.5	99.50	100.00	89.5	88.5	93.18	93.78
9: HQ335165	47.16	88.00	89	91.25	92.28	92.42	92.40	89.50	100.00	99.03	88.39	95.56
10: HQ335164	48.5	87.00	88.00	90.88	91.94	92.08	92.08	88.50	99.03	100.00	87.95	95.54
11: DQ008256	43.56	91.48	92.61	92.61	92.61	92.61	92.61	93.18	88.39	87.95	100.00	90.18
12: DQ440626	45.82	92.23	93.26	93.77	93.77	93.77	93.77	93.78	95.56	95.54	90.18	100.00

(f) Camel 61

1: Camel 61	100.00	48.34	48.18	48.18	48.18	48.34	48.34	48.34	48.18	48.34	47.87	48.03	48.03	48.68	48.50	48.34	48.03	47.55	47.48	47.64	47.64	47.80
2: U83442.1	48.34	100.00	97.51	97.45	96.88	97.16	97.01	97.05	97.05	96.98	96.83	97.20	97.64	97.17	97.05	97.11	96.04	96.48	96.70	96.95	96.95	96.86
3: U83456.1	48.18	97.51	100.00	98.33	97.89	98.20	97.98	98.02	98.02	98.02	97.86	98.24	98.68	98.11	97.92	98.11	96.25	96.66	96.85	97.10	97.10	97.07
4: U83451.1	48.18	97.45	98.33	100.00	97.83	98.17	97.96	97.93	97.99	97.93	97.83	98.21	98.59	97.96	97.86	98.05	96.04	96.32	96.54	96.86	96.86	96.89
5: U83455.1	48.18	96.88	97.89	97.83	100.00	99.46	98.33	98.43	98.43	98.43	98.33	98.65	98.65	98.68	98.58	98.77	95.71	96.00	96.13	96.47	96.47	96.35
6: U83439.1	48.34	97.16	98.20	98.17	99.46	100.00	98.61	98.71	98.71	98.71	98.61	98.93	98.93	98.96	98.86	98.99	96.08	96.27	96.46	96.81	96.81	96.68
7: U83453.1	48.34	97.01	97.98	97.96	98.33	98.61	100.00	99.84	99.84	99.78	98.62	99.06	98.68	98.62	98.49	98.55	95.78	96.20	96.20	96.51	96.51	96.48
8: U83448.1	48.34	97.05	98.02	97.93	98.43	98.71	99.84	100.00	99.94	99.87	98.71	99.15	98.77	98.71	98.59	98.65	95.82	96.23	96.23	96.54	96.54	96.51
9: U83443.1	48.18	97.05	98.02	97.99	98.43	98.71	99.84	99.94	100.00	99.87	98.71	99.15	98.77	98.71	98.59	98.65	95.82	96.23	96.23	96.54	96.54	96.51
10: U83440.1	48.34	96.98	98.02	97.93	98.43	98.71	99.78	99.87	99.87	100.00	98.71	99.15	98.77	98.71	98.59	98.65	95.82	96.23	96.23	96.54	96.54	96.51
11: U83441.1	47.87	96.83	97.86	97.83	98.33	98.61	98.62	98.71	98.71	98.71	100.00	99.37	98.62	98.62	98.43	98.43	95.69	96.10	96.32	96.57	96.57	96.48
12: U83437.1	48.03	97.20	98.24	98.21	98.65	98.93	99.06	99.15	99.15	99.15	99.37	100.00	98.99	98.87	98.74	98.81	96.01	96.51	96.61	96.92	96.92	96.83
13: U83454.1	48.03	97.64	98.68	98.59	98.65	98.93	98.68	98.77	98.77	98.77	98.62	98.99	100.00	98.81	98.74	98.84	96.32	96.67	96.95	97.20	97.20	97.17
14: U83436.2	48.68	97.17	98.11	97.96	98.68	98.96	98.62	98.71	98.71	98.71	98.62	98.87	98.81	100.00	99.12	98.99	95.82	96.35	96.42	96.67	96.67	96.64
15: U83452.1	48.50	97.05	97.92	97.86	98.58	98.86	98.49	98.59	98.59	98.59	98.43	98.74	98.74	99.12	100.00	98.99	95.75	96.17	96.20	96.51	96.51	96.48
16: U83449.1	48.34	97.11	98.11	98.05	98.77	98.99	98.55	98.65	98.65	98.65	98.43	98.81	98.84	98.99	98.99	100.00	95.85	96.23	96.35	96.73	96.73	96.64
17: U83447.1	48.03	96.04	96.25	96.04	95.71	96.08	95.78	95.82	95.82	95.82	95.69	96.01	96.32	95.82	95.75	95.85	100.00	96.04	96.35	96.73	96.73	96.54
18: U83446.1	47.55	96.48	96.66	96.32	96.00	96.27	96.20	96.23	96.23	96.23	96.10	96.51	96.67	96.35	96.17	96.23	96.04	100.00	97.36	97.61	97.61	97.68
19: U83438.1	47.48	96.70	96.85	96.54	96.13	96.46	96.20	96.23	96.23	96.23	96.32	96.61	96.95	96.42	96.20	96.35	96.35	97.36	100.00	99.22	99.22	98.93
20: U83445.1	47.64	96.95	97.10	96.86	96.47	96.81	96.51	96.54	96.54	96.54	96.57	96.92	97.20	96.67	96.51	96.73	96.73	97.61	99.22	100.00	100.00	99.25
21: U83444.1	47.64	96.95	97.10	96.86	96.47	96.81	96.51	96.54	96.54	96.54	96.57	96.92	97.20	96.67	96.51	96.73	96.73	97.61	99.22	100.00	100.00	99.25
22: U83450.1	47.80	96.86	97.07	96.89	96.35	96.68	96.48	96.51	96.51	96.51	96.48	96.83	97.17	96.64	96.48	96.64	96.54	97.68	98.93	99.25	99.25	100.00

