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Diversity and global distribution of the *Coxiella* intracellular bacterium in seabird ticks

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ABSTRACT

The obligate intracellular bacterium *Coxiella burnetii* is the etiological agent of Q fever, a widespread zoonotic disease whose most common animal reservoirs are domestic ruminants. Recently, a variety of *Coxiella*-like organisms have also been reported from non-mammalian hosts, including pathogenic forms in birds and forms without known effects in ticks, raising questions about the potential importance of non-mammalian hosts as reservoirs of *Coxiella* in the wild. In the present study, we examined the potential role of globally-distributed seabird ticks as reservoirs of these bacteria. To this aim, we tested for *Coxiella* infection 11 geographically distinct populations of two tick species frequently found in seabird breeding colonies, the hard tick *Ixodes uriae* (Ixodidae) and soft ticks of the *Ornithodoros (Carios) capensis* group (Argasidae). We found *Coxiella*-like organisms in all *O. capensis* sensu lato specimens, but only in a few *I. uriae* specimens of one population. The sequencing of *16S rDNA* and *GroEL* gene sequences further revealed an unexpected *Coxiella* diversity, with seven genetically distinct *Coxiella*-like organisms present in seabird tick populations. Phylogenetic analyses show that these *Coxiella*-like organisms originate from three divergent subclades within the *Coxiella* genus and that none of the *Coxiella* strains found in seabird ticks are genetically identical to the forms known to be associated with pathogenicity in vertebrates, including *C. burnetii*. Using this data set, we discuss the potential epidemiological significance of the presence of *Coxiella* in seabird ticks. Notably, we suggest that these organisms may not be pathogenic forms, but rather behave as endosymbionts engaged in intricate interactions with their tick hosts.

Introduction

Q fever is a widespread disease caused by *Coxiella burnetii*, an obligate intracellular bacterium (Madariaga et al., 2003; Raoult et al., 2005). This organism is highly infectious. Cattle, sheep, and goats are considered the primary reservoirs, although a variety of other mammals, including humans, may also be infected. In humans, this bacterium causes acute or chronic illness, potentially with flu-like or pneumonia-like symptoms, while in many domestic mammals, it may induce abortion. Transmission typically occurs directly, by the inhalation of infected aerosols generated from excreta of infected animals (Madariaga et al., 2003; Raoult et al., 2005; Angelakis and Raoult, 2010). Although *C. burnetii* is the sole species formally named in the genus *Coxiella* (Skerman et al., 1980), other *Coxiella*-like organisms, genetically distinct from *C. burnetii*, have occasionally been reported from non-mammalian hosts. In the past decade, an avian *Coxiella*-like organism, inducing fatal systemic infections and provisionally named *Candidatus* Coxiella avium, was described in toucans and psittacines from captive stocks in California and Oklahoma (Shivaprasad et al., 2008; Vapniarsky et al., 2012; Woc-Colburn et al., 2008). Serological surveys have also documented the presence of antibodies to *Coxiella* in more than 40 domestic and wild bird species (including pigeons, vultures, robin, or crows), suggesting that birds may be commonly infected (Enright et al., 1971; Riemann et al., 1979; To et al., 1998).

Aside from vertebrates, several tick species have been found to harbor *Coxiella*-like infections (Almeida et al., 2012; Klyachko et al., 2007; Machado-Ferreira et al., 2011; Noda et al., 1997; Reeves, 2008). Although no evidence yet exists showing that *Coxiella*-like bacteria in ticks cause disease in vertebrates, these observations raise questions about the potential importance of ticks as vectors and reservoirs of *Coxiella* in natural populations. Indeed, Reeves et al. (2006) reported *Coxiella*-like infections in several specimens of *O. capensis* s.l. from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA, suggesting that seabird ticks may be natural reservoirs of *Coxiella*. Also Wilkinson et al. (2014) recently

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Table 1
List and origin of seabird ticks used in this study. Map refers to the colony locations indicated in Fig. 1.

Tick species	Colony	Map	Year of collection	Seabird host species	*n*	Prevalence of *Coxiella* (*n* infected)
Ixodes uriae	Possession Island, Crozet Archipelago	1	2003	King penguin (*Aptenodytes patagonicus*)	20	0.0 (0)
	Hornoeya Island, Norway	2	2010	Brünnich's guillemot (*Uria lomvia*)	20	0.0 (0)
	Triangle Island, Canada	3	2010	Rhinoceros auklet (*Cerorhinca monocerata*)	14	0.5 (7)
	Bogoslof, Alaska, USA	4	2008	Tufted puffin (*Fratercula cirrhata*), Brünnich's guillemot (*Uria lomvia*)	20	0.0 (0)
	Pitchie2, Kamchatka, Russia	5	2008	Red-faced cormorant (*Phalacrocorax urile*)	20	0.0 (0)
Ornithodoros capensis s.l.	Boa Vista Island, Cape Verde	6	2008	Cape Verde shearwater (*Calonectris edwardsii*), brown booby (*Sula leucogaster*)	16	1.0 (16)
	Medes Island, Spain	7	2009	Yellow-legged gull (*Larus michahellis*)	20	1.0 (20)
	Zembra Island, Tunisia	8	2009	Yellow-legged gull (*Larus michahellis*)	20	1.0 (20)
	Juan de Nova Island, Mozambique Channel	9	2011	Sooty tern (*Onychoprion fuscatus*)	28	1.0 (28)
	Lobos de Tierra Island, Peru	10	2009	Peruvian pelican (*Pelecanus thagus*), Peruvian booby (*Sula variegata*)	5	1.0 (5)
	Pan de Azucar, Chile	11	2010–2013	Humboldt penguin (*Spheniscus humboldti*)	3	1.0 (3)

