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Borrelia, *Coxiella*, and *Rickettsia* in *Carios capensis* (Acari: Argasidae) from a brown pelican (*Pelecanus occidentalis*) rookery in South Carolina, USA

Will K. Reeves · Amanda D. Loftis · Felicia Sanders
Mark D. Spinks · William Wills · Amy M. Denison
Gregory A. Dasch

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Abstract Argasid ticks are vectors of viral and bacterial agents of humans and animals. *Carios capensis*, a tick of seabirds, infests the nests of brown pelicans, *Pelecanus occidentalis*, and other ground nesting birds along the coast of South Carolina. This tick is associated with pelican nest abandonment and could pose a threat to humans visiting pelican rookeries if visitors are exposed to ticks harboring infectious agents. We collected ticks from a pelican rookery on Deveaux Bank, South Carolina and screened 64 individual ticks, six pools of larvae, and an egg mass for DNA from *Bartonella*, *Borrelia*, *Coxiella*, and *Rickettsia* by polymerase chain reaction amplification and sequencing. Ticks harbored DNA from ''*Borrelia lonestari*'', a novel *Coxiella* sp., and three species of *Rickettsia*, including *Rickettsia felis* and two undescribed *Rickettsia* spp. DNA from the *Coxiella* and two undescribed *Rickettsia* were detected in unfed larvae that emerged in the laboratory, which implies these agents are transmitted vertically by female ticks. We partially characterize the novel *Coxiella* by molecular means.

Keywords Tick · Pelican · *Rickettsia* · *Borrelia* · *Coxiella* · South Carolina

Introduction

Argasid ticks naturally transmit arboviruses and relapsing fever spirochetes (*Borrelia* spp.), but are generally not considered natural vectors of rickettsial agents

W. K. Reeves (✉) · A. D. Loftis · A. M. Denison · G. A. Dasch
Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS G-13, Atlanta GA 30333, USA
e-mail: cui8@cdc.gov

F. Sanders · M. D. Spinks
South Carolina Department of Natural Resources, Santee Coastal Reserve, P.O. Box 37, McClellanville, SC 29458, USA

W. Wills
17A Battery Walk Ct, Columbia, SC 29212, USA

(Hoogstraal 1985). Rickettsial agents are occasionally associated with argasid ticks. For example, *Otobius megnini* (Duges) can be naturally infected with *Ehrlichia canis* but does not transmit this agent (Ewing et al. 1990). *Ornithodoros papillipes* (Birula) vertically transmits *Rickettsia sibirica* but does not transmit it to vertebrate hosts (Podboronov and Pchelkina 1989). Intracellular rickettsia-like symbionts were described in laboratory maintained *Argas arboreus* Gothe, but they are not known to infect vertebrates or cause disease (El Shoura 1990).

Carios capensis (Neumann), an ectoparasite of seabirds, was first reported from South Carolina by Keirans et al. (1992). Brown pelican colony abandonment is thought to result from tick infestation of the nests (King et al. 1977a, b; Duffy 1983). Surveys of ticks indicate that *C. capensis* is established on islands in Charleston County, and tick infestations might be associated with pelican nest abandonment (Williams et al. 1999; Reeves et al. 2002). These ticks also bite humans, are vectors of Soldado virus, and are a threat to public health when sea birds nest in urban areas (Doherty et al. 1969; Converse et al. 1975; Estrada-Pena and Jongejan 1999). Arboviruses such as Aranas Bay virus have been isolated from *C. capensis*, but the pathogenicity of these agents to wildlife or humans is unknown (Yunker et al. 1979). Other species in the *C.* (*Alectorobius*) *capensis* group bite humans, and their bites are associated with fever, headache, and local irritation (Hoogstraal and Bafort 1982; Hoogstraal et al. 1985), which can be symptoms of exposure to pathogens or allergens.

Carios capensis infests pelican nests on South Carolina's coastal islands, including Deveaux Bank. Ticks are associated with nest abandonment on this island, and deserted nests often have > 50 ticks (pers. observ.). Sea kayakers and bird watchers visit Deveaux Bank and are potentially exposed to *C. capensis*. The role of *C. capensis* in the transmission of rickettsial agents and *Borrelia* spp. are unknown, but both humans and wildlife are exposed to potentially infected ticks. We initiated a study to determine if pelican ticks harbor potentially pathogenic microbial agents.

