

**Original article****Open Access****Molecular detection of vector-borne bacterial pathogens in dromedary camels from Algeria***¹Bessas, A., ²Zaidi, S., ³Noui, A., ⁴Hezil, Dj., ²Zenia, S., and ⁵Bitam, I.¹Department of Biology, Benyoucef Benkhedda University of Algiers 1, 16000, Algiers, Algeria²Higher National Veterinary School, 16000, Algiers, Algeria³Institute of Veterinary Sciences, Ibn Khaldoun University, 14000, Tiaret, Algeria⁴Department of Biology, M'Hamed Bougara University, 35000, Boumerdes, Algeria⁵Higher School of Food Science and Agri-Food Industry (ESSAIA), 16000, Algiers, Algeria*Correspondence to: bessas.amina@gmail.com**Abstract:**

Background: In Algeria, little focus was placed on camels as hosts of tick-borne bacterial diseases. Recent studies showed a high prevalence of tick infestation in dromedaries. Transmission of various pathogenic microorganisms to camels by ticks imposes considerable economic losses to livestock and greatly impact on human and animal health. The aim of our study was to investigate the occurrence of vector-borne zoonotic bacteria in camels from Algeria.

Methodology: Blood samples were collected from 80 randomly selected camels in Laghouat province, southern Algeria. The samples were screened for *Anaplasma* spp, *Bartonella* spp, *Rickettsia* spp and *Coxiella burnetii* by qPCR. All positive samples were confirmed by standard PCR followed by sequencing. Data on age, sex, tick infestation and location of the camels were analyzed using the SPSS version 17.0 and association of these with vector-borne bacterial pathogens was determined using Chi-square (χ^2) test. P value lower than 0.05 was considered as indicative of significance.

Results: Twenty five of the 80 (31.3%) camels were positive to at least one vector-borne bacterial pathogen with *Anaplasma phagocytophilum* (22.5%, 18/80) being the most prevalent species, followed by *Anaplasma platys* (7.5%, 6/80) and *Bartonella dromedarii* (2.5%, 2/80). Only one camel was co-infected with two pathogens. All samples tested negative for *Rickettsia* spp and *Coxiella burnetii*. None of the factors (age, sex, tick infestation and study sites) was significantly associated with prevalence of vector-borne bacteria in the camels ($p > 0.05$).

Conclusion: The present study is the first report of anaplasmosis and bartonellosis in "*Camelus dromedaries*" from Algeria. Our results highlighted the need for further investigations on tickborne pathogens of camels.

Keywords: *Anaplasma phagocytophilum*, *Anaplasma platys*, *Bartonella*, tick borne bacteria, molecular detection, dromedary camels, Algeria

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Détection moléculaire des pathogènes bactériens à transmission vectorielle chez les dromadaires d'Algérie*¹Bessas, A., ²Zaidi, S., ³Noui, A., ⁴Hezil, Dj., ²Zenia, S., et ⁵Bitam, I.¹Département de Biologie, Université Benyoucef Benkhedda d'Alger 1, 16000, Alger, Algérie²Ecole Nationale Supérieure Vétérinaire, 16000, Alger, Algérie³Institut des Sciences Vétérinaires, Université Ibn Khaldoun, 14000, Tiaret, Algérie⁴Département de Biologie, Université M'Hamed Bougara, 35000, Boumerdes, Algérie⁵Ecole Supérieure des Sciences Alimentaires et des Industries Agroalimentaires (ESSAIA), 16000, Alger, Algérie*Correspondance à: bessas.amina@gmail.com

Résumé

Contexte: En Algérie, peu d'attention a été accordée aux chameaux en tant qu'hôtes de maladies bactériennes transmises par les tiques. Des études récentes ont montré une forte prévalence d'infestation par les tiques chez les dromadaires. La transmission de divers micro-organismes pathogènes aux chameaux par les tiques impose des pertes économiques considérables au bétail et a un impact considérable sur la santé humaine et animale. Le but de notre étude était d'étudier la présence de bactéries zoonotiques à transmission vectorielle chez les chameaux d'Algérie.