demonstrated the frequent presence of *Coxiella* bacteria in *O. capensis* s.l. and another seabird tick, *Ambylomma locusolum*, on islands of the western Indian Ocean using a metabarcode approach.

To clarify the potential role of seabird ticks as reservoirs and dispersers of these bacteria, we tested for *Coxiella* infection 2 tick species frequently found in high densities in seabird breeding colonies, the hard tick *Ixodes uriae* (Ixodidae) and soft ticks of the *Ornithodoros* (*Carios*) *capensis* group (Argasidae). Both ticks are exclusively associated with seabirds and have worldwide distributions (Dietrich et al., 2011). *Ixodes uriae* commonly infests seabird species in the circumpolar areas of both hemispheres. In contrast, *Ornithodoros capensis* sensu lato is a species complex currently including 8 morphospecies, which parasitize a large diversity of colonial seabirds living in tropical and temperate regions across the Pacific, Atlantic, and Indian Oceans. Both ticks are known to show some level of host specificity within colonies (see McCoy et al., 2013, for review). These ticks also harbor numerous human pathogenic organisms (Dietrich et al., 2011, 2014) and, due to the remarkable vagility of their seabird hosts, may be dispersed over considerable geographic distances (McCoy et al., 2005). For these reasons, their role as pathogen reservoirs can be significant.

In this study, we consider these issues by (i) undertaking an extensive screening for the presence of *Coxiella* infections in 11 geographically distinct populations of seabird ticks, (ii) measuring the genetic diversity of *Coxiella* isolates from these ticks through gene sequences of the *16S rDNA* and the *GroEL* genes, and (iii) characterizing the relatedness of these isolates with *C. burnetii* and other known *Coxiella*-like organisms. Based on these data, we make specific hypotheses about the role played by ticks, and notably seabird ticks, as reservoirs and vectors of *Coxiella* at broad spatial scales.

Materials and methods

Tick collection

Ticks were collected from taxonomically diverse seabirds, either from nesting material or directly from the birds (12 species belonging to 8 avian families; Table 1) and preserved in 70% ethanol. This collection encompasses 92 *O. capensis* s.l. specimens from 6 populations and 94 *I. uriae* specimens from 5 populations, all sampled 2003–2013 from distant geographic locations covering their entire distribution (including circumpolar areas of both hemispheres, as well as temperate and tropical areas of the Pacific, Atlantic, and Indian Oceans; Table 1 and Fig. 1). Although *O. capensis* s.l. represents a species complex with several described morphospecies, there is little consensus on the morphological traits that distinguish these ticks making their identification difficult, even for experienced taxonomists (Estrada-Peña et al., 2010), and recent work has called into question their previously characteristic geographic distributions (Gomez-Diaz et al., 2012). For these reasons, we refer to all *O. capensis* specimens as *O. capensis* s.l.

Screening and sequencing for Coxiella infections

DNA was extracted from ticks using the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's instructions. The quality of the extract was systematically tested by PCR amplification of a conserved region of the small ribosomal subunit *18S rRNA* encoding gene of ticks (Table 2). Using *Coxiella* sequences deposited in GenBank, *Coxiella*-specific primers were designed for 2 protein coding genes, the *16S rDNA* (encoding for the small ribosomal subunit) and the *GroEL* (Type I chaperonin) gene (Table 2). *Coxiella* infections were first screened in all tick extracts using a nested PCR assay based on the amplification of a 625-bp fragment of the *16S rDNA* gene. Positive infections were then characterized through the sequencing of both *16S rRNA* and *GroEL* gene fragments (1321–1416 bp and 619 bp, respectively), as detailed in Table 2.

We performed nested PCR amplifications as follows. The first PCR run with the external primers was performed in a 10-μL volume containing 20–50 ng of genomic DNA, 3 mM of each dNTP (Thermo Scientific), 8 mM of $MgCl_2$ (Thermo Scientific), 3 μM of each primer, 1 μL of 10× PCR buffer (Thermo Scientific), and 0.5 U of Taq DNA polymerase (Thermo Scientific). A 1-μL aliquot of the PCR product from the first reaction was then used as a template for the second round of amplification. The second PCR was performed in a total volume of 25 μL and contained