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RESEARCH ARTICLE

First molecular detection of hemotropic *Mycoplasma* spp. and molecular screening of other vector-borne pathogens in camels from the greater Cairo metropolitan area, Egypt

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ABSTRACT

In Egypt, knowledge about vector-borne bacterial pathogens in camels remains limited. To address this gap, 181 blood samples from adult one-humped camels (*Camelus dromedarius*) in the greater Cairo metropolitan area were collected from October 2021 to March 2022. Through PCR assays, four pathogens were detected, where *Anaplasmataceae* being the most common (54.7%), followed by hemotropic *Mycoplasma* spp. (29.3%), *Rickettsia* spp. (12.2%), and *Coxiella burnetii* (1.7%). Comparative sequence analysis revealed novel findings, including: 1) the identification of two distinct hemotropic *Mycoplasma* spp., one closely related to bovine *Mycoplasma* sp. (*Mycoplasma wenyonii*), and the other closely related to porcine *Mycoplasma* sp. (*Candidatus* Mycoplasma haemosuis); and 2) the detection of *Anaplasma bovis* and *Anaplasma phagocytophilum*. Additionally, *Anaplasma platys*, *Rickettsia africae*, and *Coxiella burnetii* were identified as well. It's worth noting that these vector-borne pathogens possess zoonotic potential, emphasizing the need for adopting a "One Health" approach in Egypt to safeguard the well-being of both humans and animals.

Keywords: *Anaplasmataceae*; *Camelus dromedarius*; Egypt; hemotropic *Mycoplasma* species; vector-borne pathogens.

INTRODUCTION

Anaplasmosis is a tick-borne disease caused by gram-negative, intracellular bacteria of the genus *Anaplasma,* including *Anaplasma bovis*, *Anaplasma ovis*, *Anaplasma marginale*, *Anaplasma centrale*, and *Anaplasma phagocytophilum* (Dumler *et al.*, 2001). *Anaplasma bovis* infects bovine monocytes and is transmitted by *Hyalomma* sp., *Amblyomma* sp., and *Rhipicephalus* sp. ticks (Dumler *et al.*, 2001). On the other hand, *Anaplasma platys* infects canine platelets, causing infectious canine cyclic thrombocytopenia (ICCT), and it is transmitted by *Rhipicephalus sanguineus* (Dumler *et al.*, 2001; Ramos *et al.*, 2014). Moreover, *A. phagocytophilum*, transmitted by *Ixodes persulcatus*, causing granulocytic anaplasmosis in animals and humans (Dumler *et al.*, 2001; Bakken & Dumler, 2015). Earlier studies in Egypt identified cases of camel anaplasmosis using serology (Parvizi *et al.*, 2020; Alsubki *et al.*, 2022), and reported the detection of *A. marginale* DNA (Salman *et al.*, 2022; Mahmoud *et al.*, 2023; Soliman *et al.*, 2024), *A. platys*/*A. platys*-like (Abdullah *et al.*, 2021), and *Candidatus* Anaplasma camelii (Mohamed *et al.*, 2021; Soliman *et al.*, 2024).

Hemotropic *Mycoplasma* spp. (hemoplasmas), formerly known as *Haemobartonella* and *Eperythrozoon*, are gram-negative bacteria able to cause severe hemolytic anemia (Sykes, 2010). Initially, hemoplasmas were classified under the family *Anaplasmataceae* (Kreier & Ristic, 1981). However, subsequent phylogenetic analysis resulted in their reclassification under the family *Mycoplasmataceae* (Rikihisa *et al.*, 1997). Hemoplasmas have been reported among livestock (Suzuki *et al.*, 2011), wildlife (Maggi *et al.*, 2013), pet animals (Zarea *et al.*, 2023), and humans (Steer *et al.*, 2011). The difficulty in culturing hemoplasmas *in vitro* poses a challenge for developing specific serological assays. As a result, PCR assays stand as the preferred method for diagnosing hemoplasma infections (Willi *et al.*, 2007). Globally, hemoplasmas have been exclusively recorded in dromedary camels from the southern and northwestern areas of Iran (Sharifiyazdi *et al.*, 2018; Esmaeilnejad *et al.*, 2019).

Q fever, caused by *Coxiella burnetii*, is a highly infectious zoonotic disease that affects various hosts, with cattle, sheep, and goats acting as reservoirs. Ticks transmit *C. burnetii* through transstadial and transovarian routes, excreting the bacteria in large number in their feces, where the bacteria remain viable in

the environment for extended periods. Human infections occur through inhalation of contaminated aerosols from infected animal materials. Even areas with no recent animal contact can be affected due to wind dispersal (Eldin *et al.*, 2017). In Egypt, camels have shown seropositive to *C. burnetii* (Selim & Ali, 2020), and its DNA has been detected in ticks collected from camels (Ghoneim *et al.*, 2020; Soliman *et al.*, 2024).

Rickettsia spp. are obligate intracellular bacteria in the family *Rickettsiaceae*, order *Rickettsiales*, typically transmitted via arthropod bites (Parola *et al.*, 2013). Phylogenomic analysis classifies *Rickettsia* into five groups: Spotted Fever group I (SFGI), Spotted Fever group II (SFGII), Typhus group (TG), Canadensis group (CG), and Bellii group (BG), and are mainly identified in ticks. Besides ticks, *Rickettsia* species are also identified in mosquitoes, fleas, lice, and beetles (El Karkouri *et al.*, 2022). Molecular studies in Egypt detected *Rickettsia africae* and *Rickettsia aeschlimannii* in ticks collected from dromedaries (Abdel-Shafy *et al.*, 2012; Abdullah *et al.*, 2019; Soliman *et al.*, 2024). *R. africae*, the causative agent of African tick-bite fever (ATBF), is widespread in Africa and transmitted by *Amblyomma* sp. ticks, primarily *Amblyomma hebraeum* and *Amblyomma variegatum* (Delord *et al.*, 2014). The ATBF is a common cause of fever in travellers returning from sub-Saharan Africa (Leder *et al.*, 2013), presenting with fever, eschars, lymph node enlargement, and potential complications such as purpuric cellulitis, myocarditis, and neurological syndromes (Silva-Ramos & Faccini-Martínez, 2021).

