DOI: 10.55415/deep-2022-0057.v1

Molecular Detection of *Candidatus* Coxiella mudorwiae from *Haemaphysalis concinna* in China

Authors: Mingjie Shi, Tong Qin, Zhitong Liu, Hao Feng, Yi Sun*

Affiliations:

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology, AMMS, Beijing, China

Mingjie Shi ahmusmj@163.com

Tong Qin 2358395729@qq.com;

Zhitong Liu lzt657100726@163.com

Hao Feng fhbcys@qq.com

***Corresponding to: 378176938@qq.com (Yi Sun)**

ORCiD: 0000-0002-6871-6975

DOI: 10.55415/deep-2022-0057.v1

Abstract

As one kind of obligated ectoparasites for humans and animals, ticks service as reservoir hosts for various of pathogen or nonpathogen microorganisms . Among which, *Coxiella burnetii* is known as the causative agent of Q fever, one of the most important zoonotic diseases distributed worldwide. Recently, some most closed *Coxiella*-like endosymbionts (CLEs) have been discovered in ticks, animals, even human beings. The close associations between CLEs and *C. burnetii* revealed from compared genomic analysis has aroused great interest for scientists to explore the origin of pathogenic CLEs and the possible implication on the maintaining and transmission of pathogenic *Coxiellae.* In the present study, our meta-transcript analysis has revealed that, one of CLEs with unknown pathogenicity, *Candidatus* Coxiella mudorwiae, was found infected in *Haemaphysalis concinna* evidenced with 16S rRNA gene and 3 arrays of gene transcripts including pyrophosphate--fructose 6-phosphate 1-phosphotransferase-*eda*-thiol-disulfide isomerase and thioredoxin-*greA-carB-carA-DnaJ-DnaK-grpE-ppnk*, *ropC-ropB*, *ubiA*-non canonical purine NTP pyrophosphatase-*hemK-prfA*, which suggests diverse CLEs prevail in various ticks in China, more detailed surveys are imperative to clarify the emergence of CLEs and their implication to the epidemiologic characters of Q fever.

Key Words: *Candidatus* Coxiella mudorwiae; Q fever; *Haemaphysalis concinna*; China.

DOI: 10.55415/deep-2022-0057.v1

Introduction

Coxiellae are intracellular bacteria infecting humans, vectorial arthropods and a variety of vertebrates across the world (Lang 1990). Both symbiotic and pathogenic bacteria in the family *Coxiellaceae* cause morbidity and acute disabling disease in humans and animals. *Coxiella burnetii*, the only officially recorded species in the genus *Coxiella*, has been well characterized as the agent of Q fever, which occurs sporadically and occasional outbreaks in humans worldwide annually (Angelakis and Raoult 2010). The well-known Q fever epidemic in The Netherlands during 2007–2010 has attracted much more attentions for its exceptional large epidemic (>4000 notified human cases) and serious health burden (161 to 336 million Euro) (van der Hoek and Morroy 2012, van der Hoek et al. 2013). And also, *C. burnetii* may be on the alert as a category B potential aerosolized biological weapon (Madariaga et al. 2003). To surveillance the potential health risk from *Coxiella* bacteria, intensive epidemiology surveys have revealed that diverse species including various *Coxiella*-like endosymbiont (CLE) exist within the genus *Coxiella*. The facts that various CLEs isolated from different tick species (Duron et al. 2015a) and their vertebrate hosts (Rousset et al. 2010) suggested that the natural occurrence of CLEs, is by no means fortuitous but bound to commensalism parasite in multiple tick species. As a notable role, CLEs are demonstrated to provide host ticks with some dietary supplementation, such as Vitamin B, deficient in vertebrate blood (Ben-Yosef et al. 2020). The well-characterized CLEs present close affinity with reference strains of *C. burnetii* in morphological, biological and evolutionary traits, however, they are genetically distinct. *Coxiellaceae* members were classified into a minimum of 4 highly divergent genetic clades (A–D) based on the phylogenetic analysis of 16S rRNA, lifestyle transitions, and maternally transmission traits (Duron et al. 2015b). In their taxonomic hierarchy, clade A includes all *C. burnetii* reference strains and CLEs from *Argasidae* ticks. While in clade B, CLEs of hard ticks (*Amblyomma* and *Ixodes*) and some soft ticks (*Orinthodoros*) are represented with reduced genome sized ~1M (Nardi et al. 2020). Clade C comprised of CLEs from ticks in genus *Rhipicephalus* and their relatives (Lalzar et al. 2014). The representative species, *Candidatus* Coxiella mudrowiae (*Ca.* C. mudrowiae) from *R. turanicus*, was demonstrated with larger genomes size which has a very low protein-coding content and an extremely high number of identifiable pseudogenes (Gottlieb et al. 2015). Clade C members have also been shown to be opportunistic agents of human skin infections (Gottlieb et al. 2015;

