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Coxiella Detection in Ticks from Wildlife and Livestock in Malaysia

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Abstract

Recent studies have shown that ticks harbor *Coxiella*-like bacteria, which are potentially tick-specific endosymbionts. We recently described the detection of *Coxiella*-like bacteria and possibly *Coxiella burnetii* in ticks found from rural areas in Malaysia. In the present study, we collected ticks, including *Haemaphysalis bispinosa*, *Haemaphysalis hystricis*, *Dermacentor compactus*, *Dermacentor steini*, and *Amblyomma* sp. from wildlife and domesticated goats from four different locations in Malaysia. *Coxiella 16s rRNA* genomic sequences were detected by PCR in 89% of ticks tested. Similarity analysis and phylogenetic analyses of the *16s rRNA* and *rpoB* partial sequences were performed for 10 representative samples selected based on the tick species, sex, and location. The findings here suggested the presence of *C. burnetii* in two samples, each from *D. steini* and *H. hystricis*. The sequences of both samples clustered with published *C. burnetii* sequences. The remaining eight tick samples were shown to harbor *16s rRNA* sequences of *Coxiella*-like bacteria, which clustered phylogenetically according to the respective tick host species. The findings presented here added to the growing evidence of the association between *Coxiella*-like bacteria and ticks across species and geographical boundaries. The importance of *C. burnetii* found in ticks in Malaysia warrants further investigation.

Keywords: *Coxiella*, domestic animals, ticks, zoonotic

Introduction

Ticks are effective vectors for a range of infectious diseases. A number of hard and soft tick species, including *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Hyalomma*, *Rhipicephalus*, and *Ornithodoros*, have been documented to harbor the *Coxiella burnetii* (Špitalská and Kocianová 2003, Mediannikov et al. 2010, Cooper et al. 2012). *C. burnetii* is the causative agent for Q fever, a worldwide zoonotic disease commonly affecting livestock animals such as cattle, goats, and sheep. Human infections are most likely due to contact with excreta from these animals or via the inhalation of contaminated aerosols (Tissot-Dupont and Raoult 2008). The role of ticks in transmitting Q fever to humans and animals has not been established, even though ticks are observed to be competent vectors for the transmission of *C. burnetii* to mammalian hosts in the laboratory setting (Duron et al. 2015). However, a high prevalence of *C. burnetii* in ticks in some endemic regions, such as West Africa, may indicate a role for ticks in the epidemiology of Q fever (Mediannikov et al. 2010).

A number of studies have identified a family of tick endosymbionts that are closely related to *C. burnetii*. These endosymbionts have been found in a variety of ticks species, including *Haemaphysalis* (Lee et al. 2004, Ahantarig et al. 2011, Arthan et al. 2015), *Amblyomma* (Klyachko et al. 2007, Machado-Ferreira et al. 2011), *Rhipicephalus* (Bernasconi et al. 2002), *Ixodes* (Kurtti et al. 2002), *Ornithodoros* (Almeida et al. 2012, Duron et al. 2014), and *Argas* (Reeves 2008). The most recent study based on multilocus sequence analysis of a few housekeeping genes demonstrated that all *Coxiella* strains cluster into four highly divergent clades, suggesting that *C. burnetii* evolved from a tick endosymbiont (Duron et al. 2015). Studies have indicated that these *Coxiella* endosymbionts may be important in providing for the vitamin and cofactor biosynthesis pathways and in determining the reproductive fitness of the tick hosts (Jasinskas

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et al. 2007, Zhong et al. 2007). Therefore, understanding the role of *Coxiella* endosymbionts in maintaining tick growth and survival may yield novel approaches in the control and management of tick populations.

In Malaysia, human populations living in the forested or rural regions, including farmers and the indigenous people of Malaysia, the Orang Asli, are at risk of infection from tick-borne pathogens (Audy et al. 1960, Paramasvaran et al. 2009). Unpublished seroprevalence data from our laboratory were indicative of past infections with *C. burnetii* among the Orang Asli populations. However, the prevalence of *C. burnetii* and *Coxiella*-like bacteria associated with ticks from the forests or rural areas in Malaysia has never been fully investigated. In this study, we performed molecular detection of *Coxiella* bacteria from ticks collected from wildlife and farm areas from selected sites in Malaysia.