Borreliosis, which includes Lyme borreliosis (LB) and relapsing fever (RF) transmitted by hard and soft ticks respectively (Cutler *et al.*, 2017), has limited data in Egyptian camels, with only one study detecting various *Borrelia* species (Ashour *et al.*, 2023). Bartonellosis, spread through arthropod bites and animal contact (Klangthong *et al.*, 2015), has not been extensively studied in Egyptian camels, with existing research showing no positive cases (Loftis *et al.*, 2006; Abdullah *et al.*, 2021). *Francisella tularensis*, the causative agent of tularaemia, is also poorly studied in Egyptian camels despite its potential zoonotic implications.

Vector-borne pathogens do not adhere to borders, underscoring the importance of preparedness for potential outbreaks. Inadequate surveillance leads to substantial investments of time and resources in establishing monitoring programs (Dórea *et al.*, 2016). Due to limited surveillance data on vector-borne pathogens in camels, our study aimed to identify potential vector-borne pathogens infecting camels in the greater Cairo metropolitan area. The findings of this study will enhance the understanding of the epidemiology of these pathogens in Egyptian camels.

MATERIALS AND METHODS

Sampling Sites and Sample Collection

We conducted a cross-sectional survey using a convenience sampling approach, collecting blood samples from 181 apparently healthy one-humped camels (*Camelus dromedarius*) from October 2021 to March 2022. Three sampling sites in Cairo and Giza Governates, Egypt, were selected: El-Basateen Abattoir (n = 19) in Cairo Governorate (30°00'08.9"N, 31°16'27.4"E), El-Waraq Abattoir (n = 32) (30°06'38.0"N 31°12'39.3"E), and Berkash Animal Market (n = 130) (30°08'56.8"N, 30°59'42.7"E) in Giza Governorate (Figure 1).

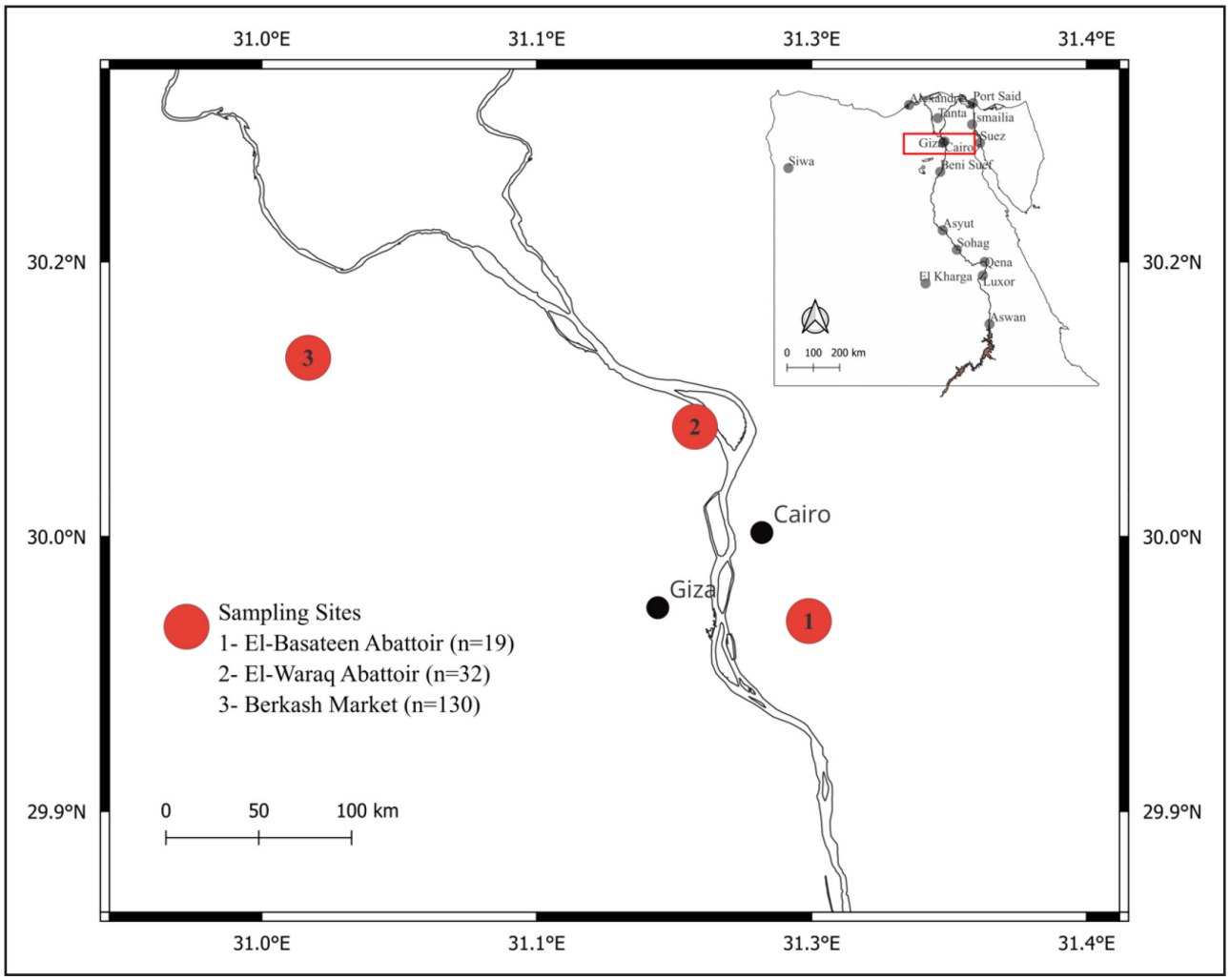

Figure 1. Egypt map showing sampling sites in this study.