DOI: 10.55415/deep-2022-0057.v1

Angelakis et al. 2016; Guimard et al. 2017; Tsementzi et al. 2018; Ben-Yosef et al. 2020). As Regarding Clade D, CLEs mainly derived from various *Haemaphysalis*, *Dermacentor* and *Amblyomma* ticks were demonstrated with presumably pathogenic *Coxiellae* responsible for horse infection (Mediannikov 2003, Seo et al. 2016). The presence of genetically divergent CLEs in many more tick species converge to support the hypothesis that these CLEs are specific endosymbionts of ticks and CLEs of clade A hosted primarily by soft ticks might have served as the ancestor of *C. burnetii.* To which, more detailed genetic information and rigorous assessment of their infection risk to vertebrates are required to describe comprehensively.

The discovery that ticks carry either *C. burnetii* or CLEs underscores the demand to insight the transmission routes of *Coxiellae* in natural cycles. Firstly, it is noteworthy that the highly virulent reference strain of *C. burnetii*, Nine Mile, was isolated from a guinea pig fed by *Dermacentor andersoni* ticks (McDade 1990, Khavkin 1991). And second, over 50 tick species had been reported carrying visual *C. burnetii* or CLEs in the microscopic observations (Duron et al. 2015a, Ullah et al. 2022). Till to now, many field studies still focused on ticks for Q fever (Pacheco et al. 2013，Körner et al. 2021) . Together with laboratory evidences, at least 10 tick species, including *D. andersoni*, have been formally proved to be competent vectors of *C. burnetii* along with their efficient transstadial or transovarial transmission abilities (Parker and Davis 1938, Smith 1940, Smith 1941, Smith 1942a, Smith 1942b, Davis 1943, Daite 1977, Siroky et al. 2010, González et al. 2020, Körner et al. 2020), however, it remains controversy whether these ticks might play any significant role in the natural spread of *C. burnetii* (Duron et al. 2015a). Furthermore, the facts of *C. burnetii* and various CLEs prevailed in different tick species suggested that these ticks can act, at least, as a reservoir for *Coxiellaceae* members. When considerations that possible contamination to host skin and persist in the environment by excreting high concentrations of *Coxiella* in ticks' feces (Körner et al. 2020), the medical significance for ticks in the environmental dissemination of both *C. burnetii* and CLEs should not be underestimated any more (Körner et al. 2021). Additionally, the noticeable presence of CLEs in salivary glands, ovaries and Malpighian tubes of various tick species in almost all the life-stages (Klyachko et al. 2007, Machado-Ferreira et al. 2011, Almeida et al. 2012), further suggests the possible transmit vertically via the egg cytoplasm or horizontally across development stages rather than being acquired by blood-sucking on infected vertebrates, which certainly alerts the risk of vertebrate

DOI: 10.55415/deep-2022-0057.v1

hosts infection, including humans.

In China, the first human case of Q fever was reported in the 1950s when patients with atypical pneumonia were then diagnosed serologically with the complement fixation test (CFT) (Zhang et al. 1951, Zhai and Liu 1957). Till to 1962, the first human case was definitively confirmed when the first Chinese strain of *C. burnetii* (Qi Yi) was isolated therein (Yu et al. 1981). Since then, near 30 seroepidemiological or molecular studies described *C. burnetii* infections in 64 cities/municipalities over 19 provinces across China (El-Mahallawy et al. 2014). Moreover, CLEs and *C. burnetii* had also been detected from ticks belonging to 5 genera including *Ixodes* (Jiao 2021), *Dermacentor* (Li 2019, Jiao 2021a), *Haemaphyslais* (Zhuang et al. 2018), *Hyalomma* (Ni, 2020) and *Rhipicephalus* (Machado-Ferreira, 2016, Jiao 2021b) and wild animals (Qi et al. 2022, Fu et al. 2022) even freshwater shrimp *Palaemonetes sinensis* (Lu et al. 2022). The presence of CLEs in these ticks confers crucial and diverse benefits to the host ticks, affecting their developments, nutrition, chemical defense, or reproduction, and also can distinctly interfere with the maintenance and/or transmission of some tick borne pathogens by tick-endosymbionts interactions, such as competition or mutual reciprocity (Brenner et al. 2021). However, the medical significance of CLEs occur in ticks from Chinese territory remains to be determined. During our meta-transcriptomic surveys for tick borne pathogens, an emerged CLE were found in *Haemaphysalis concina* collected from forest areas in Mudanjiang city, Heilongjiang province. The CLE was identified as *Ca.* C. mudrowiae in clade C, with molecular evidences from 16S rRNA, 3 arrays of transcripts including pyrophosphate--fructose 6-phosphate 1-phosphotransferase-*eda*-thiol-disulfide isomerase and thioredoxin-*greA*-*carB*-*carA*-*DnaJ*-*DnaK*-*grpE*-*ppnk*, *ropC*-*ropB*, *ubiA*-non canonical purine NTP pyrophosphatase-*hemK*-*prfA*. The discovery of *Ca.* C. mudrowiae infected in *H. concinna* in China might advance our insights in the epidemiology of Q fever and other tick borne diseases which eventually benefit us with rationale prevention and control measures based on evolutionary history.