Materials and Methods

Tick samples

Ticks were collected with the assistance from the Orang Asli from the carcasses of wild animals (wild boars and a single porcupine) obtained from routine hunting trips in the state of Selangor (3.0738° N, 101.5183° E). All site visits to the Orang Asli villages were performed with the approval from the Department of Orang Asli Development, Malaysia (JAKOA). Ticks were also collected from goats found in privately owned farms located in two locations in the state of Perak (4.5921° N, 101.0901° E), and one location in the state of Negeri Sembilan (2.7258° N, 101.9424° E), with the permission from the respective farm owners. All goat farms were managed by semi-intensive grazing system, in which the grazing area includes palm oil and rubber plantations as well as secondary forests. Once removed from the hosts, the ticks were kept alive in ziplock bags for transportation to the laboratory within 24 h and stored in –80°C until further processing. All sampling was performed during the time from July 2014 to July 2015. The collected ticks were microscopically identified and classified by life stage using published taxonomic keys (Wassef and Hoogstraal 1983, 1986, Tanskul and Inlao 1989). For DNA extraction, frozen tick samples were thawed and washed rigorously three times in 70% ethanol followed by sterile deionized water to remove possible environmental contaminants.

DNA extraction from tick samples

Mortars and pestles were soaked in 10% bleach for 1 h, thoroughly rinsed with sterile deionized water, and sterilized at 160°C overnight to ensure the absence of contaminating materials. Swabs were taken and amplification of bacterial *16s rRNA* sequences was performed to ensure no residual genomic material remained (Khoo et al. 2016). Tick samples were ground in liquid nitrogen using chilled mortar and pestle in a biosafety cabinet, using separate sets of mortar and pestle for each sample. The resulting finely ground sample was resuspended in 500 μL of sterile phosphate-buffered saline (PBS). DNA was extracted from a 200 μL aliquot of the suspension using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Mock extractions on PBS alone were performed in parallel and the resulting DNA was subjected to further screening as below.

Polymerase chain reaction for ticks and Coxiella *gene detection*

PCR amplification of partial mitochondrial *16s rRNA* sequence of selected ticks was performed using primers and protocols previously described (Black and Piesman 1994) for *Haemaphysalis* identification. *Coxiella* sp. was detected by a nested-PCR protocol to amplify the *16s rRNA* partial gene sequence using primers previously described (Duron et al. 2014) together with a separate pair of nested primers (forward: 5′-ATTTCCGGTGTAGCGGTG-3′ and reverse: 5′-ACGACAGCCATGCAGCAC-3′). Samples exhibiting the amplification of all fragments during nested PCR were considered as positive for the presence of the *Coxiella* DNA. Samples displaying no amplification of the *Coxiella*-specific *16s rRNA* fragment were subjected to additional PCR amplification of nonspecific bacterial *16s rRNA* gene as described in an earlier study (Khoo et al. 2016). The partial sequence of the *rpoB* gene from selected samples was amplified using previously described primers and protocols (Duron et al. 2015). Amplicons from representative positive samples were selected, gel purified, and sequenced based on the criteria of species, sex, and location. Mock extractions were also tested in the nested-PCR procedures, which did not exhibit any amplification of the targeted genes.

Sequence analysis

The sequences obtained were compared to the available sequences in the NCBI GenBank database (www.ncbi.nml.nih.gov/BLAST/). Primer-trimmed sequences of *Coxiella* sp. *16s rRNA* and *rpoB* genes were aligned using CLUSTALW, as implemented in MEGA6 (Tamura et al. 2013). Poorly aligned positions were removed using GBLOCKS (Talavera and Castresana 2007). Genetic variation between each sample was determined by computing pairwise nucleotide differences in MEGA6. All sequences obtained were deposited in the European Nucleotide Archive (ENA) (Accession Numbers: LT593104–LT593134 for tick mitochondrial *16s rRNAs*, LT009428–LT009437 for *Coxiella 16s rRNAs*, and LT174608–LT174617 for *Coxiella rpoB*).

Phylogenetic analysis

Phylogenies were inferred using the Bayesian Markov chain Monte Carlo method implemented in BEAST version 1.8.2 (Drummond and Rambaut 2007). The best-fit model of nucleotide substitution was selected by Akaike Information Criterion as implemented in jModelTest 2.1.7 (Posada 2008). GTR+I+G and GTR + G were selected, respectively, for *16s rRNA* and *rpoB* phylogenies.