We collected about 2 mL of blood in EDTA-coated tubes (BD Bioscience, Bergen County, NJ, USA). At abattoirs, blood was collected after incising jugular vessels, while at Berkash animal market, blood was withdrawn from jugular vessels using a syringe after careful camel restraint. We transported samples in an icebox to the Biotechnology Department, Animal Health Research Institute (AHRI), Dokki, Egypt, for further processing.

Genomic DNA Extraction

The genomic DNA was extracted from blood samples using QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instruction. In brief, we used 200 µL of whole blood for DNA extraction, and the final elution volume was adjusted to 60 µL. We assessed the quality and concentration of DNA using a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was stored at -30°C until use.

Selection of Pathogens for Analysis

We screened blood samples for the following pathogens: *Anaplasmatacea*, *Mycoplasma* spp., *C. burnetii*, *Rickettsia* spp., *Borrelia* spp., *Bartonella* spp. and *Francisella* spp., based on previously published reports (Abdel-Shafy *et al.*, 2012; Ghoneim *et al.*, 2017; Sharifiyazdi *et al.*, 2018; Abdullah *et al.*, 2021; Ashour *et al.*, 2023; Soliman *et al.*, 2024). Primer sets used in this study are listed in Table 1.

Polymerase Chain Reaction

Each PCR reaction was performed in a final reaction volume of 10 µL containing 5 µL of 2x Ampdirect® Plus (Shimadzu Corp., Kyoto, Japan), 0.05 µL of BIOTAQ™ HS DNA Polymerase (5 U/µL) (Bioline, London, UK), 0.3 µL of each primer (10 µM), 1.5 µL of template DNA, and 2.85 µL of UltraPure™ DNase/RNase-Free distilled water (Invitrogen, Waltham, MA, USA). Positive controls consisted of DNA samples previously confirmed for each pathogen, while negative controls included UltraPure™ distilled water in each PCR reaction. Thermal cycling conditions for each PCR reaction were retrieved from previous studies (Postic *et al.*, 1994; To *et al.*, 1996; Inokuma *et al.*, 2001; Zeaiter *et al.*, 2002; Criado-Fornelio *et al.*, 2003; Labruna *et al.*, 2004; Duzlu *et al.*, 2016). Subsequently, PCR products were electrophoresed on 1.5% agarose gel using a 100 bp DNA ladder.

Sequencing and Phylogenetic Analyses

We randomly selected at least 10% of positive samples for each pathogen for sequencing. Positive amplicons were purified using the NucleoSpin® Gel and PCR Clean-up kit (Macherey Nagel, Düren, Germany). The concentration of purified PCR product was measured with a NanoDrop™ 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Sanger sequencing was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequence reads were analyzed and trimmed using SnapGene® software (http://www.snapgene.com/), then assembled via MEGA X (Kumar *et al.*, 2018). Alignment against published sequences in GenBank was conducted using the BLAST search tool (https://blast.ncbi.nlm.nih.gov/Blast) to determine identity percentages. By determining the best DNA substitution model, phylogenetic analysis was performed using the maximum likelihood method with 1000 replications in MEGA X.

GenBank Accession Numbers

Accession numbers for the sequences obtained in this study were acquired by submitting coding DNA sequences via the BankIt tool (https://www.ncbi.nlm.nih.gov/WebSub/; accessed March 2024) and non-coding DNA sequences via the GenBank submission portal (https://submit.ncbi.nlm.nih.gov/subs/genbank/; accessed March 2024).

Statistical Analyses

In this study, we examined statistical associations between detected pathogens and background factors such as sampling sites, sex, and seasons. Data with low detection rates were excluded from analyses. We calculated *p*-values using Fisher's exact test or Pearson's chi-square test, considering $p < 0.05$ as statistically significant. Statistical analyses were conducted using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA).

RESULTS

Overall Detection Rates

In this study, we successfully detected the DNA of four pathogens. The most frequently detected pathogen was *Anaplasmataceae* (54.7%; 99/181), followed by *Mycoplasma* spp. (29.3%; 53/181), *Rickettsia* spp. (12.2%; 22/181), and *C. burnetii* (1.7%; 3/181). *Borrelia* spp., *Bartonella* spp., and *Francisella* spp., were not detected in this study.

Anaplasmataceae was detected in blood samples from all study sites, while *Mycoplasma* spp. was detected exclusively in samples from the Giza Governorate. Both *Anaplasmataceae* and *Mycoplasma* spp. were detected in male and female camels, as well as during both warm and cold months. *C. burnetii* and *Rickettsia* spp. were detected only in blood samples from the Berkash Market and solely

Table 1. Primer sets used for the detection of different vector-borne pathogens in camel blood samples