Méthodologie: Des échantillons de sang ont été prélevés sur 80 chameaux sélectionnés au hasard dans la province de Laghouat, dans le sud de l'Algérie. Les échantillons ont été criblés pour *Anaplasma* spp, *Bartonella* spp, *Rickettsia* spp et *Coxiella burnetii* par qPCR. Tous les échantillons positifs ont été confirmés par PCR standard suivie d'un séquençage. Les données sur l'âge, le sexe, l'infestation par les tiques et l'emplacement des chameaux ont été analysées à l'aide de la version SPSS 17.0 et l'association de celles-ci avec des pathogènes bactériens à transmission vectorielle a été déterminée à l'aide du test Chi-carré (χ^2). Une valeur P inférieure à 0,05 a été considérée comme significative.

Résultats: Vingt-cinq des 80 chameaux (31,3%) étaient positifs à au moins un agent pathogène bactérien à transmission vectorielle, *Anaplasma phagocytophilum* (22,5%, 18/80) étant l'espèce la plus répandue, suivie d'*Anaplasma platys* (7,5 %, 6/80) et *Bartonella dromedarii* (2,5%, 2/80). Un seul chameau a été co-infecté par deux agents pathogènes. Tous les échantillons ont été testés négatifs pour *Rickettsia* spp et *Coxiella burnetii*. Aucun des facteurs (âge, sexe, infestation par les tiques et sites d'étude) n'était significativement associé à la prévalence des bactéries à transmission vectorielle chez les chameaux ($p > 0,05$).

Conclusion: La présente étude est le premier rapport d'anaplasmose et de bartonellose chez les «*Camelus dromedaries*» d'Algérie. Nos résultats ont mis en évidence la nécessité de poursuivre les recherches sur les agents pathogènes transmis par les tiques chez les chameaux.

Mots clés: *Anaplasma phagocytophilum*, *Anaplasma platys*, *Bartonella*, bactéries transmises par les tiques, détection moléculaire, dromadaires, Algérie

Introduction:

Dromedaries are widespread throughout northern and eastern Africa. Algeria covers an area of 2,381,741 km², of which 87% is occupied by the Sahara where 381.882 camels live (1). One-humped camels "*Camelus dromedaries*" are an almost exclusively domesticated species that are common in arid areas. Many people of Sahara practice camel rearing for a livelihood. Camel breeding plays an important economic role in these areas, providing a wide diversity of goods (milk and meat, wool and skin) and services (agricultural activities, transport and tourism).

The hardiness of camels in arid regions has made humans more dependent on them. Despite close association between camels and humans, investigations on vector borne zoonotic bacterial pathogens infecting camels are scarce compared to other animals. In Algeria, most epidemiological surveys have focused on the research of trypanosomiasis in dromedaries (2-5). New data have confirmed that camels are susceptible to a wide range of pathogens, and can act as carriers or reservoirs of tick-borne bacterial diseases. Different *Anaplasma* species have been identified in camel populations by molecular tools such as *Anaplasma platys*, *Anaplasma ovis*, *Anaplasma phagocytophilum*, *Candidatus Anaplasma camelii* and novel genetic variants associated with *Anaplasma* strains in Iran, Morocco, Nigeria, Tunisia, Saudi Arabia, China and Pakistan (6-17).

Anaplasmosis are among emerging and potentially fatal arthropod-transmitted diseases for humans and animals (8). Camels

were found to be infected with a novel *Bartonella* spp named *Bartonella dromedarii* (18, 19). However, the epidemiological and public health importance of *Candidatus Bartonella dromedarii* in camels is not clear. *Bartonella* species infect a wide range of domestic and wild mammals. Several *Bartonella* spp are being recognized as important zoonotic pathogens that occur worldwide with presentations that range from subclinical to severe disease (20).