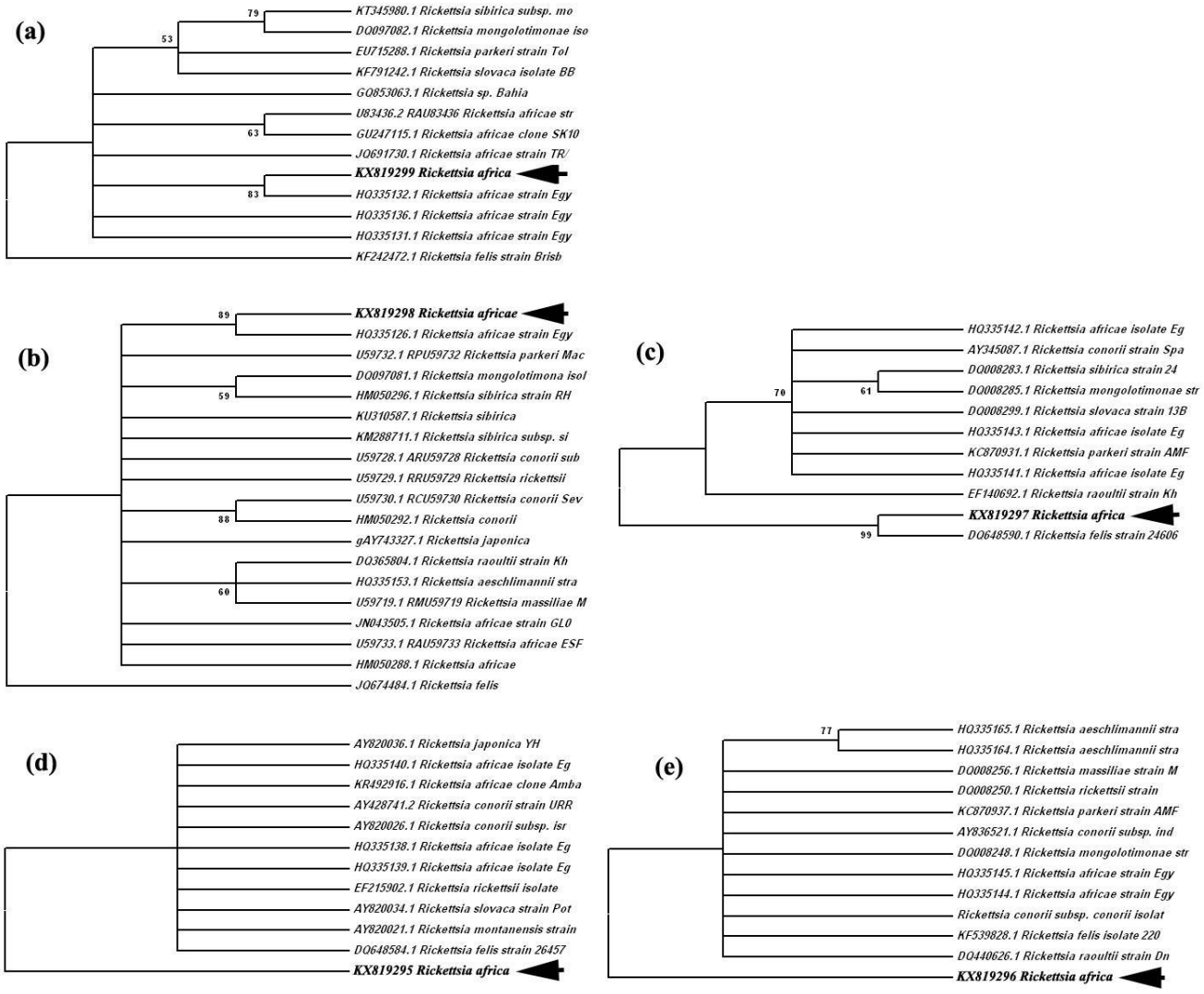

Fig 2 Phylogenetic trees of *R. africae* detected in the present study based on the sequences of two genes (*OmpA* and *gltA*) and the three intergenic spacers (*mppA*, *dksA* and *rpmE*). All sequences were aligned and Neighbor-joining trees were constructed; (a)*OmpA*, (b)*gltA*, (c)*mppA*, (d)*dksA*and(e) *rpmE*.

Epidemiological Profile on rickettsioses in the studied camels

The 61 camels investigated during the present study were divided into groups according to age, breeds and tick infestation at time of examination. The results revealed the highest prevalence of rickettsioses among aged, Abady breeds and ticks-infested camels (Tables 5 and 6).

Table 5 The prevalence of rickettsioses among camels screened by PCR with regards to age groups.

Age Groups (Years)	Total No. of Tested Camels by PCR	No. of Positive Camels by PCR	The prevalence rate (%)
< 11	16/61	4/16	25.0
11 – 13	16/61	3/16	18.7
13 – 15	6/61	2/6	33.3
15 – 17	11/61	8/11	72.7
17 – 19	5/61	4/5	80.0
> 19	7/61	4/7	57.1
Total	61	25/61	41.0

Table 6 The prevalence of rickettsioses among camels screened by PCR with regards to Breed, and Ticks Infestation.

Parameters	Breeds		Tick Infestation	
	Abady	Beshary	Ticks infested camels	Ticks free camels
No. of tested camels by PCR	37/61	24/61	14/61	47/61
No. of positive camels by PCR	21/37	4/24	6/14	19/47