Pathogen	Gene	Assay	Primer Sequences (5'- 3')		Annealing temperature (bp)	Amplicon size	Reference
			Forward	Reverse(°C)			
Anaplasmataceae	16S rRNA	PCR	GGTACCTACAGAAGAAGTCC	TAGCACTCATCGTTTACAGC	52	345	(Inokuma *et al.*, 2001)
Coxiella burnetii	*htpB*	nested PCR	GCGGGTGATGGTACCACAACA	GGCAATCACCAATAAGGGCCG	56	501	(To *et al.*, 1996)
			TTGCTGGAATGAACCCCA	TCAAGCTCCGCACTCATG	52	325	
Rickettsia spp.	*gltA*	PCR	GCAAGTATCGGTGAGGATGTAAT	GCTTCCTTAAAATTCAATAAATCAGGAT	48	401	(Labruna *et al.*, 2004)
Borrelia spp.	5S-23S IGS	PCR	CTTAGTATAAGCTTTTATACAGC	ATAGGTCAGAAACTTGAATGATACA	52	226	(Postic *et al.*, 1994)
Bartonella spp.	*groEL*	PCR	GAACTNGAAGATAAGTTNGAA	AATCCATTCCGCCCATTC	54	1188	(Zeaiter *et al.*, 2002)
Mycoplasma spp.	16S rRNA	PCR	ATACGGCCCATATTCCTACG	TGCTCCACCACTTGTTCA	60	595	(Criado-Fornelio *et al.*, 2003)
Francisella spp.	16S rRNA	PCR	GCCCATTTGAGGGGGATACC	GGACTAAGAGTACCTTTTTGAGT	60	1166	(Duzlu *et al.*, 2016)

in male camels, with both pathogens detected exclusively during warm months. Statistical analysis revealed significant variability in the detection rates of *Mycoplasma* spp. among sampling sites ($p < 0.0001$), and a seasonal influence on the detection rate of *Rickettsia* spp. ($p < 0.05$) (Table 2).

Comparative Sequencing Analyses

In this study, we sequenced a total of sixteen samples from *Anaplasmataceae*, eight from *Mycoplasma* spp., five from *Rickettsia* spp., and one from *C. burnetii*. Accession numbers and the closest matches for sequences obtained in the current work are provided in Table S1. Briefly, *Anaplasmataceae* sequences displayed 100% shared identities with *Anaplasma* sp., while *Mycoplasma* spp. sequences exhibited shared identities ranging from 99.47% to 100% with bovine *Mycoplasma* species and from 99.82% to 100% with porcine *Mycoplasma* species. *Rickettsia* spp. sequences exhibited 100% similarity with *R. africae*. The *C. burnetii* sequence displayed 100% identity with published *C. burnetii* sequences.

Phylogenetic Analyses

In the phylogram of *Anaplasmataceae*, three distinct *Anaplasma* species were identified: the first species grouped with *A. platys* isolated from dogs in the Philippines, Malaysia, and India; the second species clustered with *A. phagocytophilum* isolated from humans in South Korea, while the third species clustered with *A. bovis* isolated from livestock and ticks in China (Figure 2), marking the first confirmed occurrence of *A. bovis* and *A. phagocytophilum* in camels from Egypt. Additionally, the phylogenetic analysis of *Mycoplasma* spp., represents another novel finding, unveiling two distinct species: one belonging to the haematominutum group, clustering with *Mycoplasma wenyonii* isolated from cattle in the Philippines, Brazil, Mexico, and Cuba, supported by a robust bootstrap value

Table 2. Detection rate of different vector-borne pathogens infecting camels based on sampling sites, sexes, and seasons

Parameters	Sampling Sites			Sex		Season	
	El-Basateen Abattoir (n = 19)	El-Waraq Abattoir (n = 32)	Berkash Market (n = 130)	Male (n = 173)	Female (n = 8)	Warm Months (n = 151)	Cold Months (n = 30)
Pathogen	Number of infected Camels (%)						
Anaplasmataceae	15 (78.9%)	19 (59.4%)	65 (50%)	94 (54.3%)	5 (62.5%)	80 (53%)	19 (63.3%)
Mycoplasma spp.	n. d.	4 (12.5%)***	49 (37.7%)***	52 (30.1%)	1 (12.5%)	42 (27.8%)	11 (36.7%)
Rickettsia spp.	n. d.	n. d.	22 (16.9%)†	22 (12.7%)	n. d.	22 (14.6%)*	n. d.
Coxiella burnetii	n. d.	n. d.	3 (2.3%)†	3 (1.7%)	n. d.	3 (2%)	n. d.
Borrelia spp.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Bartonella spp.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.
Francisella spp.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.	n. d.

n: number of examined camels; n. d.: not detected; ***: $p < 0.0001$; *: $p < 0.05$; †: Data excluded from the statistical analyses due to low detection rates; "Warm months" refer to the period from October to December 2021; "Cold Months" refer to the period from February to March 2022.

