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Molecular evidence of vector-borne pathogens in dogs and cats and their ectoparasites in Algiers, Algeria

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A B S T R A C T

In Algeria, only limited information is currently available on the prevalence of emergent canine and feline vector-borne diseases. The aim of the present work was to detect by qPCR vector-associated bacteria in stray dogs and cats and their ectoparasites from Algiers.

18/117 (15.38%) dogs and 2/107 (1.87%) cats were positive for at least one vector-borne agent. *Coxiella burnetii* and *Bartonella henselae* were identified in 1/117 (0.85%) dog individually. *Ehrlichia canis* DNA was detected in 17/117 (14.52%) dogs. 1/107 (0.93%) cat was positive to *C. burnetii* and another 1/107 (0.93%) to *B. henselae*.

DNA of *Rickettsia massiliae*, *Rickettsia conorii* and *E. canis* was detected in *Rhipicephalus sanguineus*. Cat fleas were infected with *Rickettsia felis*, *B. henselae* and *Bartonella clarridgeiae*. *B. vinsonii* subsp. *berkhoffii* was identified in *Xenopsylla cheopis* collected from dogs.

The findings of this study indicate that dogs and cats from Algeria are exposed to multiple tick and flea-borne pathogens.

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1. Introduction

Vector-borne agents are increasingly recognized as important causes of morbidity and mortality in humans and domestic animals worldwide [1,2]. Companion animals, such as dogs and cats, are potential victims, reservoirs and/or sentinels of various vector-borne pathogens [3]. They are exposed to several arthropod species which are incriminated in the transmission cycles of many pathogens [4,5].

Coxiella burnetii is recognized as a worldwide zoonotic pathogen that causes Q fever [6]. Recently, many authors have highlighted the role of pets in the epidemiology of Q fever, indicating that contact with infected dogs and cats represents a risk factor for acquiring the infection [6–9]. In Algeria, *C. burnetii* infection in humans has been rarely reported [10], however no published data exist concerning the prevalence of *C. burnetii* in animals in that country.

Ehrlichia canis is a bacterium belonging to the *Anaplasmataceae* family that causes canine monocytic ehrlichiosis [2]. The disease was first described in sick dogs from Algeria, in 1935 [11]. More recently, two studies have indicated the molecular presence of *E. canis* in dogs in this country [12,13]. Recent research has demonstrated that domestic cats can also be efficient hosts of *E. canis* [14,15].

Bartonella species are emerging infectious organisms that have recently been documented in a broad range of domestic and wild mammals. In Algeria, a high prevalence of infective endocarditis is caused by *Bartonella quintana* in humans [16] and different species of *Bartonella* have been detected in fleas [17]. Investigation into the diversity of *Bartonella spp.* in Algerian reservoir animals was previously performed. To date, five *Bartonella* species (*B. vinsonii* subsp. *berkhoffii*, *B. clarridgeiae*, *B. elizabethae*, *B. rochalimae* and *B. henselae*) have been detected infecting dogs [13,18] and only one species (*B. henselae*) has been described in cats [19]. Furthermore, *Bartonella* spp. has been identified in hedgehogs (*Atelerix algirus*) and Rodents [20].

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Rickettsioses are among the oldest known vector-borne diseases. Mediterranean Spotted Fever caused by *Rickettsia conorii conorii* is endemic in Algeria [21]. Over the past ten years, thanks in particular to the use of entomological approaches, other *Rickettsia spp*, including human pathogens have been detected in ticks and fleas from Algeria [22,23]. Dogs have been considered as potential sentinels and reservoirs for *R. conorii* [24]. Cats are also involved in the cycle of SFG rickettsiae and *R. typhi*, the agent of murine typhus [25,26].

Borrelia burgdorferi sensu lato is a group of spirochete bacterial species, some of which cause lyme borreliosis, especially in humans and dogs [27]. Cats were reported to be susceptible to the infection with this agent [28]. Recently, a high rate of seropositivity for *Borrelia burgdorferi s.l.* was found in dogs from Algiers [13].

To date, information about vector-borne diseases agents circulating in Algeria remains limited. The aim of the present study was thus to assess the presence of bacteria (*C. burnetii*, *E. canis*, *Bartonella* spp., *Rickettsia* spp. and *Borrelia* spp.) of veterinary and zoonotic significance in stray dogs and cats and their ectoparasites from Algiers using rapid specific molecular tests.

2. Materials and methods

2.1. Ethic statement

Risk assessment was submitted to and approved by the ethics committee and decision board of Hygiène Urbaine d'Alger (HURBAL). HURBAL is an institution affiliated with the Algerian Ministry of the Interior, the Local Government and the Algerian Ministry of Agriculture and Rural Development. HURBAL, by decision of the Ministry of the Interior and in the context of the National Program for Rabies Control, in which the authors of the paper are not involved, was the agency which captured stray dogs and cats from Algiers. Once captured, the stray animals are housed in cages, being euthanized after expiration of the legal waiting time (7 days, in order to permit owners to claim their pets).

To facilitate fieldwork, collaborations were established with veterinary doctors and their assistants working in this establishment.

2.2. Sample collection

Between October 2010 and September 2013, spleens were collected from stray dogs and cats living in the city of Algiers, Algeria. Sampling was conducted in a room dedicated to and equipped for veterinarian activities. A necropsy was performed immediately following euthanasia of the animals. Spleen fragments were collected aseptically and stored in 70% ethanol.

