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14. ABSTRACT
We are reporting the first known isolation of the Q-fever agent, *Coxiella burnetii*, from field collected cayenne ticks, *Amblyomma cajennense*, in North America. Q-fever affects a number of domestic ungulates where it can lead to abortion in sheep and goats. There is far less known about the disease's effects on wild species, due largely to the tendency of the disease to self-resolve and long-term immunity to subsequent infections. The first recovery of *Coxiella burnetii* in North America was from the tick species *Dermacentor andersoni*. Since the original isolation *C. burnetii* has been recovered from five other North American tick species. The currently accepted mode for the majority of human infections is inhalation. The Centers for Disease Control Viral and Rickettsial Zoonoses Branch asserts the Q-fever agent as requiring as few as one organism to cause disease, via inhalation, in susceptible humans. However, with more and more isolations from ticks, evidence linking *C. burnetii* and ticks is mounting. The true role of tick species as competent vectors is still unconfirmed one way or the other. Preemptive field collections of possible vector arthropods, hosts and reservoirs can provide invaluable baseline environmental data that will prove supportive in follow-up studies and abatement efforts.

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Q-fever agent, *Coxiella burnetii, Dermacentor andersoni,* Cayenne ticks, *Amblyomma cajennense*

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Field Collection and Genetic Classification of Tick-borne Rickettsiae and Rickettsiae-like Pathogens from South Texas: *Coxiella burnetii* Isolated from Field-collected *Amblyomma cajennense*

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We are reporting the first known isolation of the Q-fever agent *Coxiella burnetii* from field-collected cayenne ticks *Amblyomma cajennense* in North America. Q-fever affects a number of domestic ungulates where it can lead to abortion in sheep and goats. There is far less known about the disease's effects on wild species, primarily because of the tendency of the disease to self resolve and to provide long-term immunity to subsequent infections. The first recovery of *C. burnetii* in North America was from the tick species *Dermacentor andersoni*. Since the original isolation *C. burnetii* has been recovered from five other North American tick species. The currently accepted mode for the majority of human infections is inhalation. The Centers for Disease Control and Prevention, Division of Viral and Rickettsial Diseases, Rickettsial Zoonoses Branch asserts the Q-fever agent as requiring as few as one organism to cause disease via inhalation in susceptible humans. However, with more and more isolations from ticks, evidence linking *C. burnetii* and ticks is mounting. The true role of tick species as competent vectors is still unconfirmed. Preemptive field collections of possible vector arthropods, hosts, and reservoirs can provide invaluable baseline environmental data that will prove supportive in follow-up studies and abatement efforts.

Key words: Coxiella burnetii; *Amblyomma cajennense*; **cayenne tick; Q-fever; tick-bone fever**

Introduction

Sir Frank MacFarlane Burnet, of the Walter and Eliza Institute in Melbourne, Australia, verified *Coxiella burnetii* as the causative agent of Brisbane abattoir "Q"-fever cases in October 1936.[1] At the time, researchers gave *C. burnetii* a *Rickettsia* genus designation[1] and *burneti* species designation in recognition of Burnet's development of the agglutination test for Q-fever.[2] Dr. Herald Cox, of the US Public Health Service's Rocky Mountain Laboratory, almost simultaneously named the agent *Rickettsia diaporica*.[3] He did this 8 days prior in fact.[3] However, because Dr. Cox rescinded his publication and designation in order to refine his work, *Rickettsia burneti* took precedence.[3] Cox's work would ultimately

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be acknowledged by the reclassification of the Q-fever agent's genus designation as *Rickettsia* to *Coxiella*.[3]

The first North American isolates came from *Dermacentor andersoni*[4] and *Amblyomma maculatum*,[5] collected from Nine Mile Creek area, Montana and Cleveland, Texas, respectively. There have been other isolations from North American tick species since then, but these data are scant, especially with regard to the vector competency for *C. burnetii*, transmission, and maintenance of Q-fever in the environment. The abortive effect on sheep and goats is documented, and it is generally accepted that humans are readily infected by aerosolized particles. However, little is mentioned about the possibility of this abortive effect on range cattle or wild ungulates. Cattle and big game hunting constitute large investments on the part of ranchers and landowners from Mexico to northern Canada. The effect of Q-fever on these investments is largely unknown, but given the pathogenicity of *C. burnetii*, it warrants investigation to develop baseline data that might prove beneficial in disease prevention. The lack of reliable data holds true for human cases, diagnosed simply as "tick-bone fever," across the United States as a result of antibiotic availability and poor local reporting practices.[6]