Camels may also be exposed to spotted fever group (SFG) *Rickettsia* spp because they may show a heavy burden of ixodid ticks. The occurrence of *Rickettsia* species such as *Rickettsia aeschlimannii*, *Rickettsia africae* and *Rickettsia sibirica mongolitimonae* in Hyalomma ticks collected from camels have been reported (21-24). In addition, *Rickettsia* spp and *Rickettsia aeschlimannii* DNA were detected in the blood of camelids (24,25). In Algeria, the detection of *Rickettsia aeschlimannii* and *Rickettsia africae* have been identified only in ticks of camels (26,27). *Coxiella burnetii* have been detected in Hyalomma tick species from Tunisia and Algeria, which raises the possibility of the involvement of this arthropod vector in the active diffusion of these bacteria among camels, other domestic animals and humans (28, 29). Moreover, previous molecular research indicated that both camels and ticks could be sources for Q fever in Egypt (30).

The most remarkable findings of some serological studies were the high antibody prevalence against *Rickettsia* spp, *Anaplasma* spp and specially *Coxiella burnetii* (25,31-36). In the south-eastern part of Algeria, a high seroprevalence of Q fever has been reported am-

ong healthy camels (29,37) but little is known about these diseases among camels in Algeria. Therefore, the present study aimed to investigate the occurrence of vector-borne bacteria (*Anaplasma* spp, *Rickettsia* spp, *Coxiella burnetii* and *Bartonella* spp) in camels from Algeria by molecular methods.

Materials and methods:

Study area

The study was carried out in Laghouat city, Algeria. Laghouat is located at the boundary between the high steppe plateaus and the Saharan region, 400 km from the Mediterranean coast (Fig. 1) at 751 m above sea level and at a latitude of 33°47' 59" North and a longitude of 2° 52' 59" East. It spreads over an area of 400 km² (38).

Study animal and sampling

A total of 80 randomly selected dromedary camels (9 males, 71 females) reared in the surroundings of the city of Laghouat served as the study animal. These one-humped camels "*Camelus dromedaries*" were apparently healthy animals belonging to 3 different herds (Fig. 1). Whole-blood samples were collected from the jugular vein of each camel using a sterile needle and immediately placed into EDTA tubes, which were transferred to an ice-box for transport, and eventually stored at -20 °C for molecular analyses.

Animal examination and data collection

All sampled animals were restrained with the help of their owners and handled humanely. Physical examination was carried out on each animal and information provided by the owners included age and sex of camels, and place of residence. The presence or absence of ectoparasites was noted.

DNA extraction

DNA was extracted from 200 µL of blood using the DNeasy Blood and Tissue Kit (Qiagen®, Hilden, Germany), according to the manufacturer's instructions. The genomic DNA was stored at -20°C under sterile conditions until used as a template in PCR assays.

PCR amplification and sequencing

To investigate the presence of vector-borne bacteria in the blood sample from camels, extracted DNA was initially tested for detection of pathogens with the real time PCR (rt-PCR) assay, and results were deemed positive if the cycle threshold (Ct) value was lower than 32. Positive samples on rt-PCR were then confirmed by conventional PCR and sequencing of the PCR products.

For *Anaplasma* spp, all samples were screened by qPCR targeting the 23S rRNA gene, and those positive were amplified by conventional PCR and the PCR products sequenced using the 23S standard PCR system as described elsewhere (39). For molecular detection and identification of *Bartonella*, genus-specific qPCR was based on the 16S-23S rRNA intergenic transcribed spacer (*ITS* gene). Positive samples were confirmed by standard PCR and sequencing of the partial sequence of the citrate synthase gene (*gltA*) as previously described (40). The blood was also subjected to qPCR with *Coxiella burnetii*-specific primers and probes to amplify the *IS1111* gene and *IS30A* spacers as previously described (41). Similarly, all extracted DNA were assessed for the presence of rickettsial DNA using *Rickettsia* genus-specific qPCR for spotted-fever group (SFG) Rickettsiae by targeting the *gltA* gene (RKND03 system) (41).