Results

Tick samples

In this study, 55 ticks were collected from four separate locations in Malaysia. The number of ticks and collected data are summarized in Table 1. From the Orang Asli village in Selangor, 34 adult ticks were collected. These ticks were identified as *Haemaphysalis hystricis* (13 males, 6 females), *Dermacentor steini* (6 males, 1 female), *Dermacentor compactus* (2 males), and *Amblyomma* sp. (6 females). All *H. hystricis* and *Dermacentor* ticks were collected from the

Table 1. *Coxiella* Detection in Ticks Collected from Various Locations in Malaysia Using *16s rRNA* Gene Amplification

Location	*Host*	*Species*	*Stage*	*Sex*	*Engorged*	*Total number*	*PCR positive for Coxiella partial 16s rRNA (n=x)*
Selangor	Wild boar	*Haemaphysalis hystricis*	Adult	F	No	6	6
				M	No	13	13
	Wild boar	*Dermacentor steini*	Adult	F	No	1	0
				M	No	6	2
	Wild boar	*Dermacentor compactus*	Adult	M	No	2	1
	Porcupine	*Amblyomma* sp.	Adult	F	Yes	5	5
					No	1	1
Perak—Farm B	Goats	*Haemaphysalis bispinosa*	Adult	F	Yes	2	2
					No	7	7
Perak—Farm T	Goats	*H. bispinosa*	Adult	F	Yes	3	3
					No	2	2
				M	No	2	2
Negeri Sembilan	Goats	*H. bispinosa*	Adult	F	No	4	4
			Nymph		No	1	1
		Total				55	49

carcasses of two wild boars. All *Amblyomma* ticks were collected from a single porcupine. Ticks (2 males, 18 females) collected from the goat farms in Perak and Negeri Sembilan were all identified as adult *Haemaphysalis bispinosa*. A single nymph was also identified as *H. bispinosa*. A number of female ticks were observed to be engorged: *Amblyomma* (5/6), *H. bispinosa* from Farm B (2/9) and Farm T (3/5) in Perak. The partial mitochondrial *16s rRNA* sequences amplified from *Haemaphysalis* ticks in Selangor and Perak were 99%–100% identical to *H. hystricis* (NCBI Accession No.: AB819197.1) and *H. bispinosa* (NCBI Accession No.: KC853419.1), respectively (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/vbz), which is consistent with the morphological identifications.

Detection of Coxiella *in tick samples*

Overall, 89% (49/55) of the ticks in this study were positive for *Coxiella 16s rRNA* genomic sequences (Table 1). All *Haemaphysalis* ticks tested, regardless of species, sex, location, or engorgement for females, exhibited positive *Coxiella* genomic sequences by PCR (40/40). *Coxiella* was also detected in all *Amblyomma* ticks tested (6/6), 2/6 of male *D. steini*, but not in the female tested (0/1). One out of the two *D. compactus* in the study was PCR positive for *Coxiella*. Nonspecific bacterial *16s rRNA* genes were amplified from all samples that tested negative for *Coxiella 16s rRNA* PCR (data not shown), indicating that the absence of the amplification of *Coxiella 16s rRNA* was not due to compromised sample DNA quality.

Analyses of tick-derived Coxiella 16s rRNA *partial sequences*

Ten representative samples, selected from nonengorged samples, were selected based on species, location of sampling, and sex (summarized in Table 2) to be sequenced for the *Coxiella* partial *16s rRNA* sequences. The similarity matrix resulting from a pairwise nucleotide comparison between all samples in this study, together with two *C. burnetii* reference strains, is presented in Table 3. All samples in this study, except for S009, S012, and S014, displayed 96.2% to 96.7% similarity to the *C. burnetii* reference strains. *Coxiella* sequences from *H. bispinosa* (N002, B002, T007, and T008) were 98.2% to 99.8% similar to each other. S002 and S006, both from *H. hystricis*, shared 99.9% sequence similarity. S014 from *H. hystricis* and S009 from *D. steini* also shared 99.9% sequence similarity to each other, but displayed only 96.2% to 96.7% similarity to other samples in this study. S012, from *D. compactus*, displayed 94.2% to 94.7% similarity to all other samples. S027, from *Amblyomma*, displayed only 96.6% to 96.9% sequence similarity to the reference strains and the other samples here.