The avoidance of the time-consuming reporting process and the unwillingness to commit patient funds to confirmatory tests likely influenced the vague diagnoses of the Alice, Texas, tick-bone fever cases. The majority of local practitioners have seen enough cases to recognize when broad-spectrum antibiotics are first order defense. Unfortunately, the only scientific data to come out of this area thus far revealed a *Rickettsia honei*-like agent in a local tick population. Researchers identified the agent by PCR only, and there was enough variance to raise questions as to whether it was truly an infective form of *R. honei*.[7] Since the organism was never sequenced, cultured, or tested to confirm identification or infectivity, there is still no concrete data as to what the causative agent of the south Texas tick-bone fever is.

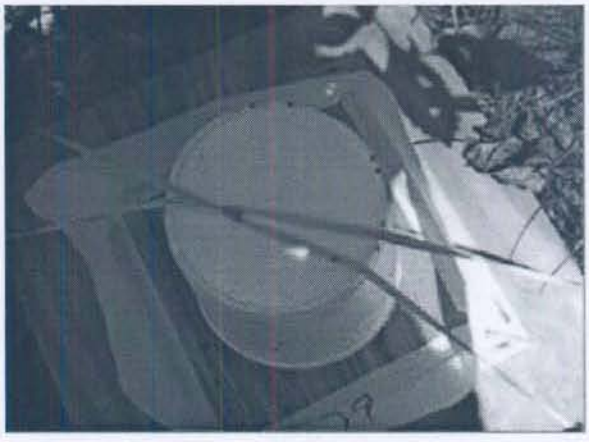

Figure 1. CO_2 trap with ticks from La Copita Ranch, Texas. Photo courtesy of D. Sanders. (In color in *Annals* online.)

Materials and Methods

Tick collections were conducted at Texas A&M's Demonstration and Research Ranch, La Copita Ranch, Alice, Texas, in October 2005 and May 2006. Collections consisted of 30 and 50 CO_2 tick traps, respectively, baited with dry ice. Personnel placed traps in habitats frequented by genera of the family Muridae (mice and rats) located within 23 m of ranch senderos. These habitats were a mix of cactus (*Opuntia* spp.), mesquite (*Prosopis glandulosa* Torr.), and white tridens (*Tridens albescens*).

Traps were, using Dr. Pete Teel's model, 30.48 cm^2 (1 ft^2) pieces of 3/8″ plywood base with quarter-round floor trim used to create a 17.78 cm^2 (7 in^2) inner square.[8] Thirty-two-ounce styrofoam cups, previously perforated along the tops, were filled with dry ice, inverted, and centered on the plywood bases within the quarter-round trim. Stretching #16 rubber bands diagonally corner to corner over the cups secure them to the plywood. Double-sided tape placed inside the quarter-round square captured and held ticks securely until removed (Fig. 1).

Traps were set by 0900 h each day. Trap locations were marked using handheld GPS units. Traps were left undisturbed for a minimum of 4 h. Following pickup all ticks

TABLE 1. Oligonucleotide Primers Used in the PCR-based Identification of Rickettsial and *Coxiella* Agents

Designator	Sequence 5′ to 3′	Length	Amplicon Size-bp
CS 162F[1] (B)	GCAAGTATCGGTGAGGATGTAATCG	25	606[1]
RpCS 877R[2] (R)	GGGGGCCTGCTCACGGCGG	19	606[1]
CS 162F[1] (B)	GCAAGTATCGGTGAGGATGTAATCG	25	381[2]
RpCS1258nR[1,2] (R)	ATTGCAAAAAGTACAGTGAACA	22	381[2]
RC 190F[1] (R)	GAGATAACGGCTGCAGGGGTA	21	740[1]
RC 190R[1] (R)	AACCGCTCCCCCTAACGTGGC	21	740[1]
RC 190NF[2] (B)	TTGGGCATTTACTTACGGTGG	21	558[2]
RC 190NR[2] (B)	GCCTGTGGTGTTATCAATCGC	21	558[2]