DOI: 10.55415/deep-2022-0057.v1

Materials and Methods

Ethics statement

Protocols for field tick collections and samples processing were reviewed and approved by the Institutional Ethics Review Board of Beijing Institute of Microbiology and Epidemiology (BIME-2020-019).

Tick collection, morphological identification and nucleotides acids extraction

A total of 213 adult ticks were harvested on vegetation in Mudanjiang, Heilongjiang province, China (44.60° N 129.59° E) randomly between April 2020 and July 2021. Identification of tick species was based on morphological characteristics following taxonomy keys for ticks in China (Teng and Liu 1991). Following species identification, the ticks were grouped by sex and each with 30 individuals. After sterilized with 70% alcohol, ticks in each pool were crushed with a sterile plastic homogenizer and total DNA and RNA were simultaneously extracted with MagMAX DNA/RNA Isolation kit (ThermoFisher, Scientific). Purified DNA/RNA was quantified by using the Qubit High Sensitivity assay (ThermoFisher Scientific) before further process.

Validation species identification by molecular markers

To verify tick species identification, we extracted genomic DNA from a proportion of the homogenates of each specimen or specimen pool. Two genes were used for tick identification: the partial 18S rRNA gene (~1100 nt) and partial COI gene (~680 nt). The former target gene was amplified using primer pairs 18S-1 (5′-CTGGTGCCAGCGAGCCGCGGYAA-3′) and 18S-2 (5′-TCCGTCAATTYCTTTAAGTT-3′), while the primer pairs LCO1490 (5′-GGTCAACAAA TCATA AAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGAC CAAAAAATCA-3′) for the latter. PCR amplifications were performed as described previously (Machida and Knowlton, 2012). For taxonomic determination, the resulting sequences were compared against the nt database as well as with all COI barcode records on the Barcode of Life Data Systems (BOLD).

Metatranscripts sequencing for *Candidatus* Coxiella mudorwiae

The RNA samples from *Haemaphysalis concinna* were reverse transcript to cDNA using PrimeScript™ IV 1st strand cDNA Synthesis Mix (Takara, Japan) and ribosomal RNA (rRNA) was removed using the Ribo-Zero Gold Kit (Illumina) following the manufacturer's instructions.

DOI: 10.55415/deep-2022-0057.v1

Subsequently, On all rRNA-depleted RNA-samples, the sequencing libraries were constructed using the KAPA Stranded RNA-Seq Kit (KAPA biosystems, Roche) with barcode adapters (Bio Scientific) according to the manufacturer's instructions. After cDNA-levels quantification by Qubit assays (ThermoFisher Scientific), equimolar amounts of nucleic acids were pooled and submitted for sequencing in each library. Further, All libraries were subjected to next-generation sequencing (NGS) by Illumina HiSeq 2500 platform on a single lane to generate the 125bp paired-end reads at the BGI Sequencing Centre (www.genomics.cn). Raw sequencing reads with low quality were excluded, trimmed with trim galore (www.bioinformatics.babraham.ac.uk/projects/trim_galore/) (Krueger et al, 2021) and then assembled de novo using Trinity v2.8.5 program. (Grabherr et al. 2011, Haas et al. 2013). The assembled scaffolds were predicted according to the open reading frames by MetaGeneMark, and CD-HIT was used to remove redundancy and obtain the initial unique gene catalog. For determination of gene abundance, the reads were realigned with the gene catalog with Bowtie 2 (Langmead and Salzberg 2012). Only genes with two or more mapped reads were deemed to be present in a sample (Qin et al. 2010). The presence of these genes in the corresponding samples was confirmed by nested RT–PCR and Sanger sequencing (data not shown). The unigenes were also aligned to the NR database (https://www.ncbi.nlm.nih.gov/) of NCBI with DIAMOND (Buchfink et al. 2015). The aligned results of each gene with e value ≤ the smallest e value × 10 were retained (Qin et al. 2010) and then processed with the Lowest Common Ancestor-based algorithm implemented in MEGAN to ensure the species annotation information of sequences (Huson et al. 2007).

