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Prevalence of select vector-borne pathogens in stray and client-owned dogs from Algiers

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ABSTRACT

Data on the prevalence of vector-borne diseases agents infecting canines in Algeria is currently lacking. The purpose of this study is to assess by serological and molecular methods the prevalence of select arthropod borne-bacterial infections in client-owned and stray dogs. Antibodies to *Anaplasma phagocytophilum* were the most prevalent at 47.7%, followed by *Borrelia burgdorferi s.l.* at 37.6%, *Ehrlichia canis* at 30.0%, *Bartonella henselae* at 32.4% and *Bartonella vinsonii* subsp. *berkhoffii* at 27%. Seroprevalence was statistically significantly higher in stray dogs than those owned by clients. Seropositivity was not associated with health status, except for *E. canis*. Molecular evaluation indicates that 17.8% of the 213 analyzed dogs were positive for *Ehrlichia* and *Anaplasma* with a prevalence of 4.2% for *E. canis*, 14.1% for *Anaplama platys* and 0% for *A. phagocytophilum*. Seven (7.1%) of the tested dogs were positive for *Bartonella* spp. with two characterized as *Bartonella rochalimae*, four as *B. henselae* and one as *B.v.* subsp. *berkhoffii*.

1. Introduction

Since the first description of *Ehrlichia canis* infections in Algerian dogs by Donatien and Lestoquard [1], the spectrum of known vector-borne bacterial infections of dogs has expanded. Members of the genera *Anaplasma*, *Bartonella*, *Ehrlichia* and *Borrelia* are now described in this host species throughout the world. In addition to the growing economic and clinical importance of these bacteria in veterinary medicine, many are considered to be zoonotic, and dogs may act as sentinels for human infections in certain instances [2].

E. canis is responsible for canine monocytic ehrlichiosis. Until recently, *E. canis* was thought to only infect dogs. Nevertheless, on the basis of the 16S rRNA gene sequence, it was suggested that *E. canis* could also be a causative agent of human monocytic ehrlichiosis [3]. Moreover in a 2006 study, *E. canis* was successfully isolated from human patients showing symptoms similar to those caused by *Ehrlichia chaffeensis* and *Ehrlichia ewingii* infections [4].

The genus *Anaplasma* includes at least two species known to infect dogs. *Anaplasma phagocytophilum*, the causative agent of granulocytic anaplasmosis [5], has been described in domestic carnivores, ruminants, horses and human around the world [6]. A variety of domestic and wild animals have also been described as reservoirs for this *Anaplasma* species [7]. *Anaplasma platys* is an organism that

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colonizes platelets and causes infectious cyclic thrombocytopenia [8]. Dogs are considered as the primary reservoir hosts for *A. platys* [9].

Borrelia burgdorferi sensu lato induces Lyme borreliosis, a major zoonotic bacterial disease. *B. afzelii*, *B. garinii* and *B. burgdorferi sensu stricto* are the most frequently circulating genospecies, and they are considered as highly pathogenic for both humans and dogs [10]. Small mammals, particularly mice, are considered to be the principal reservoir hosts of *B. burgdorferi s.l.* [11].

Numerous bacteria of the genus *Bartonella* are considered to be zoonotic agents and have been identified in various animal reservoirs. Canine bartonellosis is mainly due to *Bartonella vinsonii* subsp. *berkhoffii*. In North America, the main reservoir of this species is the coyote [12], but reservoir hosts have not be identified to date in the other countries where this *Bartonella* species has been documented. Other *Bartonella* species have also been associated with a variety of diseases in humans and dogs, such as *B. henselae*, *B. elizabethae* and *B. clarridgeiae* [13].

Different arthropods are described as vectors of these bacteria. *Rhipicephalus sanguineus*, the brown tick dog, is involved in the transmission of *E. canis* [5], *A. platys* [9] and *B.v. berkhoffii* [14]. The tick *Ixodes* is considered as the main vector of *A. phagocytophilum* [4] and *B. burgdorferi s.l.* [11] Fleas are the main vector of *B. henselae*, *B. elizabethae* and *B. clarridgeiae* [13].