The age of each animal was estimated, based on dentition and physical aspect. Information concerning sex, breed and the presence of ectoparasites was noted. Ticks, fleas and lice were collected and stored in 70% ethanol solution for later identification by genus and/or species using standard taxonomic morphological keys [1,29,30]. Dogs and cats were classified as apparently healthy or sick based on their physical condition at the time of sampling (Table 1). All samples were later processed at the National Reference Center for Rickettsial Diseases in Marseille, France.

2.3. DNA extraction

The specimens (ectoparasites and spleens) conserved in ethanol were rinsed twice for 5 min in distilled water. All experiments were conducted in a laminar flow cabinet. Each sample was incised using an individual scalpel and crushed in sterile tubes (Eppendorf; Hamburg, Germany). A total of 100 μL of DNA was extracted using the QIAamp Tissue Kit (Qiagen, Hilden, Germany) by QUIAGEN-BioRobot EZ1, according to the manufacturer's instructions. Genomic DNA was stored at −20 °C under sterile conditions until used as a template in PCR assays. The remaining piece of spleen and the ectoparasites were kept at −80 °C for additional control.

2.4. Detection of bacteria

Extracted DNA was used in qPCR amplifications to detect *C. burnetii*, *E. canis*, *Bartonella* spp, *Rickettsia* spp. and *Borrelia* spp. The final qPCR reaction mixture consisted of (5 μL) of DNA extracted with (15 μL) of mix from the Takyon PCR Kit (QIAGEN, Hilden, Germany) as previously described [31].

Table 1
Detected pathogens in dogs and cats from Algeria, as determined by quantitative PCR and information relative to positive animals.

Animal n°	Animal species	Age	Sex	Breed	Clinical status	Presence of ectoparasites	qPCR results
1	Canine	<1 year	F	Mixed-breed	H	Tick and fleas	*E. canis*
2	Canine	30 months	M	Mixed-breed	H	Tick[a] and fleas	*E. canis*
3	Canine	<1 year	F	Mixed-breed	H	Tick[a] and fleas	*E. canis*
4	Canine	4 months	M	Mixed-breed	S	Tick, fleas and lice	*E. canis*
5	Canine	8 years	M	*American Staffordshire*	S	Tick and fleas	*E. canis*
6	Canine	4 months	M	Mixed-breed	H	Tick[b] and fleas	*E. canis*
7	Canine	7 months	F	German Shepherd	H	Tick[c] and fleas	*E. canis*
8	Canine	1 year	M	Shepherd crosses	S	Tick and fleas	*E. canis*
9	Canine	18 months	M	Mixed-breed	H	–	*E. canis*
10	Canine	<1 year	F	Mixed-breed	H	Tick[b] and fleas	*E. canis*
11	Canine	–	F	Mixed-breed	H	–	*E. canis*
12	Canine	2 years	M	Mixed-breed	H	-	*E. canis*
13	Canine	1 year	F	Mixed-breed	H	Tick and fleas	*E. canis*
14	Canine	–	F	Mixed-breed	H	–	*E. canis*
15	Canine	3 years	M	Mixed-breed	H	–	Co-infection with *E. canis* and *C. burnetii*
16	Canine	–	M	Mixed-breed	H	–	*E. canis*
17	Canine	–	F	Mixed-breed	H	–	*E. canis*
18	Canine	18 months	M	Shepherd cross	H	Tick and fleas	*B. henselae*
19	Feline	2 years	M	Mixed-breed	H	–	*B. henselae*
20	Feline	<1 year	M	Mixed-breed	H	–	*C. burnetii*

M = male; F = female; H = healthy; S = sick.
[a] *Rh. sanguineus* positive by qPCR to *E. canis* and *R. massilae*.
[b] *Rh. sanguineus* positive by qPCR to *E. canis*.
[c] *Rh. sanguineus* positive by qPCR to *R. massiliae*.

Negative controls for all PCR assays consisted of DNA extracted from laboratory colonies of uninfected ticks. Positive controls incorporated DNA extracted from a diluted strain of *C. burnetii*, *E. canis*, *B. elizabethae*, *B. henselae*, *R. montanensis*, *R. typhi*, *R. conorii*, *R. massilae*, *R. felis* and *Borrelia burgdorferi* cultured in our laboratory in Marseilles. Results were recorded as positive when the cycle threshold (Ct) was lower than 36.

All samples were screened for *C. burnetii* DNA using IS30a spacers [32]. *C. burnetii*-positive samples were then confirmed by another qPCR system targeting the IS1111 [32].

For *E. canis*, the DNA extracts from spleens and ticks were amplified using a qPCR primer and probe combination based on the Ecaj 0701 gene encoding the glutaredoxin-related protein for detection of *E. canis* as previously described [33].

Molecular detection and identification of *Bartonella* genus-specific qPCR was based on the 16S-23S rRNA intergenic transcribed spacer (ITS gene) [34]. Positive samples were subsequently analyzed by a second qPCR specific for *B. henselae* targeting the heme-binding protein gene, Pap31 [35].

Samples that were qPCR-positive for *Bartonella* DNA by the ITS primers and negative for qPCR specific for *B. henselae* were then confirmed by standard PCR performed with *Bartonella*-specific primers for the citrate synthase gene (*gltA*) [34].

PCR products were purified and sequenced with *gltA* primers as described previously [34]. All obtained sequences were assembled and edited using ChromasPro (version 1.7.7). The sequences were then analyzed by Basic Local Alignment Search Tool (BLAST) and compared with sequences available in the GenBank database.