Confirmation *Candidatus* Coxiella mudorwiae infections by PCR amplification for 16S rDNA

Commercial HotStart PCR Premix Kit (Thermo Fisher Scientific) was used for PCR amplification. Nested PCR followed the works from Seo and his colleagues (Seo et al. 2016) was used to amplify the 16S rRNA of *Ca. C. mudrowiae* and other CLEs, and sequencing. Cox16SF1 (5'-CGTAGGA ATCTACCTTRTAGWGG-3') and Cox16SR2 (5'-GCCTACCCGCTTCTGGTACAA TT-3') were used to perform the first-round amplifications. And then, nested PCR was performed using the primers Cox16SF2 (5'-TGAGAACTAGCTGTTGGRRAGT-3') and Cox16SR2. All PCR amplifications were performed using the following program: pre-denaturation at 93°C for 3 min;

DOI: 10.55415/deep-2022-0057.v1

30 cycles of denaturation at 93°C for 30 s, annealing at 56°C for 30 s, and polymerization at 72°C for 1 min; and a final post-polymerization step at 72°C for 5 min. PCR products of the second amplification process were analyzed by electrophoresis, with 10 µl of the reaction mixture and a 100 bp DNA ladder (Bioneer), by using 1.5% agarose gels, for 30 min at 100 V, and visualized using UV transillumination imaging after ethidiumbromide staining. Samples yielding amplicons of the expected size were delivered to Sangon Biotechnology Co., Ltd for bi-directionally sequencing with the primers Cox16SF1 and Cox16SR1 (5'-ACTYYCCAACAGCTAGTTCTCA-3') (Seo et al. 2016).

Phylogenetic analysis

The obtained sequences were compared with the reference sequences in GenBank with the NCBI-BLAST server (http://blast.ncbi.nlm.nih.gov/blast.cgi) and multiple sequences were aligned with ClustalW with default parameters in MEGA 7.0. The phylogenetic trees of 16S rRNA, Pyrophosphate--fructose 6-phosphate 1-phosphotransferase-*eda*-Thiol-disulfide isomerase and thioredoxin-*greA-carB-carA-DnaJ-DnaK-grpE-ppnk*, *ropC-ropB*, *ubiA*-non canonical purine NTP pyrophosphatase-*hemK-prfA* of *Ca.* C. mudrowiae were constructed with the maximum likelihood method using the best-fit model of amino acid substitution (LG + I + Γ + F for all alignments) with 1000 bootstrap replicates in PhyML v.3 program(Guindon et al. 2010). All phylogenetic trees were rooted at the midpoint for clarity purposes only.

Data availability

All sequence reads generated in this project are available under the NCBI Short Read Archive (SRA) under accessions SAMN26934337–SAMN26934338 (BioProject ID: PRJNA819490).

DOI: 10.55415/deep-2022-0057.v1

Results

Prevalence of *Candidatus* Coxiella mudorwiae in *Haemaphysalis concinna*

The presence of *Coxiella*-like endosymbionts was prompted by assembly contigs derived from *H. concinna* and then confirmed by PCR amplifications targeting the 16S rRNA gene combined with sequences analysis. After annotated, all the transcripts achieved in 30 males or females *H. concinna* individuals were shown no difference which suggested unbiased CLE parasitizing in both sexes. Similar to transcripts, there were also no difference observed in the16S rRNA genes of the CLE in both sexual groups of *H. concinna*. Consequentially, the 16S rRNA gene of the CLE was shown identical to *Ca.* C. mudrowiae strain CRt (CP011126.1) with 99.72% similarity over 100% coverage. *Ca.* C. mudrowiae is known closely related to pathogenic *Ca.* C. massiliensis isolated from infected samples from human skin (Angelakis et al. 2016), one horse blood strain *Coxiella* (H-JJ-10) in Jeju Island of South Korea (Seo et al. 2016) and another human derived *Coxiella* strain manifested with a scalp eschar and neck lymphadenopathy (Guimard et al. 2017). The phylogenetic tree constructed with partial sequences of 16S rRNA genes of CLEs from 46 tick species and *C. burnetii* reference strains formed 4 distinctive branches corresponding to clade A to D of *Coxiellaece* respectively as expected. Among these branches, all *C. burnetii* reference strains were clustered in the clade A, along with other CLEs from *Ornithodoros* (*O. amblus*, *O. moubata* and *Carios capensis*) species. Meanwhile, the *Ca.* C. mudrowiae derived from *H. concinna* in the present study was shown with high similarities to *Ca.* C. mudrowiae strain CRt (CP011126.1) and *Ca.* C. mudrowiae strain CRS-CAT (CP024961.1), which formed a distinctive sub-branch fallen into clade C including other CLEs from *Rhipicephalus* and *Dermacentor* ticks. Clade C was also demonstrated well separated from other CLE clades as shown in the phylogeny tree (Figure 1 panel A and B).