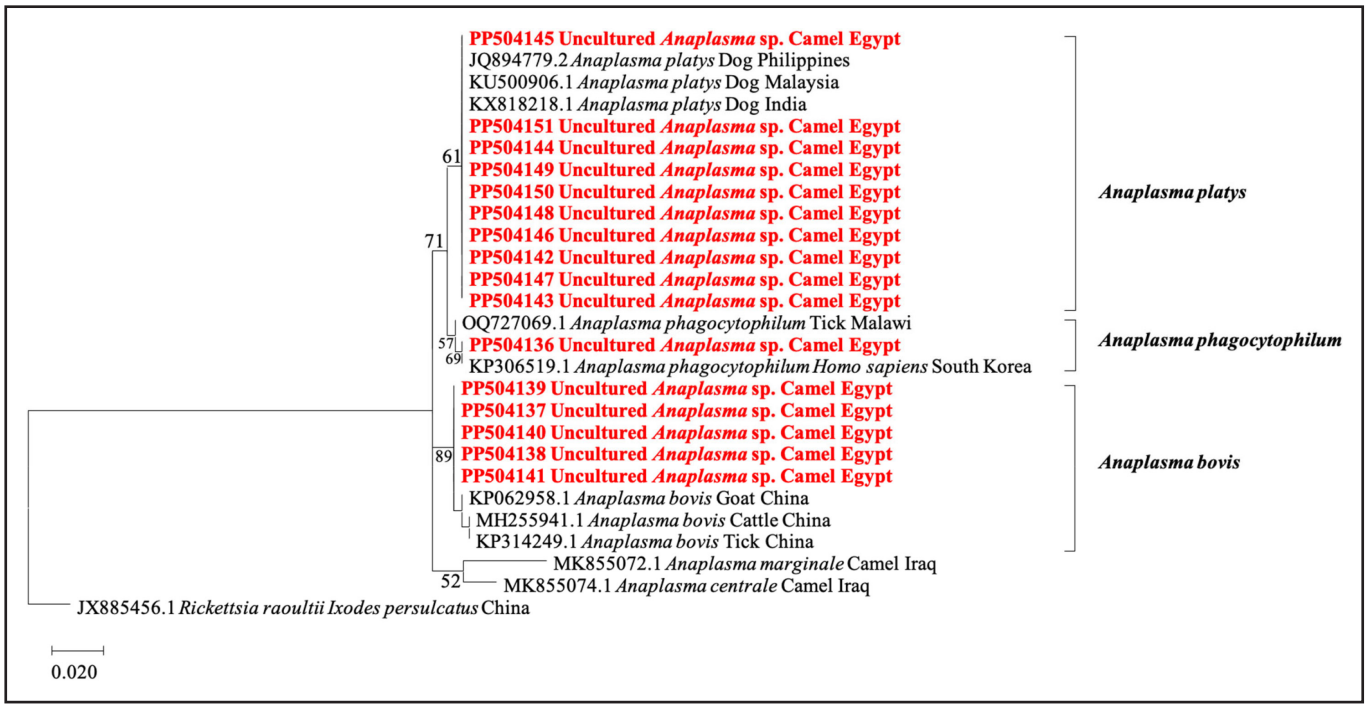

Figure 2. Phylogenetic analysis of *Anaplasmataceae* based on 16S rRNA gene. The analysis was inferred by the Maximum Likelihood method using Kimura 2-parameter model. This analysis was performed using the bootstrap analysis with 1000 replications. Sequences obtained in this study are highlighted in red boldface. *Rickettsia raoultii* (JX885456) was used as an outgroup.

of 86%, and the other from the haemofelis group, clustered with *Mycoplasma* sp. isolated from pigs in South Korea, Canada, and China, as well as camels from Iran, with a high bootstrap value of 99% (Figure 3). Furthermore, the phylogenetic analysis of *Rickettsia* spp. revealed that the sequences formed a monophyletic clade with *R. africae* isolated from ticks in South Africa, Liberia, Senegal, and Ethiopia (Figure 4). Finally, the *C. burnetii* sequence formed a monophyletic clade with *C. burnetii* isolated from ticks and livestock in African, European, and Asian countries, distinctly separated from *Coxiella*-like endosymbionts isolated from tick samples (Figure 5).

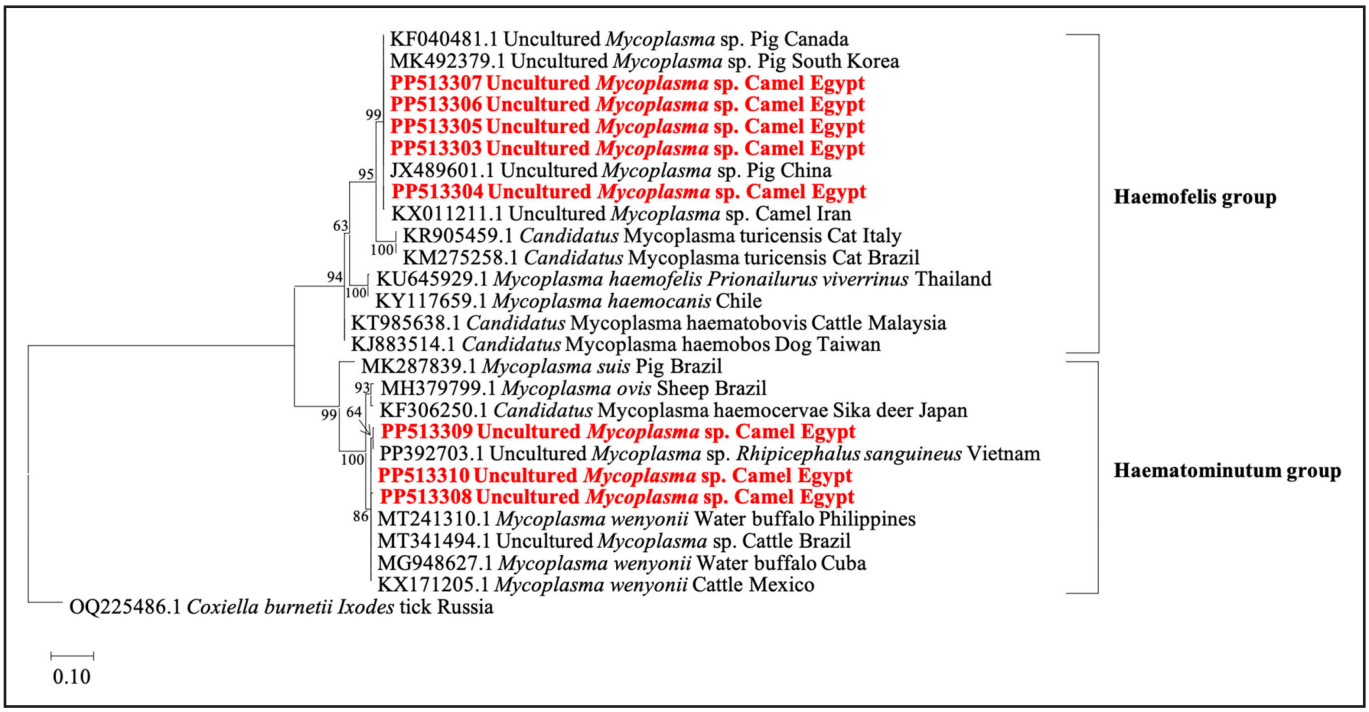

Figure 3. Phylogenetic analysis of *Mycoplasma* spp. based on 16S rRNA gene. The analysis was inferred by the Maximum Likelihood method using Kimura 2-parameter model with a discrete Gamma distribution This analysis was performed using the bootstrap analysis with 1000 replications. Sequences obtained in this study are highlighted in red boldface. *Coxiella burnetii* (OQ225486) was used as an outgroup.