Phylogeny of tick-derived Coxiella *partial* 16s rRNA *and* rpoB genes

A Bayesian-inferred phylogenetic tree was constructed based on the partial *16s rRNA* sequences from this study and *Coxiella* sequences derived from the NCBI GenBank (Fig. 1, NCBI accession numbers tabulated in Supplementary Table S2). The published sequences were selected to reflect the previously reported clustering of *Coxiella* strains into four highly divergent clades according to the tick hosts (Duron et al. 2015). Clade A, comprising various *C. burnetii* isolates and *Coxiella* of the

Table 2. Representative *Coxiella* Sequences Used in Phylogenetic Analysis

Sample number	*Tick species*	*Sex*	*Location*
N002	*H. bispinosa*	F	Negeri Sembilan
B002	*H. bispinosa*	F	Perak—Farm B
T007	*H. bispinosa*	F	Perak—Farm T
T008	*H. bispinosa*	M	Perak—Farm T
S002	*H. hystricis*	F	Selangor
S006	*H. hystricis*	M	Selangor
S014	*H. hystricis*	F	Selangor
S009	*D. steini*	M	Selangor
S012	*D. compactus*	M	Selangor
S027	*Amblyomma* sp.	F	Selangor

TABLE 3. SIMILARITY MATRIX OF *COXIELLA* PARTIAL *16S RRNA* SEQUENCES

	1	2	3	4	5	6	7	8	9	10	11	12
1 *Coxiella burnetii* RSA 493	100.0	99.9	96.4	96.4	96.4	96.2	96.7	96.7	100.0	99.9	94.8	96.7
2 *C. burnetii* CbuG Q212		100.0	96.3	96.3	96.3	96.1	96.6	96.7	99.9	99.8	94.9	96.6
3 *Coxiella* in *H. bispinosa* N002			100.0	99.8	99.8	99.7	98.3	98.4	96.4	96.3	94.3	96.6
4 *Coxiella* in *H. bispinosa* B002				100.0	99.8	99.8	98.3	98.4	96.4	96.3	94.3	96.6
5 *Coxiella* in *H. bispinosa* T007					100.0	99.7	98.3	98.4	96.4	96.3	94.3	96.6
6 *Coxiella* in *H. bispinosa* T008						100.0	98.3	98.2	96.2	96.3	94.2	96.6
7 *Coxiella* in *H. hystricis* S002							100.0	99.9	96.7	96.7	94.4	96.9
8 *Coxiella* in *H. hystricis* S006								100.0	96.7	96.7	94.5	96.8
9 *Coxiella* in *H. hystricis* S014									100.0	99.9	94.8	96.7
10 *Coxiella* in *D. steini* S009										100.0	94.7	96.7
11 *Coxiella* in *D. compactus* S012											100.0	94.7
12 *Coxiella* in *Amblyomma* sp. S027												100.0

The similarity of the *16s rRNA* sequence based on the comparison of 1165 nucleotide positions from the *Coxiella* reference and sample strains.

Ornithodoros soft ticks, as well as Clade B, consisting of *Coxiella* from *Amblyomma variegatum* and *Ixodes* sp., was clearly seen in our tree. Clade C comprising *Coxiella* from *Rhipicephalus* and Clade D comprising *Coxiella Haemaphysalis* and *Dermacentor* were also seen. However, *Coxiella* from *Amblyomma americanum* and *Amblyomma cajennense*, which hosted Clade D in the previous study, were separated from the other *Coxiella* strains (Duron et al. 2015). It is important to note that the separation between the members of Clades C and D was only partially supported (posterior probability of 0.67). Hence, the phylogenetic tree based on *16s rRNA* partial sequence alone may not be effective in resolving Clades C and D.

Coxiella from *D. steini* (S009) and *H. hystricis* (S014) were clustered with various *C. burnetii* isolates in Clade A. *Coxiella* from *H. hystricis* (S002 and S006) were clustered with the *Coxiella*-like bacteria from *Haemaphysalis longicornis*. *Coxiella* from *H. bispinosa* (N002, B002, T007, and T008) clustered with the *Coxiella* from *Haemaphysalis shimoga*. S027 from *Amblyomma* sp. clustered with *Coxiella* from *Ixodes uriae* and *A. variegatum*. The *Coxiella* endosymbionts from *Haemaphysalis*, *I. Uriae*, and *A. variegatum* appeared to form Clade B together. S012 from *D. compactus* appeared to be distinct from other *Coxiella* strains tested here.

As a complementary analysis, a separate phylogenetic tree was constructed based on the partial sequence of the *rpoB* gene (Fig. 2). The *rpoB* phylogeny showed S009 and S014 clustered with the other *C. burnetii* reference strains, which formed Clade A with the *Ornithodoros rostratus* endosymbionts. This finding was similar to the *16s rRNA* phylogenetic tree. *Coxiella* from the *Haemaphysalis* ticks (N002, B002, T007, T008, S002, and S006) and *Amblyomma* sp. (S027) clustered into Clade B along with the endosymbionts from *I. uriae* and *A. variegatum*, also consistent with the *16s rRNA* phylogenetic tree. However, *Coxiella* from *D. compactus* (S012) clustered with the *Coxiella* from *H. punctata* based on the *rpoB* phylogeny into a collapsed C and D Clade.