Using qPCR, DNA samples were screened for all spotted fever group rickettsiae (SFG) by targeting a partial sequence of the citrate synthase gene (*gltA*), RKND03 system [32].

R. massiliae and *R. conorii*-specific qPCR were conducted on positive tick DNA samples as previously described [31,36].

The fleas which were positive for *Rickettsia* spp. at the first screening were subjected to *R. felis*-specific qPCR amplification of the membrane phosphatase gene [34]. Typhus group (TG) was also tested using specific primers and an *R. typhi* probe targeting the Rpr 274 gene [37].

All spleens were screened using a *Borrelia* genus-specific qPCR targeting a fragment of the 16S rRNA gene as described [38].

3. Results

3.1. Sample collection

From October 2010 to September 2013, a total of 117 dogs and 107 cats, from animal shelters in Algiers, were sampled. These animals live in urban and rural areas, spending most of their time exclusively outdoors and do not receive any tick or flea control products. Of the 117 dogs, 59/117 (50.42%) were males and 58/177 (49.57%) were females. The canine population was predominantly crossbred dogs; the others belonged to the German shepherd, shepherd crosses, *American Staffordshire* and Pit-bull mixed breeds. Dog age ranged between 2 months and 11 years. Among the 107 cats, 58/107 (54.20%) were males and 49/107 (45.79%) were females. The cats were described as mostly belonging to mixed breeds and also to European and Siamese crossbreeds. Most cats were estimated to be less than 3 years old. Among these animals, 31/117 (26.49%) dogs and 8/107 (7.47%) cats were apparently sick. A total of 72/117 (61.53%) dogs and 65/107 (60.74%) cats were infested with at least one ectoparasite species. Mixed infestations with two or more ectoparasites were detected on 61/117 dogs (52.13%) and 18/107 cats (16.82%). Among the 117 dogs examined, 68/117 (58.12%) were found to be infested with ticks, while fleas and lice were found on 62/117 (52.99%) and 2/117 (1.71%) of dogs respectively. Of the 107 cats examined, 11/107 (10.28%) were infested with ticks, 65/107 (60.74%) with fleas and 4/107 (3.74%) with lice.

A total of 640 fleas were collected from these animals, 305/640 (47.65%) from dogs and 335/640 (52. 34%) from cats. Three species were morphologically identified, including 369/640 (57.7%) *Ctenocephalides felis* which was the most abundant, followed by 149/640 (23.3%) *Xenopsylla cheopis*, and 122/640 (19%) *Ctenocephalides canis*.

A total of 532 ticks were collected, 520/532 (97.74%) from dogs and 12/532 (2.25%) from cats. All ticks belonged to the *Rhipicephalus sanguineus* species. In addition, 48 chewing lice were collected, including 39 *Felicola subrostratus* on cats and 9 *Trichodectes canis* on dogs.

For the present study, a convenient sample of 115/532 (21.6%) ticks (103 from dogs, 12 from cats) and 225/640 (35.1%) fleas: 108 *Ctenocephalides felis* (21 from dogs, 87 from cats), 53 *Ctenocephalides canis* (49 from dogs, 4 from cats) and 64 *Xenopsylla cheopis* (62 from dogs, 2 from cats) were selected at random for molecular screening. Spleen samples collected from all 117 dogs and 107 cats were tested for the presence of pathogens.

3.2. Detection of bacteria

Using qPCR targeting the IS30a and the IS1111gene of *C. burnetii* in spleen samples, *C. burnetii* DNA was identified in 1/117 (0.85%) dog and 1/107 (0.93%) cat. Using qPCR targeting the Ecaj 0701 gene of *E. canis*, 17/117 (14.52%) dogs were positive. 10/17 (58.82%) of the positive dogs were infested with ticks and fleas and 3/17 (17.64%) were sick (Table 1). *B. henselae* DNA was amplified from the splenic tissue of 1/117 (0.85%) dog and 1/107 (0.93%) cat for both qPCR systems; genus-specific *Bartonella* spp and *B. henselae*. The dog positive for *B. henselae* harbored ticks and fleas. All animals tested negative for *Rickettsia* spp. and *Borrelia* spp. DNA.

Using qPCR targeting the RKND03 system for all spotted fever group rickettsiae (SFG), *Rickettsia* spp. DNA was detected in 29 ticks and 2 fleas. *R. massilae* DNA was identified in 28/115 (24.35%) ticks by qPCR. *R. massilae* DNA was detected in 27/103 (26.21%) ticks from dogs and in 1/12 (8.33%) ticks from cats. Only 1/115 tick (0.87%) was positive to *R. conorii*-specific qPCR. *R. conorii* DNA was detected in 1/103 (0.97%) *Rhipicephalus sanguineus* collected from dogs. Using qPCR specific for membrane phosphatase gene, we identified *R. felis* in 2/225 (0.88%) fleas. *R. felis* DNA was detected in 2/87 (2.30%) *Ctenocephalides felis* collected from cats.

21/225 (9.33%) fleas tested were positive for *Bartonella* spp. by *Bartonella* qPCR. 11/225 (4.88%) were positive by *B. henselae*-specific qPCR. *B. henselae* DNA was detected in 11/87 (12.64%) *Ctenocephalides felis* collected from cats. Sequencing of the *gltA* gene fragment from the remaining ten *Bartonella*-positive fleas revealed gene sequences matching those of *B. vinsonii* subsp. *berkhoffii* and *B. clarridgeiae*. A search in GenBank of similar partial sequences from the *gltA* gene indicated that 6/225 (2.66%) samples were identical to *B. vinsonii* subsp. *berkhoffii* (GenBank accession no. DQ360833.1, with 99% similarity) and 4/225 (1.77%) fleas were in complete homology with the sequences for *B. clarridgeiae* (accession no. FN645454.1, with 100% similarity). *B. vinsonii* subsp. *berkhoffii* DNA was detected in 6/62 (9.67%) *Xenopsylla cheopis* collected from dogs. *B. clarridgeiae* was identified in 4/87 (4.59%) *Ctenocephalides felis* collected from cats.