In North Africa, little is known about these vector-borne pathogens. A survey was conducted in Tunisia in order to determine the seroprevalence of *E. canis* and *A. phagocytophilum* infections [15]. By immunofluorescence antibody test, the authors found 54.2% and 25.2% of dogs seropositive for *E. canis* and *A. phagocytophilum*, respectively. Arthropod vectors such as fleas were sampled in Algiers. Up to 21.5% (44/204) of harvested fleas were PCR positive for *B. elizabethae*, *B. clarridgeiae* or *B. tribochorum* [16]. In other studies *Rickettsia* spp. were detected in fleas [16,17] and *Borrelia garini* in *Ixodes ricinus* [18]. In addition, molecular studies showed the presence of three *Bartonella* species (*B. clarridgeiae*, *B. elizabethae* and *B.v. berkhoffii*) in client-owned dogs [19].

In order to assess the exposure to vector-borne pathogens in dogs and subsequently assess the potential risk of infection for the population of Algiers, we investigated the prevalence of five bacterial genera or species *Anaplasma*, *Bartonella*, *E. canis* and *B. burgdorferi s.l.* in a population of stray and client-owned dogs in Algiers using serological and/or molecular techniques.

2. Materials and methods

2.1. Ethic statement

All the sampled stray dogs were caught in the context of the National Program for Rabies Control in which the authors of this paper were not involved. This program is carried out by Hygiène Urbaine d'Alger (HURBAL), which is an institution trust of the Algerian Ministry of Interior, the Local Government and the Algerian Ministry of Agriculture and Rural Development. The HURBAL director is a doctor of veterinary medicine. Two additional veterinarians were responsible for ensuring the good health of the animals that were caught. Their assistants have received training on animal welfare and methods used to capture animals. Dogs were caught carefully with a sheathed clamp. Once captured, the animals were housed in cages regularly cleaned and disinfected. They were euthanized only after expiration of the legal delay of guard (owners had seven days to claim their animal). On 23 March 2008, one of the authors (NA), was given authorization by HURBAL to collect blood samples.

2.2. Dog population and blood samples

From July 2008 to November 2010, 213 blood samples were collected from dogs of various breeds. The dogs were either patients admitted to the teaching hospital of the Algiers Veterinary School ($N = 150$) or stray dogs housed in animal shelters ($N = 63$). Dogs sampled at the Veterinary School were admitted for surgical procedures or vaccinations. Sick dogs showed a diversity of clinical signs.

Cephalic venous samples (5 ml) were collected in both EDTA tubes and tubes without an anticoagulant. The EDTA tubes were frozen at −20 °C and the dry tubes were centrifuged (3500 rpm for 10 min) before sera were frozen at −20 °C until analysis.

2.3. Serological analysis

Antibodies against *A. phagocytophilum*, *B. burgdorferi s. l.*, *B. henselae*, *B.v.* subsp. *berkhoffii* and *E. canis* were detected using a species-specific immunofluorescence antibody test (IFA).

Anti *B. henselae* and anti *B.v.* subsp. *berkhoffii* antibodies were detected using non-commercial slides following the previously described protocol of Henn et al. [20] with slight modification. The Fcwf cells have been replaced by Vero cells in our protocole. *B. henselae* strain 88.2 isolated from Algerian cat [21] and *B.v. berkhoffii* reference strain were used as antigens. The significant antibody titres were 1/50 for the two antigens.

Immunofluorescence antibody assays were performed for *A. phagocytophilum*, *E. canis* and *B. burgdorferi s.l.* using commercial slides (Megacor, Austria). Rabbit anti dog-IgG conjugate (Jackson Immunoresearch, Suffolk, UK) was used. Sera were initially screened at a dilution of 1:50 in phosphate-buffered saline and all seropositive samples were re-diluted up to 1:6400 in order to determine antibody titres. The significant antibody titres were 1/50, 1/40 and 1/50 for *A. phagocytophilum, E. canis* and *B. burgdorferi* respectively as stated by the manufacturer.

2.4. DNA extraction from canine blood and DNA amplification methods

The DNA extraction from 213 whole blood tubes was performed using the Nucleo Spin Blood Quick Pure kit (Macherey-Nagel, Germany).

DNA extracts were analyzed by classic PCR for an *Ehrlichia* spp. wide spectrum as previously described [22] then tested for the presence of *A. phagocytophilum*, *A. platys* and *E. canis* by species specific PCR [23,24] (Table 1).