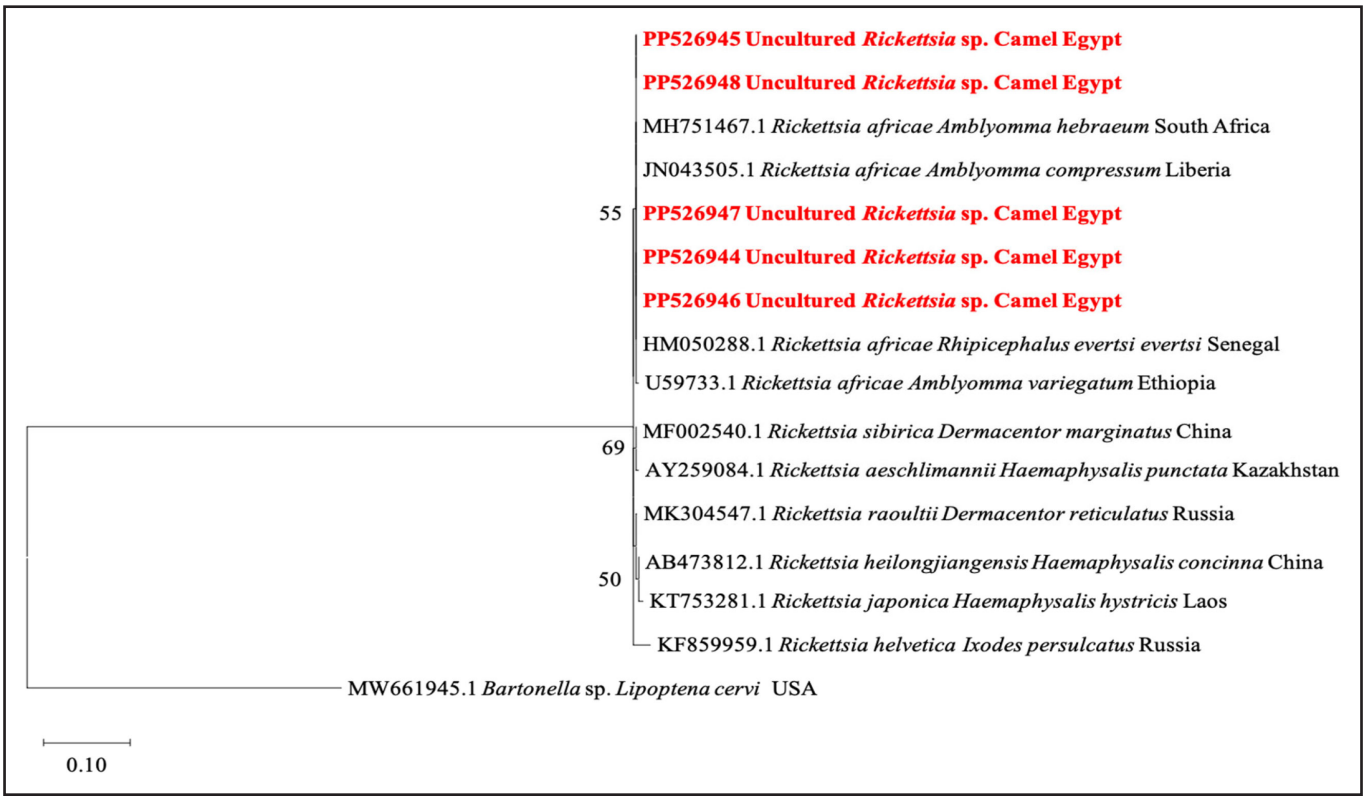

Figure 4. Phylogenetic analysis of *Rickettsia* spp. based on *gltA* gene. The analysis was inferred by the Maximum Likelihood method using Tamura 3-parameter model. This analysis was performed using the bootstrap analysis with 1000 replications. Sequences obtained in this study are highlighted in red boldface. *Bartonella* sp. (MW661945) was used as an outgroup.