Materials and methods

Carios capensis was collected from nests of the brown pelican (*Pelecanus occidentalis* Linnaeus) on Deveaux Bank Heritage Preserve, Charleston County, SC, USA (32.549° N, 80.179° W) on 21 April 2004 (1 tick) and 11 August 2004 (75 ticks). Ticks were transported to the laboratory live, identified as nymphs, males, or females, and maintained in a 76–80% humidity chamber over a saturated urea solution. Nymphal and male ticks were maintained together in tubes, but individual females were maintained in plastic vials and allowed to lay eggs. Female ticks collected on 11 August 2004 laid eggs in the laboratory and larvae emerged from these eggs. Larvae were divided into six pools of 10 larvae each for DNA extraction.

DNA was extracted from 64 individual ticks (15 females, 46 males and nymphs), 6 pools of larvae, and 1 egg mass by freezing them in liquid nitrogen and crushing them with a sterile Teflon pestle. Teflon pestles were cleaned in 10% sodium hypochlorite for 3 h, rinsed in distilled water, and autoclaved before each use. Total DNA was extracted from the pulverized remains with an IsoQuick Nucleic Acid Extraction Kit (ORCA Research Inc., Bothell, Washington) and resuspended in nuclease free water. The extract was screened for the presence of DNA from *Bartonella*, *Borrelia*, *Coxiella*, and *Rickettsia* by polymerase chain reaction (PCR)

amplification using primers described by Carl et al. (1990), Stein and Raoult (1992), Barbour et al. (1996), Yuasa et al. (1996), Roux et al. (1997), Stein et al. (1997), Fournier et al. (1998), Sekeyova et al. (1999), Houpikian and Raoult (2001), Lee et al. (2004) and Reeves (2005), and novel primer pairs in Table 1. PCR conditions consisted of an initial DNA denaturation at 95°C for 5 min; followed by 40 consecutive cycles of 1 min denaturation at 95°C, primer annealing at previously specified temperatures for 1 min, extension at 72°C for 1 min, and a final 10 min extension at 72°C. Positive and negative controls were used in all screens and consisted of genomic DNA extracts of *Bartonella henselae*, *Borrelia burgdorferi* sensu stricto, *Coxiella burnetii*, and *Rickettsia rickettsii*, or distilled water. Control reactions were always the last to be set up in PCR strips and were the last to be loaded into gels. PCR was performed using a Taq PCR Master Mix Kit (Qiagen, Valencia, California). All PCR and sequencing primer stock concentrations were 20 μmol. Each PCR reaction contained 12.5 μl of Taq PCR Master Mix Kit (Qiagen, Valencia, California), 7.5 μl of nuclease free water, 1.25 μl of each primer, and 2.5 μl of DNA extract in water. PCR products were separated by electrophoresis on 2% agarose gels and visualized under ultraviolet light with ethidium bromide. Products were purified with a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Duplicate sequencing reactions were performed with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California) using PCR primers, and excess dye was removed with a DyeEx 2.0 column (Qiagen, Valencia, California). Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, California), aligned and assembled with Seqmerge (Accelrys, San Diego, California), and compared to sequences in GenBank using the BLAST 2.0 program (NCBI, Bethesda, Maryland).

In order to reduce the possibility of contamination of the DNA extracts or PCR assays, no culturing or cultures of rickettsial agents or *Borrelia* were allowed in the laboratory where the DNA extraction or PCR were performed. All DNA extractions were preformed in a separate area of the laboratory from the PCR setup and gel electrophoresis. All PCR were set up in a containment hood to reduce airflow or contamination. The hood was UV sterilized and wiped clean with ethanol or bleach regularly. None of the agents detected nor their DNA had previously been in the laboratory where the research was conducted.

Voucher specimens of *C. capensis* are deposited in the United States National Tick Collection, Georgia Southern University, Statesboro, Georgia. DNA sequences are submitted to Genbank with the following accession numbers, ''*Borrelia lonestari*'': *fla* gene (DQ100451); *Coxiella* sp.: 16S rRNA (DQ100452), citrate synthase (*g1tA*) (DQ100453), *mucZ* (AY795081), heat shock protein (*htpB*) (DQ150581), ADP-heptose synthase (DQ249303), putative pyruvate dehydrogenase E1 component gene (DQ249304), LPS/O-antigen export permease gene (DQ217574), isocitrate dehydrogenase (*icd*) gene (DQ177442), putative pyruvate dehydrogenase E1 beta subunit gene (DQ177441), capsular polysaccharide biosynthesis protein I-like gene (DQ150580), CTP synthase (*pyrG*) gene (DQ150578), superoxide dismutase (*sod*) gene (DQ150577); 16S–23S rRNA internal spacer (DQ357511), *Rickettsia* sp. (more prevalent form): 17 kD antigenic gene (DQ105801), *rOmpB* (DQ105802); *Rickettsia* sp. (less prevalent form): 7 kD antigenic gene (DQ105801); *R. felis* 17 kD antigenic gene (DQ102709), *rOmpA* (DQ102710), *rOmpB* (DQ102711), and 16S rDNA (DQ102712) genes.