Using qPCR targeting the Ecaj 0701 gene of *E. canis*, 8/115 (6.95%) ticks were positive. *E. canis* DNA was detected in 8/103 (7.77%) *Rhipicephalus sanguineus* collected from dogs, these positive ticks were taken from 4/10 (40%) infested dogs positive by *E. canis*.

Moreover, four of these positive ticks were co-infected with *R. massilae*. All ticks tested negative for *C. burnetii*.

4. Discussion

In this study, we investigated the occurrence and diversity of canine and feline vector-borne infections in Algiers using molecular techniques. Overall, 18/177 (15.38%) dogs and 2/107 (1.87%) cats were positive for at least one vector-borne agent, including one dog co-infected with two agents *E. canis* and *C. burnetii*. We also show that ectoparasites collected from these animals were infected with several bacteria, including *R. massilae*, *R. conorii* and *E. canis* in ticks and *R. felis*, *B. henselae*, *B. clarridgeiae* and *B. vinsonii* subsp. *berkhoffii* in fleas.

To the best of our knowledge, this is the first molecular study demonstrating the presence of *C. burnetii* in dogs and cats from Algeria. *C. burnetii*, the causative agent of Q fever, is recognized as one of the most important zoonotic pathogens. In addition to domestic animals such as cattle, sheep and goats, considered to be the primary reservoirs of *C. burnetii*, pets such as dogs and cats have received attention as a potential source of human exposure [2]. Cats have been implicated in some outbreaks [39] and dogs in only one [40].

C. burnetii was detected in the spleen samples of one dog and one cat. Based on molecular results, this pathogen was also identified in dogs from Hungary [9], Japan [41], Brazil [6] and the Netherlands [42], and in cats from North America [8] and Japan [41].

In Algeria, a recent study showed that *C. burnetii* was the cause of fever in one patient, but cases are rarely documented and the prevalence of the disease is probably underestimated [10]. Our results indicate that apparently healthy dogs and cats from Algeria might serve as a source of human *C. burnetii* infection. It is possible that the positive animals lived in a rural habitat in close contact with domestic ruminants which are the most important reservoirs for this agent.

In our study, *E. canis* was the most common pathogen found in spleens of dogs. Infection with *E. canis* causes canine monocytic ehrlichiosis, first recognized as a distinct clinical entity in Algeria in 1935 [11]. Since then, ehrlichiosis has been acknowledged as an important emerging tick-borne disease in both humans and animals [43]. *E. canis*, is primarily transmitted by the brown dog tick *Rhipicephalus sanguineus*, with worldwide distribution [2]. In this work, the percentage of positivity to *E. canis* DNA in spleen samples of dogs from Algiers was 14.5%. In Algeria, *E. canis* was newly detected in blood of dogs in 7/110 (6.4%) cases in the provinces of Tizi Ouzou and Béjaïa [12] and in 10/213 (4.7%) in Algiers [13].

Previous studies have demonstrated that the spleen is the organ most likely to store *E. canis* during the subclinical phase and the last organ to harbor these organisms before elimination [44]. The authors also indicated longer persistence of *E. canis* in splenic macrophages than in blood monocytes [45]. These findings reveal that extraction and amplification of DNA from splenic aspirates is a reliable method for determining the carrier state of *E. canis* as reported here [44,45]. Recently, 27/60 (45%) spleen samples from naturally infected dogs were positive for *E. canis* by PCR assays in Brazil [46].

In our investigation, 58.8% of the positive dogs were infested with ticks. Molecular detection of *E. canis* in ticks demonstrated that 8/103 (7.77%) *Rhipicephalus sanguineus* collected from our dogs were infected. These positive ticks were collected from 4/10 (40%) infested dogs positive for *E. canis.* We report here the first direct evidence of *E. canis* in ticks from Algeria. The prevalence of *E. canis* in *Rhipicephalus sanguineus* ticks from dogs in other African countries has been reported in Cameroon at 6% [47] and in Ivory Coast at 27% [33] using molecular tools.

In the present work, one cat was qPCR-positive for *B. henselae*. This bacterium is an emerging pathogen of veterinary and medical significance [48]. Domestic cats frequently develop subclinical infection with *B. henselae*, the main agent of cat scratch disease (CSD). They are therefore considered as the principal reservoir for human infection [49,50]. The molecular prevalence of *B. henselae* in the present study (1/107; 0.9%) was similar to a study conducted on blood samples of cats in Portugal 2/649 (0.3%) [4] and in Albania by PCR 1/146 (0.7%) [51], but lower than two investigations previously reported in cats from Argentina 14/101 (11.9%) and Guatemala 32/47 (68.1) [52,53]. Recently, Whole blood samples from stray cats from Algiers, were cultured to detect the presence of *Bartonella* species. *B. henselae* was the only species isolated from 36/211 (17%) cats [19]. Because no culture method is currently recommended as a gold standard for *Bartonella* isolation, in cases where infection by these agents is suspected, molecular approach is more sensitive, especially real-time PCR [53,54]. Our result can be explained that *Bartonella* may cause persistent bacteremia in cats and therefore a high number of circulating organisms.