The prevalence rate (%)	56.8	16.7	42.8	40.4

Hematological and biochemical changes in camels with rickettsioses

The hematological and biochemical tests were applied on 61 camels of which 25 were proved *Rickettsia* infected camels by PCR and 36 were *Rickettsia* free camels. Macrocytic anemia and leucopenia were recorded in the *Rickettsia* positive camels (Table 7), while there were no significant differences in the biochemical changes between *Rickettsia* positive and negative camels (Table 8).

Table 7 Hematological parameters of Rickettsial infected camels compared with Rickettsial free camels (Mean ± SD).

Hematological Parameters	Animal Groups	
	Rickettsial Free Camels	Rickettsial Diseased Camels
RBCs ($\times10^{6}$)	5.02±0.15	5.95±0.23**
Hb (g/dl)	13.92±0.58	19.13±1.05**
PCV (%)	40.75±1.85	55.00±3.16**
MCV (fl)	80.75±1.68	90.56±2.35*
MCH (pg)	27.72±0.54	31.62±0.75**
MCVC (g/dl)	34.46±0.40	33.78±1.31
Platelets ($\times10^{3}$)	95.16±2.06	96.66±2.59
WBCs ($\times10^{3}$)	11.48±0.31	9.15±0.51**
Neutrophils (%)	81.75±0.65	83.20±0.59
Lymphocytes (%)	11.77±0.43	11.20±0.56
Monocytes (%)	5.02±0.32	4.04±0.29
Eosinophils (%)	1.55±0.11	1.54±0.14

* = significant at P< 0.05 ** = highly significant at P< 0.01

Table 8 Biochemical parameters of Rickettsial infected camels compared with Rickettsial free camels (Mean ± Standard Deviation; SD).