Discussion and Conclusion

In the present study, we detected the presence of *Coxiella* genomic sequence in a number of tick species, including *H. bispinosa*, *H. hystricis*, *D. compactus*, *D. steini*, and *Amblyomma* sp., collected from wildlife and goats in Malaysia. The results are consistent with reports identifying *Coxiella*-like bacteria or endosymbionts in a number of *Haemaphysalis*, *Dermacentor*, and *Amblyomma* tick species (Lee et al. 2004, Jasinskas et al. 2007, Klyachko et al. 2007, Liu et al. 2013, Arthan et al. 2015, Duron et al. 2015). Several female ticks of *Haemaphysalis* and *Amblyomma* sampled here were fully engorged, hence it is possible that any *Coxiella* DNA observed may have originated from host blood. However, the detection of *Coxiella* sequences in most of the nonengorged ticks suggests the association of *Coxiella* linked to the ticks themselves and not the animal host. Thus, only nonengorged tick samples were chosen as representative samples for sequencing and further analyses to avoid potential confounding effects from host blood.

In pairwise nucleotide comparisons of the eight *Coxiella*-like bacteria sequenced here, *Coxiella* from the same genus of ticks, such as *Haemaphysalis*, exhibited greater similarity to each other, suggesting that genetic variation is host dependent. Although Duron et al. (2015) have reported that *Coxiella* endosymbionts from *Haemaphysalis* belong to Clade D, our findings suggested that *Coxiella*-like bacteria from *H. hystricis*, *H. bispinosa*, *H. shimoga*, and *H. longicornis* are clustered together with *Coxiella* endosymbionts from *A. variegatum*, *I. uriae*, and the *Amblyomma* sp. (S027) in Clade B. It is important to note, however, that the *Haemaphysalis* hosts for the *Coxiella* strains clustered in Clade B in this study are primarily found in the Asian or Southeast Asian regions, which were not represented in the study by Duron et al. (2015). *H. punctata*, the only *Haemaphysalis* representative in Duron's study, commonly occupies the European continent. Since *Coxiella*-like bacteria are likely to have coevolved with the tick hosts, the geographical separation and the subsequent evolutionary history of ticks could have resulted in the genetic differences as shown in the phylogenies. This observation is also mirrored in the separation of the *Coxiella* strains from *Amblyomma* ticks into different clades (Duron et al. 2015). The *Coxiella* from *A. americanum* and *A. cajennense*, both dominantly found in the Americas, are clustered in Clade D. *A. variegatum*, which is prevalent in Africa, belongs to Clade B.

In this study, the phylogenies based on the *16s rRNA* or *rpoB* sequences individually were insufficient to separate the members of Clades C and D. These discrepancies may have

