



SHORT COMMUNICATION

First molecular evidence of *Coxiella* spp. from *Rhipicephalus sanguineus* ticks in Cebu, Philippines

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Özet

Ybanez AP. Filipinler, Cebu'dan elde edilen *Rhipicephalus sanguineus* kenelerinden *Coxiella* spp. türlerinin moleküler olarak ilk belirlenmesi. **Eurasian J Vet Sci, 2014, 30, 1, 35-38**

Coxiella türleri kene ısırması ile geçebilen patojenik etkindir. Mevcut araştırma Filipinler, Cebu'da *Rhipicephalus sanguineus* kenelerinden *Coxiella* türlerinin varlığını moleküler tekniklerle belirlemek için yapıldı. Otuzaltı köpekten elde edilen toplam 164 adet kene Nested Polimeraz Zincir Reaksiyonu (PCR) ile kısmi 16S ribozomal RNA gen bölgesi çoğaltılarak analiz edildi. Beş adet köpekten elde edilen 9 adet kene pozitif (%5.5) bulundu. Sekans analizi sonucunda *Coxiella burnetii* (%96.3) ve *Coxiella* sp. endosymbiont (%98.1-100) ile benzer *Coxiella* spp.'ya ait DNA fragmentleri tespit edildi. Belirlenen DNA fragmentleri birbirleri ile de %98.3-100 oranında benzerdi. Bu çalışma Filipinler Cebu'da *Rhipicephalus sanguineus* kenelerinden elde edilen *Coxiella* spp. varlığını gösteren ilk araştırmadır.

Anahtar kelimeler: *Coxiella* sp., *Rhipicephalus sanguineus*, Filipinler

Abstract

Ybanez AP. First molecular evidence of *Coxiella* spp. from *Rhipicephalus sanguineus* ticks in Cebu, Philippines. **Eurasian J Vet Sci, 2014, 30, 1, 35-38**

Coxiella species are known to be potentially pathogenic tick-borne organisms. This study was aimed to investigate the presence of *Coxiella* sp. in *Rhipicephalus sanguineus* ticks in Cebu, Philippines by molecular based techniques. A total of 164 ticks were collected from 36 dogs and analyzed by a Nested Polymerase Chain Reaction (PCR) for the amplification of partial 16S ribosomal RNA gene region. Nine ticks (5.5%) obtained from 5 dogs were found positive. Sequencing revealed partial DNA fragments of *Coxiella* spp. which were 96.3% and 98.1-100% identical to *Coxiella burnetii* and *Coxiella* sp. endosymbiont, respectively. The detected DNA fragments also shared 98.3-100% identities with each other. This study is the first report on the existence of *Coxiella* spp. from *Rhipicephalus sanguineus* ticks in Cebu, Philippines.

Keywords: *Coxiella* sp., *Rhipicephalus sanguineus*, Philippines



Coxiella is a genus of gram-negative, obligate, intracellular bacteria which has similar characteristics with *Legionella* and *Francisella* spp. of the order Legionales (Williams et al 2010). The genus is best known for *Coxiella burnetii* which causes Q fever, a zoonotic disease affecting humans and animals with a worldwide distribution (Maurin and Roullet 1999, Arricau-Bouvery et al 2005). The pathogen may be carried by tick vectors including *Rhipicephalus sanguineus*. This vector can also be infected with an endosymbiont *Coxiella* sp. (Bernasconi et al 2002), which can be potentially pathogenic (Weller et al 1998). There are limited studies on the distribution of *Coxiella* spp. in Southeast Asian countries, including the Philippines. The present study aimed to give epidemiological information and molecular evidence about the presence of *Coxiella* sp. in *R. sanguineus* ticks in Philippines.

A total of 164 ticks were collected from 36 dogs in 5 veterinary clinics/hospital in Cebu, Philippines (GPY Veterinary Animal- Consolation Branch, GPY Veterinary Animal-Tres Branch, AZYP Pet Doctor’s Veterinary Center, Pet Science Veterinary Center, Veterinary Teaching Hospital of the College of Veterinary Medicine, Southwestern University) in 2010. The samples were stored in 70% ethanol until morphological identifications. Morphological identifications were performed using a binocular compound microscope and guided by established tick identification guide/keys (Walker et al 2000, Walker et al 2003). After identification, Genomic DNA extractions were performed on individual ticks by using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA), and final elution was adjusted to 200 µL with TE buffer. The extracted genomic DNA’s were stored at -30°C until PCR analysis. PCR

Table 1. Percent identities of detected partial 16S rRNA fragments.

Clone	Isolate/Clone					
	<i>Coxiella</i> sp. endoymbiont	<i>Coxiellaburnetii</i>	D23C (%)	D23E (%)	D25B (%)	D25C (%)
D23C	99.4-100	96.3	--	100	99.4	98.3
D23E	99.6-100	96.3	100	--	99.4	98.3
D25B	98.7-100	96.3	99.4	99.4	--	98.5
D25C	98.1-98.8	95.5	98.3	98.3	98.5	--