We report for the first time the detection of *B. henselae* and *B. clarridgeiae* in *Ctenocephalides felis* collected from cats in Algeria. The prevalence rate of *B. henselae* and *B. clarridgeiae* DNA was 12.64% and 4.59% respectively. This finding is consistent with reports in several countries worldwide [55–57]. In Algeria, *B. clarridgeiae* was detected in the blood of dogs [18] and in fleas collected from hedgehogs [17].

In this study, we detected *B. henselae* in the spleen of one dog. Domestic dogs may be excellent epidemiological sentinels for *Bartonella* infection in humans [58]. In accordance with our findings, *B. henselae* was molecularly amplified from the splenic tissue of dogs [59,60]. Recently, *B. henselae* was identified in 4/96 (4.16%) dog *blood samples* in Algeria by PCR [13].

Molecular analysis of ectoparasites from our dogs revealed that *B. vinsonii* subsp. *berkhoffii* was detected in 6/62 (9.67%) *Xenopsylla cheopis*. These positive fleas belong to dogs that were negative for *Bartonella* spp. *Bartonella vinsonii* subsp. *berkhoffii* was earlier detected infecting Algerian dogs [18]. *Xenopsylla cheopis*, the Oriental rat flea, is distributed worldwide and is suspected to transmit several *Bartonella* species [61]. In Algeria, *B. tribocorum* and *B. elizabethae* were detected in *Xenopsylla cheopis* collected from rodents [17]. We detected for the first time the presence of *B. vinsonii* subsp. *berkhoffii* in *Xenopsylla cheopis*.

In our study, all animals tested negative for *Rickettsia* spp. Molecular research of these bacteria in ectoparasites showed positives results. We identified *R. felis* DNA in 2/87 (2.30%) *Ctenocephalides felis* collected from cats. In Algeria, the molecular presence of *R. felis* was detected in *Ctenocephalides canis* and *Xenopsylla cheopis* from rodents [23]. Lately, *Ctenocephalides felis* has also been shown to harbor this emerging pathogen (data submitted to parasites and vectors [62]. In previous studies, *R. felis* DNA was detected in *Ctenocephalides felis* of cats in Thailand [55], in Malaysia [57] and in Ethiopia [56].

We also reported an infection rate of 24.4% for *R. massiliae* and 11% for *R. conorii* in *Rh. sanguineus* ticks. These two spotted fever group rickettsiae were amplified from *Rh. sanguineus* by molecular tools in Algeria [63,64] and in Tunisia [65]. Overall, we confirm that dogs and cats can act as hosts for ectoparasites infected with several rickettsial agents.

An outdoor housing, contact with other hosts and the non-use of preventive or therapeutic anti-ectoparasite applications were found to be correlated with PCR-positivity to vector-borne infections [5], which is associated with a higher exposure of stray dogs and cats of our study to arthropod vectors and the agents they might transmit.

5. Conclusion

This is the first study to investigate exposure to multiple vector-borne pathogens in stray cats from Algeria. This is also the first

time that *C. burnetii* has been described in animals from Algeria. The occurrence of these agents in the country, with zoonotic character, emphasizes the need to alert the veterinary community, owners and public health authorities to the risk of infection. Control measures, including chemoprophylaxis against the ectoparasite vectors, should be implemented to prevent infection of domestic carnivores, other vertebrate hosts and people.

This paper has provided interesting new information on zoonotic pathogens emerging from companion animals. Additional epidemiological data using larger numbers of domestic animals from more localities is necessary in order to determine the actual prevalence and distribution of these diseases in Algeria, as well to allow the analysis of differences between risk factors. Further investigations are also warranted in order to isolate these species and to determine their clinical significance.

Conflict of interests

The authors declare that they have no competing interests.