Transcript genes of *Candidatus* Coxiella mudorwiae in *Haemaphysalis concinna*

RNA samples from *H. concinna* yielded an average 10.96Gb of data including 7.1- 7.6 × 10^7 or so 150-base pair-end reads from male and female ticks. Clean reads were subsequently assembled into contigs and compared to the NCBI Genomes database after host subtraction and quality filtering, resulting in 43 or 63 contigs adults from males and females of *H. concinna*, respectively. A prediction of ORFs was also implemented for comparison with the protein database of the *Ca.* C. mudrowiae and other CLEs available through BLASTP. As results, 16 transcripts of *Ca.* C. mudrowiae were identified from both sexes of *H. concinna*, which including

DOI: 10.55415/deep-2022-0057.v1

pyrophosphate--fructose 6-phosphate 1-phosphotransferase, *eda*, thiol-disulfide isomerase, *greA*, *carA*, *carB*, *DnaJ*, *DnaK*, *grpE*, *ppnK* and *rpoC*, *rpoB*, *ubiA*, non-canonical purine NTP pyrophosphatase, *hemK* and *prfA*. Aligned sequences of the 16 genes or transcripts were listed in Table 1 with their identities and coverages of putative amino acids of top hit proteins of CLEs. Among them, the 10 genes or transcripts including pyrophosphate--fructose 6-phosphate 1-phosphotransferase, thiol-disulfide isomerase and thioredoxin, *eda*, *greA*, *carA*, *carB*, *DnaJ*, *DnaK*, *grpE*, *ppnK* were shown with over 78% identities on more than 97% coverages with those of *Ca.* C. mudrowiae stain CRt (CP011126.1). Meanwhile the rest six transcripts of *rpoC-rpoB*, *ubiA*, non-canonical purine NTP pyrophosphatase*, hemK* and *prfA* were demonstrated with higher identities, ranged from 77.26% to 94.17%, on100% coverages with those of *Ca.* C. mudrowiae stain CRt (CP011126.1). The results also supported a conclusion that the CLE from *H. concinna* should be identified as *Ca.* C. mudrowiae (Figure 2), which belongs to clade C of family *Coxiellae* which has a genome reduction and prominent gene decay because of the loss of selection on gene functions (Gottlieb et al. 2015).

Discussion

Q fever is a widespread zoonotic disease by *C. burnetii*, a ubiquitous intracellular bacterium. However, due to its highly polymorphic clinical manifestation, Q fever is difficult to prevent, diagnose, and treat in humans and animals. (Duron et al. 2015b). Human exposure to *C. burnetii* may result in asymptomatic to mild infection, but also in acute or chronic disease. Although rarely fatal, the disease may lead to and can be highly debilitating, even under treatment (Raoult et al. 2005). This post infection fatigue syndrome is associated with raised, impaired cellular responses, but with apparently no viable *Coxiella*, low or negligible antibody levels and clinical expression of a long lasting fatigue complex involving many body systems (Marmion et al. 2009). Therefore, Q fever presents as high socioeconomic burden and significant challenges for public health. To prevent and control the possible infections in humans and animals, a great amount of pioneering studies have been carried out to elucidate the pathogenicity, epidemiology, diagnosis and treatment aspects of *C. burneti* and more than 40 tick species have been found to naturally infect *C. burnetii* or CLEs (Duron et al. 2015b). Our next-generation sequencing (NGS) approaches have also revealed that *Ca.* C. mudrowiae, and not *C. burnetii*, prevailed in *H. concinna* populations,