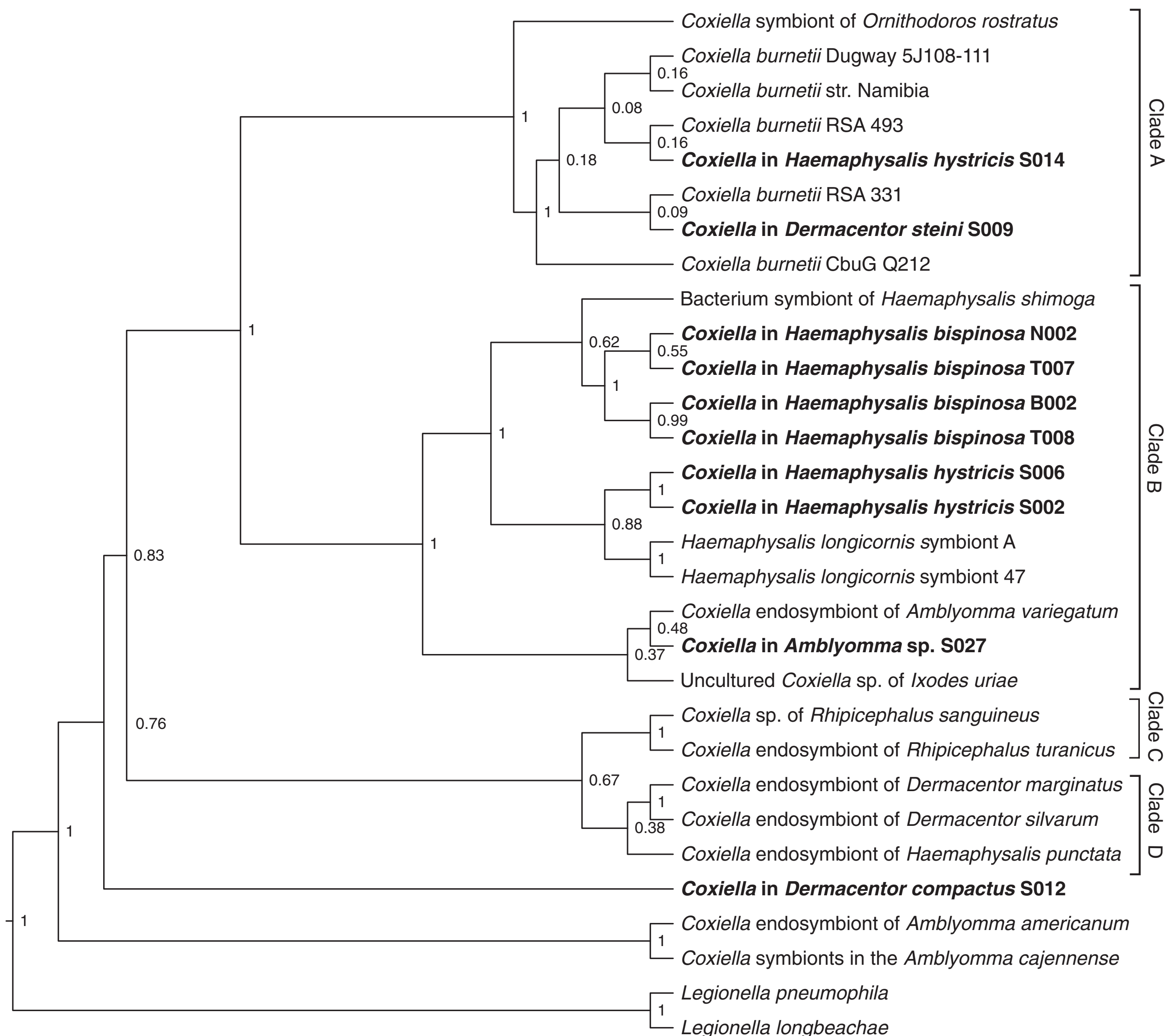

FIG. 1. Phylogenetic tree of *Coxiella* sp. based on partial *16s rRNA* sequences. Bayesian-inferred phylogenetic tree of *Coxiella* sp. based on 1165 aligned nucleotides of the *16s rRNA* sequence. The posterior probabilities are displayed adjacent to each node. Samples from this study are highlighted in *bold*. Clades labeled are indicative of the clustering of various *Coxiella* strains previously described by Duron et al. (2015). Sequences from *Legionella pneumophila* and *Legionella longbeachae* were included as the outgroup.

arisen due to the use of individual genes in the phylogenetic analyses here as opposed to the multilocus approach of the previous study, which used five concatenated gene sequences in the phylogenetic analyses (Duron et al. 2015). The single-gene analyses used here may also explain the inconsistencies observed for the *Coxiella* from *D. compactus* (S012) in the *16s rRNA* and *rpoB* phylogenies. The genetic relationship of this strain will need to be confirmed with additional analyses, including the use of the multilocus sequencing approach.

The *Coxiella* sequences obtained in this study were directly sequenced from PCR amplicons, which excluded the possibility for investigating coinfections of multiple species of *Coxiella*, including coinfections of *C. burnetii* and *Coxiella*-like bacteria. Recent studies have shown that coinfections of pathogens and endosymbionts in individual ticks were common especially in *Ixodes ricinus* ticks (Moutailler et al. 2016). At the time of writing, the authors were unaware of studies investigating the coinfections of *Coxiella* species in individual ticks, which merits further investigation as it will be important to study the functional interactions between potentially symbiotic *Coxiella* strains and the pathogenic *C. burnetii.*

Several studies have investigated the presence of *Coxiella*-like bacteria or endosymbionts in different tick species based on the detection of the *16s rRNA* gene. *A. americanum*, *A. cajennense*, *O. rostratus*, and *Ornithodoros capensis* appeared to display high frequency for the presence of *Coxiella* bacteria (Clay et al. 2008, Machado-Ferreira et al. 2011, Almeida et al. 2012, Duron et al. 2014). On the contrary, other tick species, such as a number of *Haemaphysalis* and *Rhipicephalus* species, showed variable or low prevalence of