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References

[1] F. Dantas-Torres, D. Otranto, Dogs, cats, parasites, and humans in Brazil: opening the black box, Parasit. Vectors 7 (2014) 22.
[2] L. Wei, P. Kelly, K. Ackerson, J. Zhang, H.S. El-Mahallawy, B. Kaltenboeck, et al., First report of *Babesia gibsoni* in Central America and survey for vector-borne infections in dogs from Nicaragua, Parasit. Vectors 7 (2014) 126.
[3] E. Claerebout, B. Losson, C. Cochez, S. Casaert, A.C. Dalemans, C.A. De, et al., Ticks and associated pathogens collected from dogs and cats in Belgium, Parasit. Vectors 6 (2013) 183.
[4] C. Maia, C. Ramos, M. Coimbra, F. Bastos, A. Martins, P. Pinto, et al., Bacterial and protozoal agents of feline vector-borne diseases in domestic and stray cats from southern Portugal, Parasit. Vectors 7 (2014) 115.
[5] J. Kamani, G. Baneth, K.Y. Mumcuoglu, N.E. Waziri, O. Eyal, Y. Guthmann, et al., Molecular detection and characterization of tick-borne pathogens in dogs and ticks from Nigeria, PLoS Negl. Trop. Dis. 7 (3) (2013) e2108.
[6] M.A. Mares-Guia, T. Rozental, A. Guterres, R. Gomes, D.N. Almeida, N.S. Moreira, et al., Molecular identification of the agent of Q fever – *Coxiella burnetii* – in domestic animals in State of Rio de Janeiro, Brazil, Rev. Soc. Bras. Med. Trop. 47 (2) (2014) 231–234.
[7] M.M. de Rooij, B. Schimmer, B. Versteeg, P. Schneeberger, B.R. Berends, D. Heederik, et al., Risk factors of *Coxiella burnetii* (Q fever) seropositivity in veterinary medicine students, PLoS ONE 7 (2) (2012) e32108.
[8] K. Cairns, M. Brewer, M.R. Lappin, Prevalence of *Coxiella burnetii* DNA in vaginal and uterine samples from healthy cats of north-central Colorado, J. Feline Med. Surg. 9 (3) (2007) 196–201.
[9] S. Hornok, B. Denes, M.L. Meli, B. Tanczos, L. Fekete, M. Gyuranecz, et al., Non-pet dogs as sentinels and potential synanthropic reservoirs of tick-borne and zoonotic bacteria, Vet. Microbiol. 167 (3–4) (2013) 700–703.
[10] E. Angelakis, O. Mediannikov, C. Socolovschi, N. Mouffok, H. Bassene, A. Tall, et al., *Coxiella burnetii*-positive PCR in febrile patients in rural and urban Africa, Int. J. Infect. Dis. 28 (2014) 107–110.
[11] A.L.A. Donatien, Existence en Algérie d'une rickettsia du chien, Bull. Soc. Pathol. Exot. 28 (1935) 418–419, Ref Type: Generic.
[12] M. Dahmani, A. Loudahi, O. Mediannikov, F. Fenollar, D. Raoult, B. Davoust, Molecular detection of *Anaplasma platys* and *Ehrlichia canis* in dogs from Kabylie, Algeria, Ticks Tick Borne Dis. 6 (2) (2015) 198–203.
[13] N. Azzag, E. Petit, C. Gandoin, C. Bouillin, F. Ghalmi, N. Haddad, et al., Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers, Comp. Immunol. Microbiol. Infect. Dis. 38 (2015) 1–7.
[14] L.S. de Oliveira, L.C. Mourao, K.A. Oliveira, A.M. da Matta, A.C. de Oliveira, M.R. de Almeida, et al., Molecular detection of *Ehrlichia canis* in cats in Brazil, Clin. Microbiol. Infect. 15 (Suppl 2) (2009) 53–54.
[15] I.A. Braga, L.G. dos Santos, D.G. de Souza Ramos, A.L. Melo, G.L. da Cruz Mestre, D.M. de Aguiar, Detection of *Ehrlichia canis* in domestic cats in the central-western region of Brazil, Braz. J. Microbiol. 45 (2) (2014) 641–645.
[16] A. Benslimani, F. Fenollar, H. Lepidi, D. Raoult, Bacterial zoonoses and infective endocarditis, Algeria, Emerg. Infect. Dis. 11 (2) (2005) 216–224.
[17] I. Bitam, J.M. Rolain, V. Nicolas, Y.L. Tsai, P. Parola, V.A. Gundi, et al., A multi-gene analysis of diversity of Bartonella detected in fleas from Algeria, Comp. Immunol. Microbiol. Infect. Dis. 35 (1) (2012) 71–76.
[18] T. Kernif, M. Aissi, S.E. Doumandji, B.B. Chomel, D. Raoult, I. Bitam, Molecular evidence of Bartonella infection in domestic dogs from Algeria, North Africa, by polymerase chain reaction (PCR), Am. J. Trop. Med. Hyg. 83 (2) (2010) 298–300.
[19] N. Azzag, N. Haddad, B. Durand, E. Petit, A. Ammouche, B. Chomel, et al., Population structure of *Bartonella henselae* in Algerian urban stray cats, PLoS ONE 7 (8) (2012) e43621.
[20] I. Bitam, J.M. Rolain, T. Kernif, B. Baziz, P. Parola, D. Raoult, Bartonella species detected in rodents and hedgehogs from Algeria, Clin. Microbiol. Infect. 15 (Suppl 2) (2009) 102–103.