In all the experiments, distilled water was included as negative control. The positive

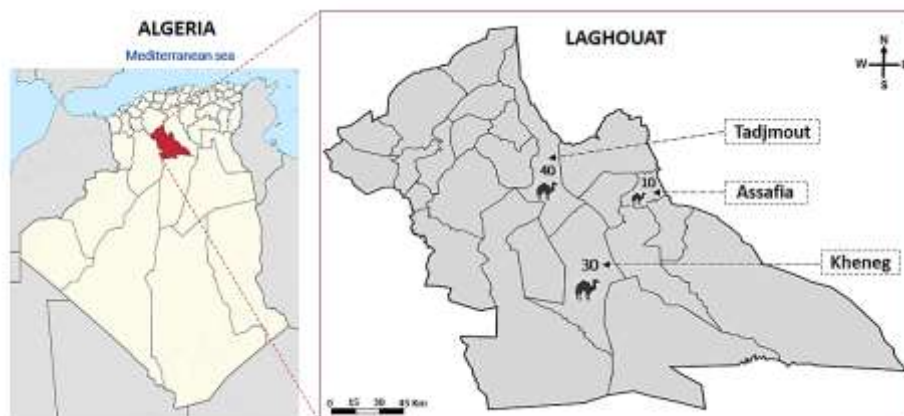


Fig. 1: A map of Laghouat city showing study sites and the number of sampled camels

controls included DNA extracted from a dilution of cultured strains of *Anaplasma phagocytophilum* (for the detection of *Anaplasma* spp), *Bartonella elizabethae* (for the detection of *Bartonella* spp), *Coxiella burnetii* (for the detection of *Coxiella burnetii*) and *Rickettsia montanensis* (for the detection of *Rickettsia* spp).

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago, IL). Association of variables (sex, age, tick infestation and location of the animals) with the prevalence of vector-borne bacterial pathogens was done using Chi-square (χ^2) test. P values lower than 0.05 were considered as indicative of significance.

Results:

Following qPCR assay, 24 (30.0%) of the 80 camels were positive for *Anaplasma* spp, and 2 (2.5%) were positive for *Bartonella* spp. Only 1 (1.3%) camel was co-infected with these two agents (Table 1). All surveyed camels were negative for *Coxiella burnetii* and *Rickettsia* spp DNA. Sequencing and BLAST analyses of *Anaplasma* spp showed 100% identity with several genotypes of *Anaplasma phagocytophilum* (GenBank accession numbers CP015376.1, CP006618.1, NR_076399.1 etc) and 100% identity with *Anaplasma platys* (GenBank accession no. CP046391.1). A BLAST search of *gltA* sequences identified *Bartonella* strains which were very close to a newly proposed species, *Bartonella dromedarii* (99.59% similarity with GenBank accession numbers KJ909817.1, KJ909815.1, KJ909814.1 etc).

None of the factors (age, sex, tick infestation and location sites of camels) was significantly associated with the prevalence of

vector-borne bacteria pathogens in the camel ($p>0.05$). Table 2 represents the molecular studies showing the prevalence of vector-borne bacterial pathogens in camel across the world.

Discussion:

The objective of the current study is to determine epidemiological role of camels and their zoonotic potential in the transmission of vector-borne bacteria pathogens in Algeria. In total, 31.3% of the dromedary camels studied were positive for at least one agent, including one animal co-infected with *Anaplasma platys* and *Bartonella* spp. In this study, the association between occurrence of these bacteria pathogens in the camels and selected factors (age, sex, tick infestation and study sites) was not statistically significant ($p>0.05$).

To the best of our knowledge, this study is the first report of camel anaplasmosis in Algeria. A total of 24 (30.0%) of 80 camels studied were positive to *Anaplasma* spp, 18 (22.5%) were identified as *Anaplasma phagocytophilum* and 6 (7.5%) as *Anaplasma platys*. The DNA of *A. phagocytophilum* and *A. platys* were previously identified in bovine blood samples in Algeria (39). *Anaplasma platys* infects mainly dogs, and canine infections with this agent have also been reported in Algeria by molecular methods (42,43). Furthermore, antibodies against *A. phagocytophilum* were found in dogs and horses from Algeria (43,44). In previous studies, the PCR prevalence of *A. platys* ranged from 3.33% to 61.11% among camels in many countries (13,15,45,46), and this agent was also detected in Rhipicephalus ticks collected from Bactrian camels in China (15).