[1]Conserved in-group sequences. [2]Nested sequences: (B) Billings *et al.*,[7] (R) Regnery *et al.*[9]

TABLE 2. Tick Trapping Success for 2005 and 2006

Date	Species	Life Stage	Sex	Count
October 2005	*Amblyomma maculatum*	Adult	Male	2
	A. maculatum	Adult	Female	8
	Aponomma sp.	Adult	Female	1
	Amblyomma cajennense	Nymph		139
May 2006	*A. cajennense*	Adult	Male	11
	A. cajennense	Adult	Female	6
	A. cajennense	Nymph		1458

and fleas were removed using BioQuip® Featherweight broad-tip forceps, placed in screw cap glass vials containing 100% EtOH, and labeled according to trap number (BioQuip, Rancho Dominguez, CA).

Nymphal pools, by trap site, were ground in saline buffer. Adult ticks were individually processed by trap site. The tick DNA solution (5 μL) from QIAamp DNA mini kit (for tissue) (QIAGEN, Valencia, CA) was amplified using a Perkin Elmer (Waltham, MA) thermocycler and 50 μL mix [0.2 mmol/L deoxyribonucleotide triphosphate (dNTP), 1X buffer (1 mmol/L $MgCl_2$), 1 mmol/L $MgCl_2$, 2.6% dimethylsulfoxide (DMSO), 200 mmol/L each primer, 2U TAQ polymerase]. Primer sets used were from Billings *et al.*[7] and Regnery *et al.*[9] specific for *gltA* and *rompA* genes (Table 1). Sequenced products were compared using GenBank BLAST of relevant reported sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Over the 2-year period, 1597 nymphs, 14 females, and 13 male *Amblyomma* spp. ticks were collected (Table 2). Nine nymphal pools, two female ticks, and three male ticks were positive for *C. burnetii*, with one female tick also positive for a *R. honei*-like rickettsia (Table 3). For 2005, 14 pools of *A. cajennense* nymphs were tested; four were found to be positive for a field infection rate of 28.5%. Of the 29 *A. cajennense* nymphal pools tested in 2006, four were positive, resulting in a field infection rate of 13.8%. There were eight male

TABLE 3. PCR Results: Rickettsial and *Coxiella* DNA Isolates

Year	Species	Life Stage	Sex	Positive Pools	Agent
2005	*A. cajennense*	Nymph		4	*Coxiella burnetii*
2006	*A. cajennense*	Nymph		4	*C. burnetii*
2006	*A. cajennense*	Adult	Male	3	*C. burnetii*
2006	*A. cajennense*	Adult	Female*	2	*C. burnetii*

*One female was copositive for a *Rickettsia honei*-like species. Sequencing suggests an uncharacterized species.

and seven female *A. cajennense* pools tested in 2006, with three and two positive, respectively, giving infection rates of 37.5% and 28.5%, respectively.

Discussion

Exhaustive literature searches failed to turn up a previous isolation of *C. burnetii* from field-collected *A. cajennense* ticks. In addition, to our knowledge, Q-fever has not been clinically confirmed from the La Copita area, and, without supporting clinical data, there is no way of knowing what the tick-bone fever agent was. However, with the discovery of C. *burnetii* being the prominent infectious agent in the tick population, in addition to the lack of clinical data, it is possible *C. burnetii* is the pathogen causing human disease in south Texas.

Again, to our knowledge, there has yet to be a definitive identification of the agent associated with the tick-bone fever cases from the La Copita area. The situation warrants further investigation; specifically, what agents exist in the area, which agents are truly infectious, and vector competency testing. Answers to these would elucidate what agents pose a true health hazard and which are simply endosymbionts; insight to the transmission cycle would also be provided. The relative absence of the *R. honei*-like agent and *C. burnetii* from the greater portion of the ticks collected suggests these forms are not symbiotic with the tick species collected. However, we cannot at this time say whether they are pathogenic to humans, livestock, or wildlife.

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Conflicts of Interest

The authors declare no conflicts of interest.

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