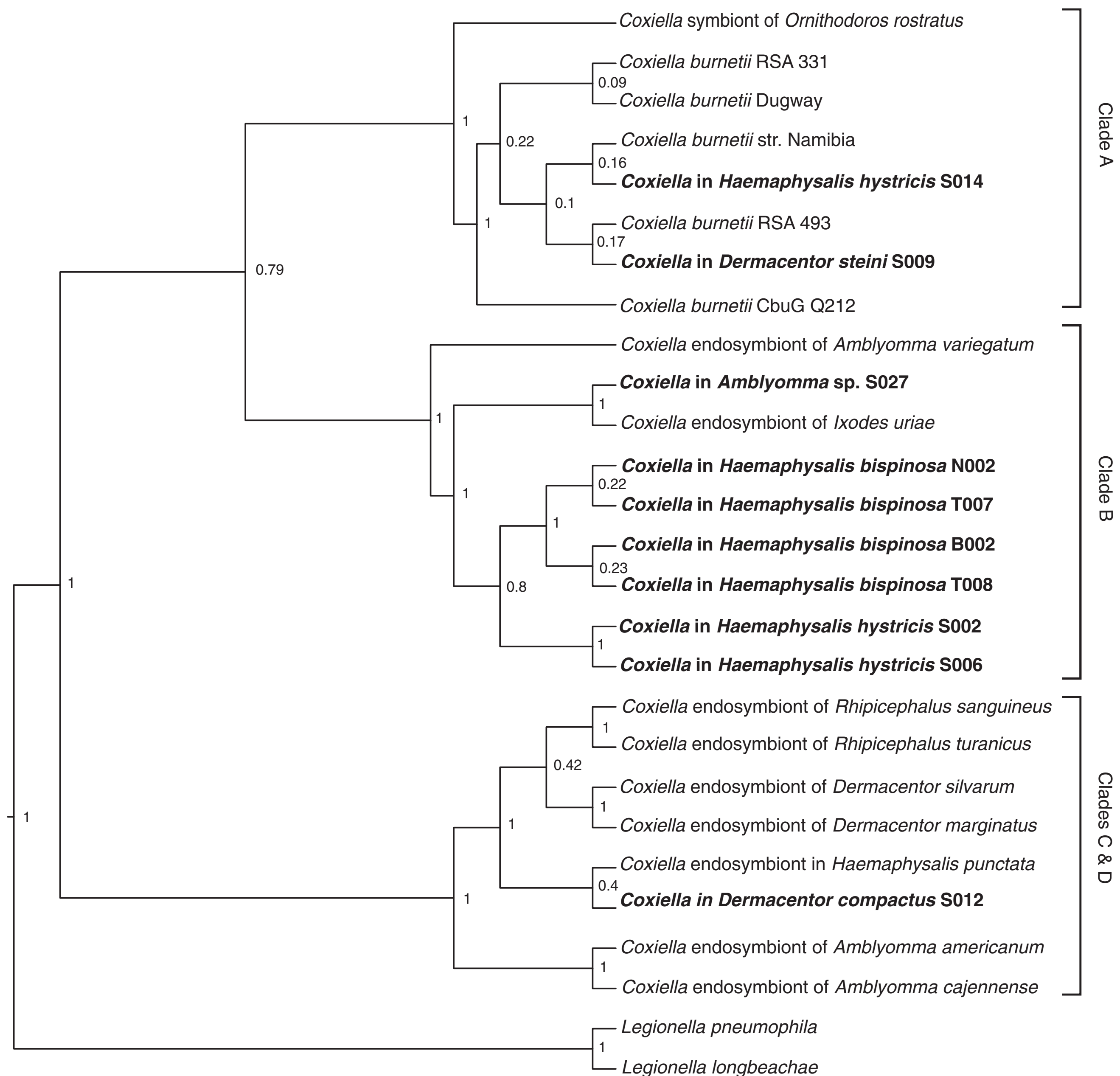

FIG. 2. Phylogenetic tree of *Coxiella* sp. based on partial *rpoB* sequences. Bayesian-inferred phylogenetic tree of *Coxiella* sp. based on 485 aligned nucleotides of the *rpoB* sequence. The posterior probabilities are displayed adjacent to each node. Samples from this study are highlighted in *bold*. Clades labeled are indicative of the clustering of various *Coxiella* strains previously described by Duron et al. (2015). Sequences from *L. pneumophila* and *L. longbeachae* were included as the outgroup.

