Comparative Immunology, Microbiology and Infectious Diseases 45 (2016) 23–28

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

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

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ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 15 January 2016
Accepted 19 January 2016

Keywords:
Dog
Cat
Tick
Flea
Coxiella burnetii
Ehrlichia canis
Bartonella spp.
Rickettsia spp.
qPCR
Algeria

ABSTRACT

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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http://dx.doi.org/10.1016/j.cimid.2016.01.002
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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*.

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