Table 1 PCR primers used to screen total DNA extracts of *Carios capensis* from Deveaux Bank, South Carolina 2004

Primer (gene)	Nucleotide sequence 5′–3′	Annealing temperature	Organism detected	Reference reaction conditions
Cox-F-pry2 (*PryG*)	TTATTTACCAACGTTCCTGAGCCG	60	*Coxiella* spp.	This paper
Cox-R-pry2 (*PryG*)	TTTATCCCGAGCAAATTCAATTATGG	60	*Coxiella* spp.	This paper
PDHA461F (Pyruvate dehydrogenase, E1 component)	ATGGTGAGACGGGAGAACCC	60	*Coxiella* spp.	This paper
PDHA461R (Pyruvate dehydrogenase, E1 component)	TGTCCTGTCTTGTCTTCGCG	60	*Coxiella* spp.	This paper
PDHE639F (Dehydrogenase, E1 component, beta subunit, putative)	CACTGGTCGAAGCCGTCAAT	55	*Coxiella* spp.	This paper
PDHE639R (Dehydrogenase, E1 component, beta subunit, putative)	ATGTCGCCACCTTCACGTAAT	55	*Coxiella* spp.	This paper
PDHE692F (Dehydrogenase, E1 component, beta subunit, putative)	CCCTTAACCATCCGCGCTAT	60	*Coxiella* spp.	This paper
PDHE692R (Dehydrogenase, E1 component, beta subunit, putative)	AGTGTACATCGGTGTGGCCA	60	*Coxiella* spp.	This paper
HEPT678F (ADP heptose-synthase, putative)	TTTTGCTGATATCGAACGCG	55	*Coxiella* spp.	This paper
HEPT678R (ADP heptose-synthase, putative)	CACGATCCACAAAGCTCGAAA	55	*Coxiella* spp.	This paper
HEPT1655F (ADP heptose-synthase, putative)	GCTTGTTGCAGCGCTATCAG	60	*Coxiella* spp.	This paper
HEPT1655R (ADP heptose-synthase, putative)	TTCCATGCGCGCTTGTAAC	60	*Coxiella* spp.	This paper
OANT704F (LPS/O-antigen export permease)	TTATCGACCGACTAAAGGCCA	60	*Coxiella* spp.	This paper
OANT704R (LPS/O-antigen export permease)	GCACCCTTGCCTCGATAAAC	60	*Coxiella* spp.	This paper
OANT703F (LPS/O-antigen export permease)	AAATACGCAGGCGCTACAAAC	60	*Coxiella* spp.	This paper
OANT703R (LPS/O-antigen export permease)	TTGTTGGATATCGCGCACC	60	*Coxiella* spp.	This paper
CAPI844F (Capsular polysaccharide biosynthesis protein)	ATTTAGTGGGTTTCGCGCAT	60	*Coxiella* spp.	This paper
CAPI844R (Capsular polysaccharide biosynthesis protein)	CATCAGCATACGTTTCGGGAA	60	*Coxiella* spp.	This paper

Results and discussion

Both positive and negative controls produced the expected results. None of the agents detected in this study were used as controls.

Rickettsial infections are often rare, with 1–10% of wild ticks being infected (e.g., Guedes et al. 2005). DNA from *Rickettsia felis* was detected in 1 of 64 *C. capensis*. Rickettsial species identification was based on sequence similarity between amplicons from the *Rickettsia* in *C. capensis* and *R. felis* sequences in GenBank, including the complete genome (GenBank#CP000053). PCR amplicons were similar to those of *R. felis*, including a 100% similarity of 394 bp of the 17 kD antigenic gene to both an isolate of *R. felis* (GenBank#AF195118) and to the complete genome of *R. felis*, a 100% similarity of the rOmpA gene to the complete genome of *R. felis* (GenBank#CP000053), a 99% similarity of 755 bp to both the rOmpB gene of an isolate of *R. felis* (GenBank#AF182279) and to the complete genome of *R. felis* (GenBank#CP000053), and a 99% similarity of 1356 bp of 16S rRNA to an *R. felis* (GenBank#L28944) isolate from a patient and to the complete genome of *R. felis* (GenBank#CP000053).