Biochemical Parameters	Animal Groups	
	Rickettsial Free Camels	Rickettsial Diseased Camels
Total Protein (g/dl)	7.70±0.79	7.58±0.17
Albumin (g/dl)	2.37±0.34	2.43±0.22
Globulin (g/dl)	5.16±0.39	5.05±0.27
Albumin/Globulin Ratio	0.54±0.08	0.61±0.11
GOT (AST; IU/L)	58.40±6.59	53.30±4.95
GPT (ALT; IU/L)	47.54±5.63	35.70±2.88
ALP (IU/L)	55.97±4.73	55.61±3.98
Creatinine (mg/dl)	2.47±0.16	2.42±0.06
Urea (mg/dl)	72.37±4.80	45.57±2.86

DISCUSSION

Globalization, international trade, urbanization, climate change, travel and animals' mobility are factors that led to rapid extension of the zoogeographical range of many tick species, subsequently, tick-borne diseases (**Shaw *et al*., 2003; Harrus and Baneth, 2005**). Therefore, researches on ricketsiae are exceeded because of their public health implication, zoonotic importance and worldwide distribution (**Parola and Raoult, 2001; Dantas-Torres *et al*., 2012; Kernif *et al*., 2012b; Parola *et al*., 2013**).

In Egypt, few studies have been undertaken on the epidemiology of rickettsioses infection in camels as reservoirs of *Rickettsia* spp. The main objective of this study is to evaluate the clinical, hematological, and biochemical profiles of camel rickettsioses and their molecular diagnostic investigations to confirm the previously detected and/or novel genotypes of *Rickettsia* in Egypt.

The main clinical signs observed in the 61 studied camels were similar to those mentioned by **Wernery *et al.* (2001)** who reported some clinical characteristic of rickettsiosis as lethargy, emaciation, recumbency and enlarged edematous lymph nodes that agreed with the findings of the present study. Concerning the apparently healthy camels, the results agreed with other reportsstated thatno statistically significant differences were found between clinically healthy and sick animals (**Kelly *et al*., 1992; Solano-Gallego *et al*., 2006; Ortuno *et al*., 2009; Riveros-Pinilla *et al*., 2015**).

In the present study, it was found that *H. dromedarii* was the most abundant tick species on camels, while other *Hyalomma* spp recorded very low infestation rate in agreement with previous findings recorded by **Abdel-Shafy (2000) ; Diab *et al*. (2001); El-Kammah *et al*. (2001); Abdel-Shafy *et al*. (2012); Abdullah *et al*. (2016a).**

Gimenez staining technique of camels'blood and tick hemolymph staining revealed that the prevalence of *Rickettsia* spp. was 0 % and 10.1 %, respectively (Table 2). The negative results of Gimenez staining technique in camel blood films may return to the low numbers of rickettsiae circulating in the blood and had probably cleared from blood (**Breitschwerdt *et al.*, 1990; Parola *et al*., 2005**). While, hemolymph staining was successful as a field test for detection of *Rickettsia* in ticks which kept ticks undamaged, so that the infected ticks can be used in other purposes (**Gimenez, 1964**). However, the susceptibility of the Gimenez stain to other bacterial agents than *Rickettsia* justified the magnified prevalence percentage of infection in ticks by staining technique and needed to be confirmed by PCR; the more specific technique (**Parola *et al*., 2013; Guillemi *et al*. 2015**).

Here, PCR technique was carried out on 61 camels' blood samples and their ticks using *OmpA* and *gltA* genes; SFP specific primers (**Parola *et al*., 2013; Guillemi *et al*., 2015**). The results revealed that twenty-five camels, from Cairo, Giza and Sinai provinces and one tick (*H. marginatum*) from Sinai province, were positive for *Rickettsia* spp. infection. The samples from positive animals and ticks were additionally screened by intergenic spacers (*mppA*, *dksA* and *rpmE*) amplification and sequencing. The prevalence of *Rickettsia* spp. in camels was 41.0 % and 1.01 % in *Hyalomma* spp. (Table 2). In the previous studies, *Rickettsia* spp. were identified in camel blood film stain from Dubai (**Wernery *et al*., 2001**) and 18.8% of camel blood samples by PCR from Nigeria (**Kamani *et al*., 2015**). On the other hand, **Mentaberre *et al*. (2013)** reported that 83 % of camels were found infected with *Rickettsia* spp. serologically by ELISA. However, the detection of SFG *Rickettsia* spp. in the present study indicated the importance role of camels in the persistence of *Rickettsia* in the nature than previously thought (**Wernery and Kaaden, 2002; Kamani *et al*., 2015**). Furthermore, our results of *Rickettsia* positive ticks were similar to those were previously reported by **Niebylski *et al*. (1999)**, **Levin *et al*. (2009)** and **Socolovschi *et al*. (2009a)** that the naturally infection rate of ticks with rickettsiae almost is < 1% because of the lethal effects of *Rickettsia*. In the previous studies carried out in Egypt, SFG were detected in *Rh. sanguineus* and *Hyalomma* species at Sinai by immunostaining and PCR (**Lange *et al*., 1992**) while **Loftis *et al*. (2006a, b)** detected *R. aeschlimannii* in *Hyalomma* spp. by PCR. Moreover, **Abdel-Shafy *et al.* (2012)** were the first to report *R. africae* in *H. dromedarii*, *H. impeltatum* and *H. marginatum*, and *R. aeschlimannii* in *H. impeltatum* and *H. marginatum* collected from camels in Sinai.