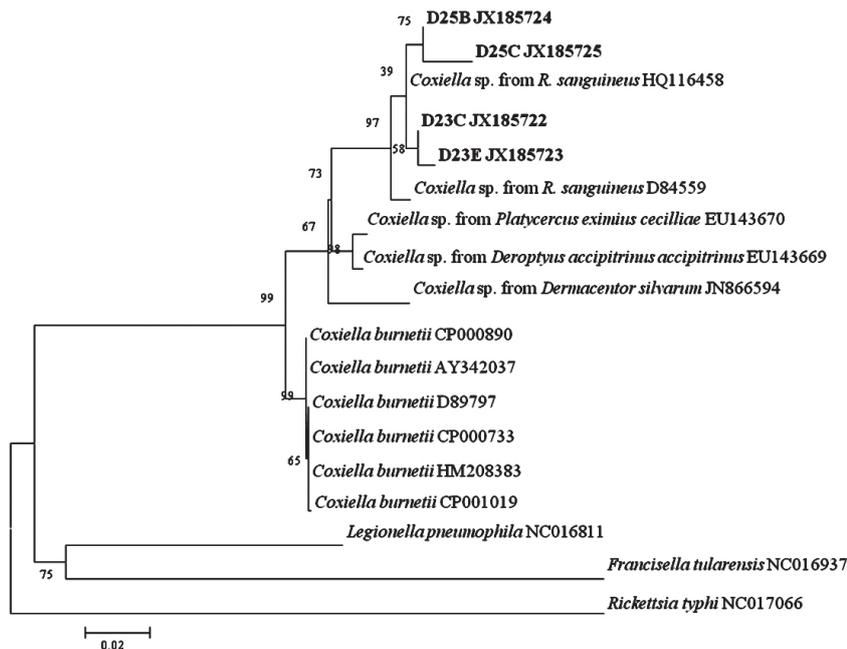


Figure 1. Phylogenetic relationship of *Coxiella* spp. detected from *R. sanguineus* in the Philippines (D25B, D25C, D23C, D23E) with other closely related bacteria based on the 16S rRNA gene. Sequences were compared using MEGA 5.05 by maximum likelihood method (kimura-two parameter). The numbers at the nodes represent the percentage of 1000 bootstrap resamplings. *Rickettsia typhi* was used as out-group.





was performed using a final volume of 25 μ L. Genomic DNAs from individual tick samples were analyzed by Nested PCR for the amplification of the 16S rRNA gene. The Semi Nested PCR protocol was performed in two rounds with the primers fd1 and Rp2 (1st round PCR), (Weisberg et al 1991) and fd1 and GA1UR (2nd round PCR), (Warner and Dawson 1996). The primer set fd1/Rp2 is used to amplify several eubacterial species, while the primer set fd1/GA1UR is usually used to amplify a 426 bp-fragment of the genus *Anaplasma* and *Ehrlichia*. The reference genomic DNA of *Anaplasma platys* was provided from France (Beaufils et al. 2000) and used as positive control, while distilled water was used as negative control in all PCR amplifications. The amplification products were analyzed by electrophoresis in 1.5% agarose gel. The positive amplification products were gel purified by QIAquick PCR purification kit (Qiagen, USA). Direct sequencing method was performed by using the same internal primers for most of the PCR amplicons. In cases where obtained nucleotide sequences were of low quality, amplicons were cloned by TOPO TA cloning (Invitrogen, USA) using the procedures recommended by the manufacturer. Subsequently, colony PCR and sequencing were performed by using the primers provided by the kit (M13Forward and M13Reverse). The BLAST search (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to initially compare the sequence data obtained in this study with those found in the GenBank Data Base. Percent identities were computed without considering the gaps. The multiple sequence alignment analysis was performed by using MUSCLE program (Edgar 2004) utilizing the default parameters. Phylogenetic analysis was performed by using maximum likelihood method (kimura-two parameter) employed in MEGA 5.05 (Tamura et al 2011). The tree stability was estimated by bootstrap analysis for 1,000 replications.

PCR results revealed a total of 9 ticks (5.5%) showing similar bands slightly higher than the positive control. Subsequent sequencing of 4 amplicons revealed lengths of 476-477 bp, which were 50-51 bp longer than the target length. BLAST search results revealed 96.3% and 98.1-100% identities to *Coxiella burnetii* and *Coxiella* sp. endosymbiont, respectively. The detected DNA fragments also shared 98.3-100% identities with each other (Table 1). Phylogenetic analysis revealed that the detected *Coxiella* spp. from the ticks in the Philippines formed a clade with the *Coxiella* sp. endosymbionts in *R. sanguineus* from USA and Marshall Islands. The clade was supported by a high bootstrap value (Figure 1). The partial *Coxiella* spp. sequences obtained in this study were registered at Genbank with accession numbers JX185722, JX185723, JX185724, and JX185725.

The molecular detection of *Coxiella* sp. DNA fragments in the Philippines adds new knowledge on the diversity of microorganisms found in *R. sanguineus* ticks in the country, in which DNA fragments of *Ehrlichia canis* (JN391409) and *Anaplasma platys* (JN121382) have also been detected (Ybañez et

al 2012). Although the natural history of the *Coxiella* spp. in the present study remains to be determined, its relatedness with the potentially pathogenic endosymbiont *Coxiella* sp. and *C. burnetii* presents a public health concern. *C. burnetii* has been detected in Philippine chicken eggs (Tatsumi 2006). Recent attempt to detect *C. burnetii* by indirect immunofluorescence from humans in the country have failed (Camer et al 2003). However, one tourist from Spain was found infected with the pathogen after a 60-day visit to the rural areas in the Philippines (Ta et al 2008). Therefore, it is possible that the patient was exposed to the pathogen during the visit. Q fever may present an asymptomatic or mildly symptomatic seroconversion to fatal clinical signs which can lead to death (Maurin and Raoult 1999). *C. burnetii* can be transmitted by ticks via a bite or feces to birds, rodents and ruminants (Lang 1990). Thus, clinicians should consider possible infection with *Coxiella* spp. especially when there is probable exposure to *R. sanguineus*, a tick species found ubiquitous all throughout the Philippines which can parasitize humans (Bermúdez et al 2012).

In conclusion, this is the first report of molecular detection and characterization of *Coxiella* spp. from *R. sanguineus* ticks in the Philippines.

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