DOI: 10.55415/deep-2022-0057.v1

which supported the hypothesis that diverse CLEs rather than presumptive *C. burnetii*, predominate in most tick species investigated thus far (Andreotti et al. 2010,Williams-Newkirk et al. 2014, Qiu 2014). The reality that ticks carry both *C. burnetii* and CLEs further emphasizes the demand to distinguish *Coxiellae* members clearly, which is necessary to improve our understanding of the epidemiology and evolutionary history of Q fever. Factually, the infection of *C. burnetii* in ticks might be confirmed rigorously through an array of impressive assays, including hemolymph tests, isolation in cell-lines (Vero) (Glazunova et al. 2005) and multiple-locus variable number tandem repeat (MLVA) analysis (Arricau-Bouvery et al, 2005), multispacer sequence typing (MST) (Svraka et al. 2006) and SNP genotyping (Huijsmans et al. 2011, Pacheco et al. 2013). Nevertheless, many studies has not been done so rigorously which led to the frequent misidentifications of CLEs. Some strains, primarily assumed as *C. burnetii* visually, actually be misidentification results of CLEs. *Coxiella*-like endosymbiont in *A. americanum*, for instance, had been repeatedly misidentified as *C. burnetii* in the older literatures (Parker 1944, Philip 1955), but recent sequence-based detections had confirmed that it is actually a CLE (CP007541) of clade D characterized with smaller genome size (Smith et al. 2015). In our studies, total 16 function genes or transcripts beyond 16S rRNA of *Ca.* C. mudrowiae were achieved, although the percent identities of some genes are quite low compared to reference strain (Table 1), which may be explanted by the possible sense mutations or different strain from various localities. The limitation of our study is obvious since we failed to achieve full genome of *Ca*. C. mudrowiae and identify the CLE unambiguously. Future attempts to isolate *Ca.* C. mudrowiae from *H. concinna* would benefit us much to reach the goal.

Overall, heritable endosymbiotic bacteria are of ecological and evolutionary importance to the particular arthropod species, as they potentially confer crucial and diverse benefits to the host by affecting its development, nutrition provisioning, chemical defense, or reproduction (Smith et al. 2015). The high frequency of CLEs transfer occurring at the tick-vertebrate interface may enhance or decrease the probability of infections with not only *C. burnetii* but also with other tick-borne pathogens (Clay et al. 2008). For examples, *Wolbachia* spp. has recently been found as defensive endosymbionts to interfere with the replication and transmission of various pathogens in their arthropods hosts including mosquitoes, flies and others (Hamilton and Perlman, 2013, Brownlie and Johnson 2009), which eventually limit the vector competence of these blood-sucking

DOI: 10.55415/deep-2022-0057.v1

arthropods (LePage et al. 2013, Moreira et al. 2009). Although most of CLEs described to date are confined to infect ticks, however, whether commensalism CLEs parasitized in ticks limit their vector competence to transmit pathogenic organisms including *C. burnetii* remains to be determined. Under natural circumstances, most CLEs in ticks pose much lower infection risk to vertebrates than *C. burnetii* dose, which may be explained by the facts that genomic reduction or conjugation machinery might take place in the evolutionary history of CLEs, which exchange genetic information, for instance, Dot/Icm, Type IVB secretion system (T4BSS) via horizontal gene transfer (HGT) (Brenner et al. 2021). And thus, most *Coxiella* progenitors have evolved into avirulent CLEs with reduced genome and devoid of known virulence genes (Duron et al. 2015a) (CLE of *A. americanum*) or intermingled pathogens retaining virulent genes more or less (*C. burnetii* or pathogenic CLEs). The infectious *Ca.* C. mudrowiae was also demonstrated with much biological activities in *H. concinna* along with transcription evidences from 16 genes, which were involved in many essential physiological processes, including energy metabolism (e.g. pyrophosphate--fructose 6-phosphate 1-phosphotransferase), intercellular vesicles formation or transportation (e.g. *DnaJ*, *DnaK*, *grpE*), and potential intracellular invasions. Whether the transcriptions of these genes participate the complicated interactions with pathogens or other endosymbionts parasite in host ticks remains to be clarified. In the other side, the occurrence of CLE, such as *Ca.* C. mudrowiae, also suggested a feasible CLEs based approach to modify pathogen transmission in arthropods, which means our interesting research efforts in a direction for intervention of pathogens' transmission (Ahantarig et al. 2013). Therefore, it is of necessary to describe the diversity of CLEs, characterize more fully their genetic relatedness, and assess their potential to cause infections in vertebrates.

In conclusion, our finding of *Ca.* C. mudrowiae infected in *H. concinna* has revealed that studies on CLEs can advance our understanding of CLE diversity and epidemiology of Q fever. *Ca.* C. mudrowiae and *C. burnetii* are closely related, but differ in their transmission ecology and infectiousness. This phenotypic diversity makes the evolution of genus *Coxiella* a topic of peculiar interest, because there are clearly transitions between avirulent and virulent symbionts residing in confined intracellular environments.