Coxiella-like bacteria (Bernasconi et al. 2002, Arthan et al. 2015). Our findings showed that all *H. hystricis* examined harbored *Coxiella 16s rRNA* sequence, whereas the prevalence was only 17% in *H. hystricis* ticks in previous reports (Arthan et al. 2015). The reason for this discrepancy is unclear. It is, however, important to note that *Coxiella*-like bacteria were not detected in all *Haemaphysalis* tick species examined in the prior study (Arthan et al. 2015). Similar observations were made in our previous attempt to study the bacterial communities in *Haemaphysalis* ticks in Malaysia (Khoo et al. 2016). We observed nonuniform relative abundance of *Coxiella* in all of the *Haemaphysalis* ticks tested. These findings suggest that the role of the *Coxiella*-like bacteria or endosymbiont may not be equally important across all tick species. Since the bulk of current studies into the physiological role of *Coxiella* endosymbiosis in ticks are investigated in *A. americanum*, extra precautions must be exercised when extrapolating the findings to other tick species (Klyachko et al. 2007, Zhong et al. 2007, Smith et al. 2015).

Overall, the findings here are consistent with the current hypothesis in which the association between *Coxiella* and ticks is universal across species and geographical boundaries (Duron et al. 2015). There are yet to be any reports of human or animal infections with *Coxiella*-like bacteria from ticks.

However, avian infections of *Coxiella*-like bacteria have been reported even though there is no established link to ticks (Shivaprasad et al. 2008, Vapniarsky et al. 2012). Therefore, the potential of these *Coxiella*-like bacteria causing zoonotic infections merits further investigations.

Our findings suggest the possibility of *C. burnetii* infections in *H. hystricis* and *D. steini* ticks found in the forests of Malaysia, based on the clustering of the derived *Coxiella* sequences with other *C. burnetii* isolates in Clade A. To the best of the authors' knowledge, there is yet to be any report on the presence of *C. burnetii* infections in *H. hystricis* and *D. steini* ticks. Previous studies have identified the presence of *C. burnetii* in *R. sanguineus* ticks collected from dogs visiting a veterinary hospital in Malaysia (Watanabe et al. 2015). Wildlife animals such as wild boars and rodents have been previously implicated as reservoirs to infectious agents of livestock animals, including *Brucella suis*, *C. burnetii*, *Anaplasma phagocytophilum*, and *Leptospira interrogans* (Meng et al. 2009, Meerburg and Reusken 2011, Silaghi et al. 2014). The role of ticks in transmitting infection between multiple host animals and maintaining the reservoir of these pathogens merits investigation. The findings here also underscore the need for extensive prevalence studies of *C. burnetii* in wildlife within this region. Q fever outbreaks in livestock have been reported in Malaysia as recently as 2009 (Bina Rai et al. 2011, Norina et al. 2011). In humans, Q fever and the seroprevalence of *C. burnetii* have been reported in rural areas in Malaysia and Thailand (Tay et al. 1998, Suputtamongkol et al. 2003, Bina Rai et al. 2011, Blacksell et al. 2015). Unpublished serological studies performed on the Orang Asli in our laboratory have also indicated past *C. burnetii* exposure in this population. Hence, the presence of *C. burnetii* in wildlife animals and ticks is a potential health threat to both humans and livestock in this region.

In summary, the present study shows the presence of *Coxiella*-like bacteria, and possibly *C. burnetii*, in a number of tick species found on wildlife and domesticated goats from the rural areas in Malaysia. Our findings add to the growing evidence of the universality of *Coxiella* as tick-associated bacteria across various tick species and geographical locations. The possible presence of *C. burnetii* also suggests that ticks and the wildlife in Malaysia are potential reservoirs for the pathogen, signifying the need for improved surveillance activity to safeguard humans and livestock from the health risks of Q fever.

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Author Disclosure Statement

The authors declare that they have no competing interests. B.L.P. is a military service member of the U.S. Government. This work was prepared as part of his official duties. The opinions and assertions contained herein are those of the author(s) and are not to be construed as official or reflecting the views of the Department of the Navy, Department of Defense, or the U.S. Government. Title 17 U.S.C. §105 provides that ''Copyright protection under this title is not available for any work of the United States Government.'' Title 17 U.S.C. §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.

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