Rickettsia felis was previously known as symbiote of *Ctenocephalides felis* (Bouché) (Siphonaptera: Pulicidae) and is the causative agent of a ''murine-typhus like disease'' in humans (Williams et al. 1992). *Rickettsia felis* is maintained in cat fleas by vertical transmission, but horizontal transmission to fleas from infected vertebrates has not been demonstrated (Wedincamp and Foil 2002). This agent is known primarily from Pulicidae of Old World origin, but *Anomiopsyllus nudata* Baker, a Nearctic flea, has been reported to harbor *R. felis* (Stevenson et al. 2005). Durden et al. (1999) suggest that *R. felis* is established in South Carolina, because it was detected in fleas from the neighboring state of Georgia.

The susceptibility of pelicans to *R. felis* and the source of the *Rickettsia* in *C. capensis* is unknown. While pets are not allowed on Deveaux Bank, we noted dog footprints in close proximity to human footprints among the pelican nests. In South Carolina, dogs are frequently infested with *C. felis* (Durden et al. 1999), and fleas infected with *R. felis* could drop off on the island and later feed on a pelican. Alternately, *C. capensis* could naturally harbor *R. felis*. The infection rates of *R. felis* in laboratory colonies of *C. felis* ranges from 2.5 to 63% (Wedincamp and Foil, 2002). If 2.5% of the ticks on Deveaux Bank were infected, we would expect to find one in 50 ticks to harbor *R. felis*. Further research is needed to determine the relationship between argasid ticks and *R. felis*.

Two undescribed *Rickettsia* spp. were detected in two and 14 ticks, respectively. The 17 kD antigenic genes were amplified and sequenced from both *Rickettsia* spp. This gene is highly conserved within species of *Rickettsia*. The sequences were unique when compared to each other and to those in GenBank. The PCR amplicon from the more prevalent *Rickettsia* sp. was 97% similar to *Rickettsia* sp. ARANHA genotype (GenBank# AY360215). The amplicon from the less prevalent *Rickettsia* sp. was 96% similar to that of *R. australis* (GenBank# M74042). A 797 bp fragment of the rOmpB gene was successfully amplified from the more prevalent *Rickettsia* sp. This amplicon shared a 91% similarity to *Rickettsia massiliae* (GenBank# AF123714) but could not be compared to the *Rickettsia* sp. ARANHA genotype, because a similar region of the gene has not been sequenced. DNA from the more prevalent *Rickettsia* sp. was detected in the pool of larvae that emerged from eggs laid by

females in the laboratory. The presence of DNA from *Rickettsia* in tick larvae suggests that it is vertically transmitted by transovarial transmission. We did not sample all females, egg masses, or larvae and cannot determine the filial or transovarial infection rates. DNA from a novel *Coxiella* sp. in the ticks interfered with the citrate synthase (*glt*A) PCR assay designed for *Rickettsia* (Roux et al. 1997), and we were unable to amplify DNA from *Rickettsia* with these primers.

An undescribed *Coxiella* sp. was detected in all ticks. *Coxiella burnetii* is the sole species named in the genus *Coxiella* (Skerman et al. 1980). The DNA sequences of the agent detected in *C. capensis* were not identical to any strain of *C. burnetii*. Initial identification of this agent was based on the 16S rDNA and *muc*Z amplicon sequences, which shared a 98% and 94% similarity to sequences from the *C. burnetii* genome (GenBank# AE016965-AE016828). Amplification of DNA from *Coxiella* from all ticks, including egg masses and unfed larvae, provides evidence of transovarial and transstadial transmission of this agent. The potential pathogenicity of this novel agent for vertebrates is unknown. Endosymbiotic *Coxiella* spp. are known from both argasid and ixodid ticks (e.g., Lee et al. 2004; Mediannikov et al. 2003; Noda et al. 1997; Roshdy 1968), but the nature of the symbiotic relationship between the bacterium and its tick host is unknown. Likewise, the phylogenetic relationships between *C. burnetii* and the symbiotic *Coxiella* spp. are unknown. We summarize the similarity between genes sequenced from this new *Coxiella* sp. and *Coxiella burnetii* in Table 2. The genes sequenced from this agent are unique but most closely related to *C. burnetii* and share a 93–98% similarity to this agent. No phylogeny was derived for these genes, because *C. burnetii* is the only recognized species in the genus *Coxiella* that could be compared to these sequences. With the exception of the 16S rRNA gene, which is highly conserved, all other bacterial sequences were not similar to other agents other than *C. burnetii*.