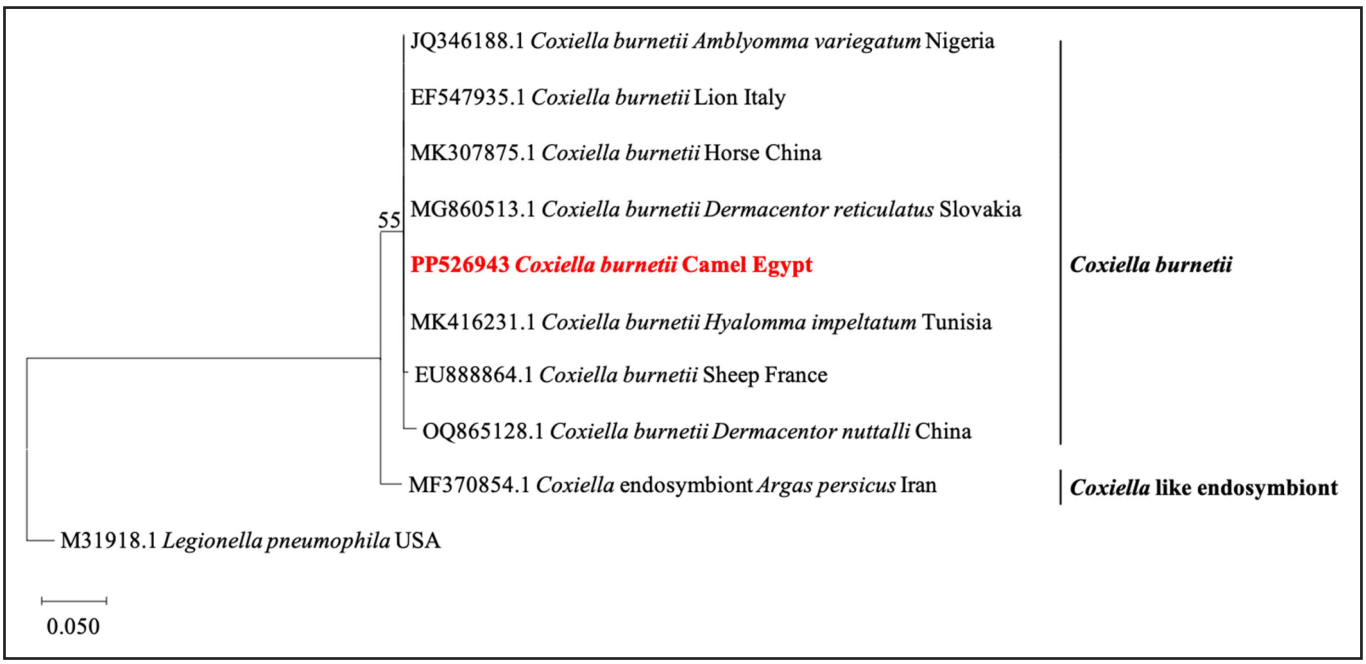

Figure 5. Phylogenetic analysis of *Coxiella burnetii* based on *htpB* gene. The analysis was inferred by the Maximum Likelihood method using Tamura 3-parameter model. This analysis was performed using the bootstrap analysis with 1000 replications. Sequences obtained in this study are highlighted in red boldface. *Legionella pneumophila* (M31918.1) was used as an outgroup.

DISCUSSION

This study revealed the first confirmation of *Mycoplasma* spp. infection in camels from Egypt. Comparative analysis of 16S rRNA sequences identified two distinct *Mycoplasma* species: one closely related to *M. wenyonii* of the haemominutum group and the other related to a porcine *Mycoplasma* species of the haemofelis group. The widespread bovine hemoplasma *M. wenyonii* (Tagawa *et al.*, 2008; Nouvel *et al.*, 2019; Altay *et al.*, 2022; Thongmeesee *et al.*, 2022; Erol *et al.*, 2023; Kamani *et al.*, 2023) causes bovine infectious anaemia, with clinical signs including anaemia without haemoglobinuria, limb and udder oedema, and reduced milk production (Nouvel *et al.*, 2019). Transmission routes of *M. wenyonii* are unclear, but it has been detected in blood-sucking arthropods (Hornok *et al.*, 2011; Song *et al.*, 2012; Thongmeesee *et al.*, 2022) and in calves born from infected cows (Hornok *et al.*, 2011; Sasaoka *et al.*, 2015). The porcine *Mycoplasma* species, named *Candidatus* Mycoplasma haemosuis, shares genetic similarities with *Candidatus* Mycoplasma turicensis (Fu *et al.*, 2017) and has been detected in pigs from China (Fu *et al.*, 2017), South Korea (Seo *et al.*, 2019), and Germany (Stadler *et al.*, 2020; Ade *et al.*, 2022). *Ca.* M. haemosuis is associated with fever, anemia, and skin alterations in pigs (Stadler *et al.*, 2020), and undergoes vertical transmission within pig herds (Ade *et al.*, 2022).

To our knowledge, *M. wenyonii* had not been previously detected in dromedary camels globally. However, this study represents the second instance of *Ca.* M. haemosuis detection among camels, with the first reported case from southern Iran (Sharifiyazdi *et al.*, 2018). Although previous research from Egypt reported camel infections with hemoplasmas (Eissa *et al.*, 2024), their findings lacked publication of *Mycoplasma* sequences, hindering validation. The detection of bovine and porcine hemoplasmas in camel blood samples suggests a lack of strict host specificity, consistent with reports of similar findings in other animal hosts (Zhuang *et al.*, 2009; Mascarelli *et al.*, 2016). The lack of detailed information regarding vectors and the pathogenic potentials of *Mycoplasma* species infecting camels necessitates intensive investigations which may be challenging due to the inability of *Mycoplasma* species to grow *in vitro*.

Anaplasmataceae was the most commonly detected pathogen in our study, present in 54.7% (99/181) of examined camels, a higher rate than previously reported by (Abdullah *et al.*, 2021) (6.7%; 10/149) and (Mohamed *et al.*, 2021) (29%; 29/100). Representative *Anaplasmataceae* sequences (n = 16) exhibited significant similarity and clustered with reference isolates of *A. platys*, *A. phagocytophilum*, and *A. bovis*. El-Baky and Allam (El-Baky & Allam, 2018) reported the identification of *A. phagocytophilum* and *A. bovis* in camels in Egypt, but their findings lacked publication of *A. phagocytophilum* and *A. bovis* representative sequences. Therefore, to our knowledge, our study represents the first phylogenetic analysis of *A. phagocytophilum* and *A. bovis* in camels in Egypt.