Sequencing and phylogenetic analyses were performed on *OmpA* and *gltA* genes and intergenic spacers (*mppA*, *dksA* and *rpmE*) amplified from camels and *Hyalomma* spp. The present results revealed that the Egyptian obtained *R.*

africae records; KX819299, KX819298, KX819297, KX819295 and KX819296, were highly similar to the reference counterparts; HQ335132.1, HQ335126.1, HQ335143.1, HQ335138.1 and HQ335144.1, which were obtained previously from Sinai province in Egypt (**Abdel-Shafy *et al*., 2012**). Nonetheless, the topology inferred from non-coding intergenic spacers, illustrates a relationship between Egyptian isolates and other *Rickettsia* spp. The presence of obtained strains in a separate clade in the NJ trees of *dksA* and *rpmE* sequences (Fig.2d, e) were in accordance to the fact that non-coding intergenic spacers are able to identify a single *Rickettsia* spp. (**Fournier *et al*., 2004**). Therefore, the present results suggested the novelty of the obtained strain of *R. africae* in *H. marginatum* collected from camels in Sinai province. These results were in agreement with **Abdel-Shafy *et al*. (2012)** who were the first to identify *R. africae* in *H. dromedarii*, *H. impeltatum* and *H. marginatum* from Sinai province. Moreover, *R. africae* was detected in *H. dromedarii* on camels from Algeria (**Kernif *et al*., 2012a**), while in Israel it was detected in *H. dromrdarii*, *H. impeltatum*, *H. excavatum* and *H. turanicum* (**Kleinerman *et al*., 2013**). Although, *R. africae* in South Africa was associated only with *Amblyomma spp.* (**Parola and Raoult, 2001; Parola *et al*., 2005**). The present study confirmed that *Hyalomma* spp. have a potential role as a vector for *R. africae* (ATBF) in North Africa (**Abdel-Shafy *et al*., 2012; Kernif *et al*., 2012a**).

Regarding the phylogenetic analyses of camels *Rickettsia* spp., which were positive by *OmpA* amplification, the similarity percent to other reference *Rickettsia* strains published by Fournier et al. (1998) was 48.68 % with *R. africae* isolate U83436.2 (Table 4). The results revealed that the *Rickettsia* spp. detected in camel (no. 61) from Sinai province was closely matching to *R. africae*. Furthermore, the present Egyptian isolates were clustered in a separate clade with higher similarity to the reference counterparts (HQ335132.1, HQ335136.1 and HQ335131.1) which were obtained previously from Sinai province in Egypt, as well (**Abdel-Shafy *et al*., 2012**) (Fig.3). These results suggested that the presence of *R. africae* strain in camel. Accordingly, this is the first molecular detection of *Rickettsia* DNA in camels in Egypt. In addition, *Hyalomma* spp. are the main camel ticks in Egypt and North Africa (**Abdel-Shafy, 2000; Diab *et al*., 2001; El-Kammah *et al*., 2001; Abdel-Shafy *et al*., 2012**). The present study confirmed that *Hyalomma* spp. have a potential role as a vector for *R. africae* in camels. Moreover, the detection of *R. africae* in *H. marginatum* and in its camel from Sinai province indicated that *R. africae* can act as an emerging pathogen in Sinai province.

Concerning the ageand breed susceptibility, the highest infection rate of rickettsioses was recorded in age groups of 17 to 19 years and Abady camel breed, respectively as shown in tables (5 and 6). Though there were limited data on age or breed susceptibility in camels, other studies were applied on dogs and horses concluded the absence of statistical association between infection rate with *Rickettsia* spp. and age, sex and breed **Riveros-Pinilla *et al.* (2015)**. Concurrently, Cunha et al. (2014) observed that older animals were more reactive with *Rickettsia* than younger animals, which may be due to the prolonged and/or repetitive exposure of older animals to ticks infected with *Rickettsia* spp. and/or senile lower immunity. Hence, the present study revealed a significant difference among age groups.