Concerning *A. phagocytophilum*, very few studies have reported its occurrence in dromedaries. PCR analyses showed that 34.2%

Table 1: Prevalence of vector-borne bacterial pathogens with respect to demographic characteristics of camels

Camels	No sampled	Total of positive results	OR (or χ^2)	p-value	<i>Anaplasma phagocytophilum</i>	<i>Anaplasma platys</i>	<i>Bartonella dromedarii</i>
Sex							
Male	9	4 (44.4)	1.503	0.45	1 (11.1)	*2 (22.2)	*2 (2.5)
Female	71	21 (29.5)			17 (23.9)	4 (5.6)	0
Age group (years)							
1-3	17	8 (47)	2.975 ⁺	0.2259	6 (35.3)	2 (11.7)	0
4-9	40	12 (30)			9 (22.5)	3 (7.5)	1 (2.5)
≥ 10	23	5 (21.7)			3 (13)	1 (4.3)	1 (4.3)
Tick infestation							
No	5	0	0.18	0.317	0	0	0
Yes	75	25 (33.3)			18 (24)	6 (8)	2 (2.5)
Study sites							
Kheneq	30	9 (30)	1.92 ⁺	0.3829	5 (16.6)	3 (10)	2 (6.6)
Assafia	10	5 (50)			5 (50)	0	0
Tadjmout	40	11 (27.5)			8 (20)	3 (7.5)	0
Total	80	25 (31.3)			18 (22.5)	6 (7.5)	2 (2.5)

*One camel coinfecting by *Anaplasma platys* and *Bartonella dromedarii*.; OR=Odds ratio; + = Chi-square (χ^2)

Table 2: Prevalence of vector-borne bacterial pathogens in camel from molecular studies around the world

Pathogens	Country	Number of animal tested	Number of animal positive	Prevalence (%)	Molecular assay used	Reference
Anaplasma spp						
<i>(Candidatus Anaplasma Camelii)</i>	Saudi Arabia	100	26	26.0	PCR/Sequencing	16
	Iran	100	6	6.0	PCR/Sequencing	50
	Morocco	106	42	39.6	PCR/Sequencing	14
	Iran	200	30	15.0	PCR/Sequencing	8
	Kenya	249	171	68.7	PCR/high-resolution melting (PCR-HRM)/Sequencing	51
	Nigeria	176	71	40.3	PCR/Sequencing	52
	Kenya	296	233	78.7	PCR/high-resolution melting (PCR-HRM)/Sequencing	53
<i>Anaplasma platys</i>	China	279	20	7.2	PCR/Sequencing	15
	Nigeria	36	22	61.1	PCR/reverse line blotting (PCR-RLB)	13
	Saudi Arabia	170	9	5.3	PCR/Sequencing	45
	Iran	60	2	3.3	PCR/Sequencing	46
Novel <i>Anaplasma</i> sp. strains genetically related to <i>A. platys</i>	Tunisia	226	40	17.7	PCR/Sequencing	17
<i>Anaplasma phagocytophilum</i>	Iran	207	71	34.2	Polymerase chain reaction (PCR)/nested PCR	11
	Saudi Arabia	170	1	0.6	PCR/Sequencing	45
<i>Anaplasma ovis</i>	Tunisia	412	5	1.2	PCR/Sequencing	6
Novel Genetic Variants Associated to <i>Anaplasma ovis</i>	Iran	100	2	2.0	PCR/Sequencing	10
Bartonella spp						
<i>Bartonella henselae</i>	Tunisia	412	15	3.6	PCR/Sequencing	48
<i>(Candidatus Bartonella dromedarii)</i>	Iran	106	18	17.0	PCR/Sequencing	19
Rickettsia spp						
<i>R. africae</i>	Egypt	61	25	41.0	PCR/Sequencing	54
Rickettsial species	Tunisia	293	8	2.7	PCR/Sequencing	24
<i>R. aeschlimannii</i>			2	0.7		
<i>R. monacensis</i>			4	1.4		
<i>R. helvetica</i>			1	0.3		
<i>R. massiliae</i>			1	0.3		
<i>R. africae</i>			1	0.3		
Coxiella burnetii						
	Iran	130	14	10.8	PCR	55
	Saudi Arabia	82	13	15.9	PCR	56
	Egypt	113	52	46.0	PCR/Sequencing	30
	Tunisia	412	0	0	PCR	28
	Algeria	184	0	0	qPCR	29
	Kenya	296	10	3.4	PCR/high-resolution melting (HRM) analysis/Sequencing	53