[21] P. Parola, C.D. Paddock, C. Socolovschi, M.B. Labruna, O. Mediannikov, T. Kernif, et al., Update on tick-borne rickettsioses around the world: a geographic approach, Clin. Microbiol. Rev. 26 (4) (2013) 657–702.
[22] I. Bitam, Vectors of rickettsiae in Africa, Ticks Tick Borne Dis. 3 (5–6) (2012) 382–386.
[23] I. Bitam, B. Baziz, T. Kernif, Z. Harrat, P. Parola, D. Raoult, Molecular detection of *Rickettsia typhi* and *Rickettsia felis* in fleas from Algeria, Clin. Microbiol. Infect. 15 (Suppl 2) (2009) 255–256.
[24] M.L. Levin, L.F. Killmaster, G.E. Zemtsova, Domestic dogs (*Canis familiaris*) as reservoir hosts for *Rickettsia conorii*, Vector Borne Zoonotic Dis. 12 (1) (2012) 28–33.
[25] M.M. Nogueras, I. Pons, A. Ortuno, J. Miret, J. Pla, J. Castella, et al., Molecular detection of *Rickettsia typhi* in cats and fleas, PLoS ONE 8 (8) (2013) e71386.
[26] F. Segura, I. Pons, J. Miret, J. Pla, A. Ortuno, M.M. Nogueras, The role of cats in the eco-epidemiology of spotted fever group diseases, Parasit. Vectors 7 (2014) 353.
[27] B. Skotarczak, Why are there several species of *Borrelia burgdorferi sensu lato* detected in dogs and humans? Infect. Genet. Evol. 23 (2014) 182–188.
[28] I. Krupka, R.K. Straubinger, Lyme borreliosis in dogs and cats: background, diagnosis, treatment and prevention of infections with *Borrelia burgdorferi sensu stricto*, Vet. Clin. North Am. Small Anim. Pract. 40 (6) (2010) 1103–1119.
[29] A.P. Whitaker, Fleas (Siphonaptera), 1/16th ed., Royal Entomological Society, 2007, pp. 178.
[30] A.R. Walker, A. Bouattour, Ticks of Domestic Animals in Africa: A Guide to Identification of Species, Bioscience Reports, Edinburgh, 2007, Ref Type: Generic.
[31] C. Socolovschi, P. Reynaud, T. Kernif, D. Raoult, P. Parola, Rickettsiae of spotted fever group, *Borrelia valaisiana*, and *Coxiella burnetii* in ticks on passerine birds and mammals from the Camargue in the south of France, Ticks Tick Borne Dis. 3 (5–6) (2012) 355–360.
[32] C. Socolovschi, T. Kernif, D. Raoult, P. Parola, Borrelia, Rickettsia, and Ehrlichia species in bat ticks, France, 2010, Emerg. Infect. Dis. 18 (12) (2012) 1966–1975.
[33] C. Socolovschi, J. Gomez, J.L. Marie, B. Davoust, P.M. Guigal, D. Raoult, et al., *Ehrlichia canis* in *Rhipicephalus sanguineus* ticks in the Ivory Coast, Ticks Tick Borne Dis. 3 (5–6) (2012) 411–413.
[34] H. Leulmi, C. Socolovschi, A. Laudisoit, G. Houemenou, B. Davoust, I. Bitam, et al., Detection of *Rickettsia felis*, *Rickettsia typhi*, *Bartonella* species and *Yersinia pestis* in Fleas (Siphonaptera) from Africa, PLoS Negl. Trop. Dis. 8 (10) (2014) e3152.
[35] E. Angelakis, V. Roux, D. Raoult, J.M. Rolain, Real-time PCR strategy and detection of bacterial agents of lymphadenitis, Eur. J. Clin. Microbiol. Infect. Dis. 28 (11) (2009) 1363–1368.
[36] Y. Bechah, C. Socolovschi, D. Raoult, Identification of rickettsial infections by using cutaneous swab specimens and PCR, Emerg. Infect. Dis. 17 (1) (2011) 83–86.
[37] G. Walter, E. Botelho-Nevers, C. Socolovschi, D. Raoult, P. Parola, Murine typhus in returned travelers: a report of thirty-two cases, Am. J. Trop. Med. Hyg. 86 (6) (2012) 1049–1053.
[38] P. Parola, G. Diatta, C. Socolovschi, O. Mediannikov, A. Tall, H. Bassene, et al., Tick-borne relapsing fever borreliosis, rural senegal, Emerg. Infect. Dis. 17 (5) (2011) 883–885.
[39] T.J. Marrie, H. Durant, J.C. Williams, E. Mintz, D.M. Waag, Exposure to parturient cats: a risk factor for acquisition of Q fever in Maritime Canada, J. Infect. Dis. 158 (1) (1988) 101–108.
[40] F. Buhariwalla, B. Cann, T.J. Marrie, A dog-related outbreak of Q fever, Clin. Infect. Dis. 23 (4) (1996) 753–755.
[41] T. Komiya, K. Sadamasu, H. Toriniwa, K. Kato, Y. Arashima, H. Fukushi, et al., Epidemiological survey on the route of *Coxiella burnetii* infection in an animal hospital, J. Infect. Chemother. 9 (2) (2003) 151–155.
[42] H.I. Roest, C.B. van Solt, J.J. Tilburg, C.H. Klaassen, E.K. Hovius, F.T. Roest, et al., Search for possible additional reservoirs for human Q fever, The Netherlands, Emerg. Infect. Dis. 19 (5) (2013) 834–835.
[43] N. Maazi, A. Malmasi, P. Shayan, S.M. Nassiri, T.Z. Salehi, M.S. Fard, Molecular and serological detection of *Ehrlichia canis* in naturally exposed dogs in Iran: an analysis on associated risk factors, Rev. Bras. Parasitol. Vet. 23 (1) (2014) 16–22.