Ethics approval and consent to participate

No human participants involving the present study, protocols for field tick collections and samples processing were reviewed and approved by the Institutional Ethics Review Board of Beijing Institute of Microbiology and Epidemiology (BIME-2020-019).

Consent for publication

All authors contributed to the manuscript and approved the submitted version.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This study was supported by State Key Program of Infectious Diseases of China (2017ZX10303404) and National key research and development program (2022YFC2305001).

Authors' contributions

Designed the study: Y Sun, performed field and lab-work: Mingjie Shi and Tong Qin, Performed bioinformatics work: Y. Sun, Mingjie Shi, Zhitong Liu. Wrote the manuscript Y. Sun, Mingjie Shi, T. Qin and H. Feng. All authors read and approved the manuscript.

Acknowledgements

We thank all the staff in the tick-borne disease department in Mudanjiang Forestry Central Hospital and Balin Clinics for their help with sample collections.

Reference

Lang, G.H. (1990) Coxiellosis (Q fever) in animals. In Q FEVER (Vol. 1) The Disease (Marrie, T.J., ed.), pp. 23–48, CRC Press.

Angelakis, E. and Raoult, D. (2010) Q Fever. Vet. Microbiol. 140, 297–309

van Asseldonk, M., Prins, J., & Bergevoet, R.H. (2013). Economic assessment of Q fever in the Netherlands. *Preventive Veterinary Medicine*, 112 1-2, 27-34 . DOI:10.1016/j.prevetmed.2013.06.002

van der Hoek, W. and Morroy, G. (2012) Epidemic Q fever in humans in the Netherlands. In *Coxiella burnetii*: Recent advances and new perspectives in research of the Q fever bacterium (Toman, R. et al., eds), pp. 329–364, Springer

Madariaga, M.G., Rezai, K., Trenholme, G.M., & Weinstein, R.A. (2003). Q fever: a biological weapon in your backyard. *The Lancet. Infectious Diseases*, 311, 709-21. DOI:10.1016/S1473-3099(03)00804-1

Duron, O., Sidi-Boumedine, K., Rousset, É., Moutailler, S., & Jourdain, E. (2015b). The importance of ticks in Q fever transmission: what has (and has not) been demonstrated? *Trends in parasitology*, 31 11, 536-552 . DOI:10.1016/j.pt.2015.06.014

Rousset, E. (2010) Q Fever. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. p. 13, Office International des Epizooties (OIE) – World Organization for Animal Health.

Ben-Yosef, M., Rot, A., Mahagna, M., Kapri, E., Behar, A., & Gottlieb, Y. (2020). *Coxiella*-like endosymbiont of *Rhipicephalus sanguineus* is required for physiological processes during ontogeny. *Frontiers in Microbiology*, 11.493. DOI:10.3389/fmicb.2020.00493

Duron, O., Noël, V., McCoy, K.D., Bonazzi, M., Sidi-Boumedine, K., Morel, O.J., Vavre, F., Zenner, L., Jourdain, E., Durand, P., Arnathau, C., Renaud, F., Trape, J., Biguezoton, A.S., Cremaschi, J., Dietrich, M., Léger, E., Appelgren, A., Dupraz, M., Gómez-Díaz, E., Diatta, G., Dayo, G., Adakal, H., Zoungrana, S., Vial, L., & Chevillon, C. (2015a). The recent evolution of a maternally inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*. *PLoS Pathog*. 11, e1004892 DOI:10.1371/journal.ppat.1004892.

Gottlieb, Y., Lalzar, I., & Klasson, L. (2015). Distinctive genome reduction rates revealed by genomic analyses of two *Coxiella*-like endosymbionts in ticks. *Genome Biology and Evolution, 7*, 1779 - 1796. DOI:10.1093/gbe/evv108.

Guimard, T., Amrane, S., Prudent, E., El Karkouri, K., Raoult, D., & Angelakis, E. (2017). Case report: scalp eschar and neck lymphadenopathy associated with bacteremia due to *Coxiella*-like bacteria. *The American Journal of Tropical Medicine and Hygiene*, 97(5), 1319-1322 . DOI:10.4269/ajtmh.17-0251.