A. phagocytophilum has been sporadically reported in camels in Tunisia, Saudi Arabia, the United Arab Emirates, and China (Ben Said *et al.*, 2014; Alanazi *et al.*, 2020; El Tigani-Asil *et al.*, 2021; Zhao *et al.*, 2023), with human cases documented globally (Gaowa *et al.*, 2014; Lee *et al.*, 2018; Hing *et al.*, 2019). The potential of camels in transmitting *A. phagocytophilum* to uninfected tick vectors needs to be evaluated. Studies on *A. bovis* in camels are scarce, with variable detection rates across different regions (Belkahia *et al.*, 2015; Zhao *et al.*, 2023; Ma *et al.*, 2024). Recent findings suggest the zoonotic potential of *A. bovis* (Lu *et al.*, 2022, 2019), prompting further exploration into its role in human infections in Egypt. Some *Anaplasmataceae*-positive samples exhibited similarities to *A. platys,* originally recognized as a canine pathogen, which has been previously detected in dogs and livestock, including camels, in Egypt (Abdullah *et al.*, 2021; AL-Hosary *et al.*, 2021; Abdel-Shafy *et al.*, 2022; Hegab *et al.*, 2022), suggesting a broader host range than previously thought. The pathogenicity of *A. platys* in camels remains unknown. Zoonotic transmission of *A. platys* from dogs to humans has been documented in the USA (Breitschwerdt *et al.*, 2014), highlighting the need for continued investigation of this pathogen in Egypt.

In our study, *C. burnetii* was detected in 1.7% (3/181) of examined camels. The detection of this pathogen suggests its potential circulation within camel populations, thereby posing risks to both livestock and human health. The detection of *C. burnetii* among livestock poses a health hazard for slaughterhouse workers, who can become infected due to aerosol contamination

during slaughter procedures (Mioni *et al.*, 2020). Previous studies detected *C. burnetii* in 5.4% of ticks collected from camels in Egypt, including several ixodid tick species (*H. dromedarii*, *A. variegatum*, *H. anatolicum anatolicum* and *R. pulchellus) suggesting their involvement in epidemiology of C. burnetii* (Ghoneim *et al.*, 2020; Soliman *et al.*, 2024). Comparative sequencing analysis of the *htpB* gene of *C. burnetii* isolate revealed its clustering in a monophyletic clade with globally reported *C. burnetii* isolates, distinctly separated from *Coxiella*-like endosymbionts. This result suggests that *C. burnetii* detected in camels is likely zoonotic as well as pathogenic for livestock and humans. However, further studies are needed to elucidate this hypothesis.

Research on rickettsiosis in Egyptian camels is limited. Our study evaluated *Rickettsia* spp. infection rates, revealing a lower prevalence of 12.2% (22/181) compared to a previous report of 41% (25/61) (Abdullah *et al.*, 2019). Sequencing analysis of the *gltA* gene revealed the similarity of our isolates with *R. africae*, consistent with its previous detection in *Hyalomma* spp. ticks in Egypt (Abdel-Shafy *et al.*, 2012; Abdullah *et al.*, 2019). This result suggests the potential role of *Hyalomma* spp. ticks in maintaining *R. africae* circulation among camels in Egypt. Given the known human-biting behaviour of *Hyalomma* spp. ticks (Kassiri & Nasirian, 2021), there's a possibility of *R. africae* transmission to humans by these ticks, necessitating further investigation.

Our analysis revealed significant variations in *Mycoplasma* spp. cases across sampling locations, with a notable concentration in blood samples from the Berkash Animal Market, a prominent Center for animal trade in Egypt. This market, identified as a high-risk area for Rift Valley Fever Virus (RVFV) transmission (Napp *et al.*, 2018), serves as a potential hotspot for pathogen dissemination due to trade activities. In this study, *Rickettsia* spp. was only detected during warm months. Previous studies in Egypt have detected *Rickettsia* spp. in ticks of the genus *Hyalomma* (Abdullah *et al.*, 2019; Abdel-Shafy *et al.*, 2012). These ticks thrive in hot seasons, supported by prior observations in Egypt (Asmaa *et al.*, 2014; Hassan *et al.*, 2017; Essa *et al.*, 2022) and Iran (Nourollahi Fard *et al.*, 2012). Recent increases in the number of hot days per year in Egypt, as predicted by (Hamed *et al.*, 2022), suggest a progressively warmer climate, possibly leading to year-round tick activity. This could account for positive detections of *Anaplasmataceae* and *Mycoplasma* spp. in both warm and cold months despite no significant differences in detection rates.

This study faced limitations in obtaining a substantial sample size from female camels and lacked comprehensive data on management practices. Therefore, future research activities should encompass larger sample sizes spanning diverse geographical regions, including both male and female camels managed under various conditions. Such efforts are imperative for gaining a deeper understanding of the infection dynamics of vector-borne bacterial pathogens in Egyptian camels.

CONCLUSION

This study marks the first detection of bovine and porcine *Mycoplasma* species among camels in Egypt, along with the detection of *A. bovis* and *A. phagocytophilum*, urging deeper exploration of their health implications. Notably, *A. platys*, *A. bovis*, *A. phagocytophilum*, *R. africae*, and *C. burnetii* possess zoonotic potential, elevating the risk of interspecies transmission, particularly due to the close bond between pastoralists and their camels. Additionally, veterinarians and slaughterhouse workers face potential exposure to these zoonotic pathogens. This underscores the importance of adopting a "One Health" approach in Egypt, promoting collaboration between the veterinary and human health sectors to safeguard the well-being of both animals and humans.

Ethics approval and consent to participate

Animal owners were encouraged to participate in this study by providing them with detailed study objectives, and a verbal agreement was obtained before sample collection. All protocols for the use of animal samples were approved by Obihiro University of Agriculture and Veterinary Medicine (Permit ID: 22-23).

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper have no conflict of interest.

Data availability

All data generated in this study are included in this article and its supplementary file.

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