The infection rate with rickettsioses was relatively higher in ticks-infested camels (42.8 %) than in ticks-free camels (40.4 %), as shown in table (6). The present results indicated that camels infested by ticks were at high risk to be positive for *Rickettsia* spp. because *Hyalomma* spp. were reported as the principle vector of rickettsioses in Egypt. However, some camels infested by ticks were negative for rickettsioses in the present study, this may be attributed to the fact that attached ticks were free from *Rickettsia* spp. or were infected with *Rickettsia* but they recently attached to these camels and yet to transmit the infection to their hosts. Furthermore, the ticks-free camels (at the time of examination) which were proved positive for *Rickettsia*, might be infested previously by ticks, which possibly had been removed manually and/or received various acaricide treatments.

Moreover, hematological and biochemical profiles in studied camels were recorded as shown in tables (7 and 8). The presented results revealed that macrocytic anemia and leucopenia were recorded in *Rickettsia* positive camels. The leucopenia recorded in this study may be attributed to the decrease in monocytes and lymphocytes. While, no significant differences were reported in biochemical changes between *Rickettsia* positive and negative camels. However, the available data on hematological and biochemical parameters in camels are limited, previous experimental studies were applied on dogs recorded anemia and early leukopenia during the course of disease followed by progressive leukocytosis and severe thrombocytopenia (**Gasser *et al*., 2001; Elchos and Goddard, 2003; Parola *et al*., 2005; 2013**). Similarly, **Scorpio *et al*. (2008)** reported no specific hematologic or biochemical differences between seronegative and seropositive dogs.

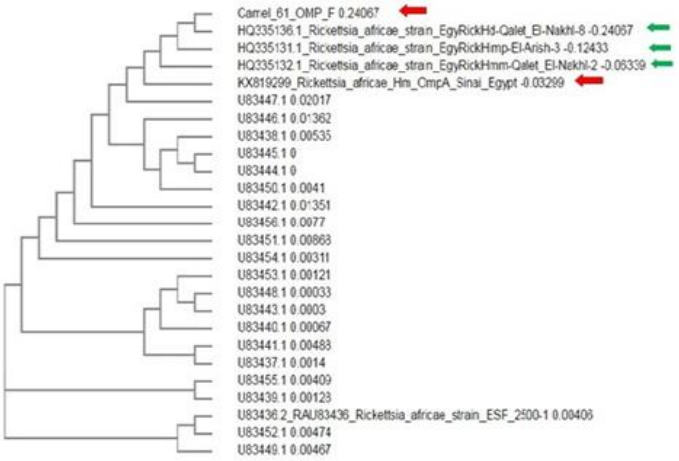

Fig 3 Cladogram of current molecular epidemiological status of the Egyptian *Rickettsia* spp. isolates that compare the ones obtained during the present study from Camels and its tick (*H. marginatum;* red arrows), with other *Rickettsia* spp. records of local (green arrows) and international isolates within Genbank database dependent on alignment of *OmpA* genes sequences constructed by the Clustal omega multiple alignments software utilizing NJ equation.

CONCLUSION

A novel strain of *R. africae* was detected in *H. marginatum* picked from camel from Sinai province that was dissimilar from previous Egyptian isolates by molecular characterization. This is the first detection of *Rickettsia* DNA in camels by PCR in Egypt with the prevalence rate 41.0 %. Moreover, the detection of *R. africa* in *H. marginatum* and its camel from Sinai indicated that *R. Africa* act as an emerging pathogen in Sinai province. Rickettsioses has tobe included during examination of imported animals as exotic diseases as well as the differential diagnosis of non-specific febrile illness of camels. Further, the detection of tick-borne *Rickettsia* in camels and their ticks not only indicates that camels' populations in Egypt are at risk, but also presents possible zoonotic implications in human populations since *Hyalomma* spp. were known to be aggressive to bite human, which likely can facilitate the transmission of *Rickettsia* to human. In conclusion, our data indicates that camels may play a role in persistence of*Rickettsia*in Egypt. Thus, further investigations are warranted to better understand the epidemiological dynamics of *Rickettsia*; survivalwithin vector populations, host species, and the horizontal transmission between vector and host species.

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