(71/207) of camels harbored *A. phagocytophilum* in Iran (11). More recently, DNA of this pathogen was identified in only one camel among 170 tested (0.6%) in Saudi Arabia (45). In Tunisia, seropositivity to *A. phagocytophilum* was detected in camels by indirect immunofluorescence (IFA) (12). *Anaplasma phagocytophilum* is an emerging tick-borne zoonotic pathogen of increased interest worldwide which has medical as well as veterinary importance. This bacterium is the causative agent of human granulocytic anaplasmosis associated with high mortality in humans. *Anaplasma platys* could also possibly have a zoonotic potential (47). Anaplasmosis has been described to be a subclinical disease in Arabian one-humped camels (*Camelus dromedarius*) (15). The presence of dogs in the camel farms explains the positivity of the camels to anaplasmosis in our study. In addition, all of the positive camels in our study harbored ticks. Therefore, they can be directly affected by tick-borne pathogens and can play the role of reservoir for further transmission to humans and other animals. New species of *Anaplasma* have been identified infecting dromedaries in other studies (8,14,16).

The DNA of *Bartonella* spp was found in only two camels with a prevalence of 2.5%. Sequence comparisons analysis showed a high homology (99.59%) to *Bartonella dromedarii*. In a previous study, Rasis and colleagues (18) confirmed the presence of a novel species of *Bartonella* in camelids, which has been named *Bartonella dromedarii* sp. Nov. Lately, *Candidatus Bartonella dromedarii* was identified in camels from Iran (19) with a prevalence of 17% (18/106). More studies are required to evaluate precisely the risk factors, transmission routes, and the ability to infect humans of this *Bartonella* species. Other studies reported the presence of two species of *Bartonella* (*B. rochalimae* and *B. bovis*) in *Hyalomma dromedarii* ticks collected from camels (20). A recent study reported the occurrence of novel *B. henselae* genotypes closely related to those isolated from humans in Tunisian dromedaries (48). The identification of new *Bartonella* variants in camels suggests a continuous evolution of strains' diversity which is related to a complex maintenance of this bacterium in nature, as was observed in other mammals (49). The camels in our study could have been in contact with wild canids and felids as well as rodents which are the animals most frequently found in the Algerian desert.

In the present study, all tested samples yielded negative results for *Rickettsia* spp and *Coxiella burnetii*. Nevertheless, these findings do not exclude the presence of these agents in camels since we worked on a small sample and in only one region. Limited resources, low levels of regulation, poor hygiene,

high mobility of animals and herders, heavy infestation by ectoparasites, the close human to camel contact and lack of consistent veterinary care can justify the probable role of camelids as a significant source for zoonotic diseases in Algeria.

Conclusion:

We report here the first molecular detection of *Anaplasma* spp and *Bartonella* spp in dromedary camels suggesting a possible involvement of camelids as hosts or reservoirs in the transmission cycle of these agents in arid and Saharan areas in Algeria. Therefore, further extensive molecular surveys on large number of samples covering many localities in the country are needed in order to correctly address the prevalence and geographical distribution of vector-borne and zoonotic diseases in camels. This in turn will help in designing and implementation of effective preventive and control measures.

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