[44] S. Harrus, M. Kenny, L. Miara, I. Aizenberg, T. Waner, S. Shaw, Comparison of simultaneous splenic sample PCR with blood sample PCR for diagnosis and treatment of experimental *Ehrlichia canis* infection, Antimicrob. Agents Chemother. 48 (11) (2004) 4488–4490.
[45] G. Baneth, S. Harrus, F.S. Ohnona, Y. Schlesinger, Longitudinal quantification of *Ehrlichia canis* in experimental infection with comparison to natural infection, Vet. Microbiol. 136 (3–4) (2009) 321–325.
[46] K.C. de Sousa, M.R. Andre, H.M. Herrera, G.B. de Andrade, M.M. Jusi, L.L. dos Santos, et al., Molecular and serological detection of tick-borne pathogens in dogs from an area endemic for *Leishmania infantum* in Mato Grosso do Sul, Brazil, Rev. Bras. Parasitol. Vet. 22 (4) (2013) 525–531.
[47] L.M. Ndip, R.N. Ndip, S.N. Esemu, D.H. Walker, J.W. McBride, Predominance of *Ehrlichia chaffeensis* in *Rhipicephalus sanguineus* ticks from kennel-confined dogs in Limbe, Cameroon, Exp. Appl. Acarol. 50 (2) (2010) 163–168.
[48] Y.L. Tsai, C.C. Lin, B.B. Chomel, S.T. Chuang, K.H. Tsai, W.J. Wu, et al., Bartonella infection in shelter cats and dogs and their ectoparasites, Vector Borne Zoonotic Dis. 11 (8) (2011) 1023–1030.
[49] B.B. Chomel, H.J. Boulouis, S. Maruyama, E.B. Breitschwerdt, *Bartonella* spp. in pets and effect on human health, Emerg. Infect. Dis. 12 (3) (2006) 389–394.
[50] M.G. Pennisi, F. Marsilio, K. Hartmann, A. Lloret, D. Addie, S. Belak, et al., *Bartonella species* infection in cats: ABCD guidelines on prevention and management, J. Feline Med. Surg. 15 (7) (2013) 563–569.
[51] C. Silaghi, M. Knaus, D. Rapti, I. Kusi, E. Shukullari, D. Hamel, et al., Survey of *Toxoplasma gondii* and *Neospora caninum*, haemotropic mycoplasmas and other arthropod-borne pathogens in cats from Albania, Parasit. Vectors 7 (2014) 62.
[52] G.L. Cicuttin, D.F. Brambati, M.F. De Gennaro, F. Carmona, M.L. Isturiz, L.E. Pujol, et al., *Bartonella* spp. in cats from Buenos Aires, Argentina, Vet. Microbiol. 168 (1) (2014) 225–228.
[53] Y. Bai, M.F. Rizzo, D. Alvarez, D. Moran, L.F. Peruski, M. Kosoy, Coexistence of *Bartonella henselae* and *B. clarridgeiae* in populations of cats and their fleas in Guatemala, J. Vector Ecol. 40 (2) (2015) 327–332.
[54] R. Staggemeier, D.A. Pilger, F.R. Spilki, V.V. Cantarelli, Multiplex SYBR(R) green-real time PCR (qPCR) assay for the detection and differentiation of *Bartonella henselae* and *Bartonella clarridgeiae* in cats, Rev. Inst. Med. Trop. Sao Paulo 56 (2) (2014) 93–95.
[55] P. Parola, O.Y. Sanogo, K. Lerdthusnee, Z. Zeaiter, G. Chauvancy, J.P. Gonzalez, et al., Identification of *Rickettsia* spp. and *Bartonella* spp. in from the Thai-Myanmar border, Ann. N. Y. Acad. Sci. 990 (2003) 173–181.
[56] B. Kumsa, P. Parola, D. Raoult, C. Socolovschi, Molecular detection of *Rickettsia felis* and *Bartonella henselae* in dog and cat fleas in Central Oromia, Ethiopia, Am. J. Trop. Med. Hyg. 90 (3) (2014) 457–462.
[57] A.S. Mokhtar, S.T. Tay, Molecular detection of *Rickettsia felis*, *Bartonella henselae*, and *B. clarridgeiae* in fleas from domestic dogs and cats in Malaysia, Am. J. Trop. Med. Hyg. 85 (5) (2011) 931–933.
[58] P.P. Diniz, B.A. Morton, M. Tngrian, M. Kachani, E.A. Barron, C.M. Gavidia, et al., Infection of domestic dogs in Peru by zoonotic Bartonella species: a cross-sectional prevalence study of 219 asymptomatic dogs, PLoS Negl. Trop. Dis. 7 (9) (2013) e2393.
[59] D.G. Ohad, D. Morick, B. Avidor, S. Harrus, Molecular detection of *Bartonella henselae* and *Bartonella koehlerae* from aortic valves of Boxer dogs with infective endocarditis, Vet. Microbiol. 141 (1–2) (2010) 182–185.
[60] S.G. Friedenberg, N. Balakrishnan, J. Guillaumin, E.S. Cooper, K. Lewis, D.S. Russell, et al., Splenic vasculitis, thrombosis, and infarction in a febrile dog infected with *Bartonella henselae*, J. Vet. Emerg. Crit. Care (San Antonio) (2015).
[61] S.A. Billeter, L. Colton, S. Sangmaneedet, F. Suksawat, B.P. Evans, M.Y. Kosoy, Molecular detection and identification of *Bartonella species* in rat fleas from northeastern Thailand, Am. J. Trop. Med. Hyg. 89 (3) (2013) 462–465.
[62] H. Leulmi, A. Aouadi, I. Bitam, A. Bessas, A. Benakhla, D. Raoult, et al., Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria, Parasit. Vectors 9 (1) (2016) 27.
[63] I. Bitam, P. Parola, K. Matsumoto, J.M. Rolain, B. Baziz, S.C. Boubidi, et al., First molecular detection of *R. conorii*, *R. aeschlimannii*, and *R. massiliae* in ticks from Algeria, Ann. N. Y. Acad. Sci. 1078 (2006) 368–372.
[64] M. Khaldi, C. Socolovschi, M. Benyettou, G. Barech, M. Biche, T. Kernif, et al., Rickettsiae in arthropods collected from the North African Hedgehog (*Atelerix algirus*) and the desert hedgehog (*Paraechinus aethiopicus*) in Algeria, Comp. Immunol. Microbiol. Infect. Dis. 35 (2) (2012) 17–122.
[65] F. Khrouf, Y. M'Ghirbi, A. Znazen, J.M. Ben, A. Hammami, A. Bouattour, Detection of Rickettsia in *Rhipicephalus sanguineus* ticks and *Ctenocephalides felis* fleas from southeastern Tunisia by reverse line blot assay, J. Clin. Microbiol. 52 (1) (2014) 268–274.