DNA from a *Borrelia* sp. was detected in 2 of 59 ticks. A 324 bp amplicon of the flagellin (*fla*) gene was 99% similar to that of ''*Borrelia lonestari*'' (GenBank# AY237713). The fla gene is conserved and used in the identification of *Borrelia* spp. (Barbour et al. 1996); however, we feel that definitive species identification of an unexpected *Borrelia* spp. from a tick is best made by further characterization of additional genes or culturing of the agent by a laboratory that specializes in spirochete isolations. We did not further characterize this agent but present its presence because that data might be of interest to spirochete workers.

The only known vector of ''*B. lonestari*'' is an ixodid tick, *Amblyomma americanum* (Linnaeus) (Varela et al. 2004). *Amblyomma americanum* will feed on ground nesting birds, and *C. capensis* could have acquired ''*B. lonestari*'' while feeding on infected birds. We did not look for this tick on Deveaux Bank; however, it was reported to be widely distributed in South Carolina (Williams et al. 1999; Reeves et al. 2002) and on coastal islands (Whitlock et al. 2000). While *C. capensis* is not currently known to transmit *Borrelia* sp. to humans (Estrada-Pena and Jongejan 1999), this tick will bite people.

We conclude that *C. capensis* harbors potentially pathogenic bacterial agents in the genera *Borrelia, Coxiella*, and *Rickettsia*. DNA from *Bartonella* was not detected by PCR amplification. The larvae of *C. capensis* will bite humans, and possibly domestic animals, that visit pelican rookeries (Estrada-Pena and Jongejan 1999). Visitors to Deveaux Bank or similar islands could be exposed to infected ticks. These ticks might transmit pathogens to visitors or their pets. In addition, brown pelicans are exposed to infected ticks, and the pathogenicity of the associated organisms to

Table 2 Gene sequence similarities between *Coxiella burnetii* and the novel *Coxiella* sp. from *Carios capensis*, collected from South Carolina, 2004

Gene from *Coxiella* sp.	Length of fragment and percent similarity to *Coxiella burnetii* and other undescribed *Coxiella*
16S rDNA	1274 bp 97% to *Ornithodoros moubata* symbiote A (AB001521), 98% to *C. burnetii* (AE016828), 95% to *Haemaphysalis longicornis* symbiont 66 (AY342036), 94% to *Coxiella* sp. (*Rhipicephalus sanguineus* symbiont) (D84559), 94% to symbiont of *Haemaphysalis concinnae* (AF521888), 93% to *Legionella* sp. OUB41 (AB058918)
16s–23s rDNA intergenic region	722 bp 95% to *C. burnetii* genome (AE016828)
GltA	379 bp 94% to *C. burnetii* genome (AE016828)
MucZ	792 bp 94% to *C. burnetii* genome (AE016828)
Icd	612 bp 93% to *C. burnetii* genome (AE016828)
Sod	258 bp 98% to *C. burnetii* genome (AE016828)
HtpB	287 bp 97% to *C. burnetii* genome (AE016828)
PryG	501 bp 96% to *C. burnetii* genome (AE016828)
Pyrute dehydrogenase, E1 component	646 bp 95% to *C. burnetii* genome (AE016828)
Dehydrogenase, E1 component, beta subunit, putative	595 bp 95% to *C. burnetii* genome (AE016828)
ADP Heptose-synthase, putative	762 bp 94% to *C. burnetii* genome (AE016828)
LPS/O-antigen export permease	646 bp 95% to *C. burnetii* genome (AE016828)
Capsular polysaccharide biosynthesis protein	601 bp 93% to *C. burnetii* genome (AE016828)

pelicans is unknown. Some *Coxiella* and *Rickettsia* species are infectious in aerosolized dust, and islands with heavy tick infestations might produce infectious aerosol plumes that are a threat to public health.

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