Nardi ,T., Olivieri, E., Kariuki, E., Sassera D., Castelli, M. (2020) Sequence of a Coxiella endosymbiont of the tick *Amblyomma nuttalli* suggests a pattern of convergent genome reduction in the *Coxiella* genus. *Genome Biol. Evol.* 13(1),1-7. DOI:10.1093/gbe/evaa253

Seo, M., Lee, S., VanBik, D., Ouh, I., Yun, S., Choi, E., Park, Y., Lee, S., Kim, J.W., Cho, G., Kwon, O., & Kwak, D. (2016). Detection and genotyping of *Coxiella burnetii* and *Coxiella*-like bacteria in horses in South Korea. *PLoS ONE, 11*. (5):e0156710. DOI:10.1371/journal.pone.0156710

Angelakis, E., Mediannikov, O., Jos, S., Bérenger, J., Parola, P., & Raoult, D. (2016). *Candidatus* Coxiella massiliensis Infection. *Emerging Infectious Diseases*, 22, 285 - 288. DOI: 10.3201/eid2202.150106

Khavkin, T. (1991). Q fever studies in the U.S.S.R. In Q fever: The Biology of *Coxiella burnetti* (Williams, J.C. and Thompson, H.A., eds), pp. 311–326, CRC Press

McDade, J.E. (1990). Historical aspects of Q fever. In Q FEVER (Vol. 1) The Disease (Marrie, T.J., ed.), pp. 5–22, CRC Press

Ullah, Q., Jamil, T., Saqib, M., Iqbal, M., & Neubauer, H.K. (2022). Q Fever-a neglected zoonosis. *Microorganisms*, 10,1530. DOI:10.3390/microorganisms10081530

Körner, S., Makert, G.R., Ulbert, S., Pfeffer, M., & Mertens-Scholz, K. (2021). The prevalence of *Coxiella burnetii* in hard ticks in Europe and their role in Q fever transmission revisited—A systematic review. *Frontiers in Veterinary Science*, 8, 655715. DOI:10.3389/fvets.2021.655715

Pacheco, R.D., Echaide, I.E., Alves, R.N., Beletti, M.E., Nava, S., & Labruna, M.B. (2013). *Coxiella burnetii* in ticks, Argentina. *Emerg. Infect. Dis., 19*, 344 - 346. DOI: 10.3201/eid1902.120362.

Parker, R.R. and Davis, G.E. (1938). A filter-passing infectious agent isolated from ticks. II. Transmission by *Dermacentor andersoni*. *Public Health Rep.* 53, 2267–2276. Corpus ID: 75146461.

Smith, D.J.W. (1940) Studies in the Epidemiology of Q fever. 3.Transmission of Q fever by the

tick *Haemaphysalis humerosa*. *Aust. J. Exp. Biol. Med. Sci.* 103–118. DOI:10.1038/ICB.1940.11

Smith, D.J.W. (1941). Studies in the epidemiology of Q fever. 8. The transmission of Q fever by the tick *Rhipicephalus sanguineus*. *Aust. J. Exp. Biol. Med. Sci.* 19, 133–136. DOI:10.1038/ICB.1941.21

Smith, D.J.W. (1942a). Studies in the epidemiology of Q fever. 10. The transmission of Q fever by the tick *Ixodes holocyclus* (with notes on tick-paralysis in Bandicoots). *Aust. J. Exp. Biol. Med. Sci.* 20, 213–217. Corpus ID: 209544688

Smith, D.J.W. (1942b) Studies in the epidemiology of Q fever. 11. Experimental infection of the ticks *Haemaphysalis bispinosa* and *Ornithodoros* sp. with *Rickettsia burneti*. *Aust. J. Exp. Biol. Med. Sci.* 20, 295–296. DOI:10.1038/ICB.1942.47

Davis, G.E. (1943). American Q fever: experimental transmission by the argasid ticks *Ornithodoros moubata* and *O. hermsi*. *Public Health Rep.* 58, 984–987. DOI:10.2307/4584503

Daiter, A.B. (1977). Transovarial and transspermal transmission of *Coxiella burnetii* by the tick *Hyalomma asiaticum* and its role in Q rickettsiosis ecology. *Parazitologiya* 11, 403–411. Corpus ID: 25934840

Siroky P, Kubelova M, Modry D, Erhart J, Literak I, Spitalska E, & Kociánová, E. (2010). Tortoise tick *Hyalomma aegyptium* as long term carrier of Q fever agent *Coxiella burnetii*—evidence from experimental infection. *Parasitology Research*, 107, 1515-1520. doi: 10.1007/s00436-010-2037-1

González, J., González, M.G., Valcárcel, F., Sánchez, M.E., Martín-Hernández, R., Tercero, J.M., & Olmeda, Á.S. (2020). Transstadial transmission from nymph to adult of *Coxiella burnetii* by naturally infected *Hyalomma lusitanicum. Pathogens*. 9:884. doi: 10.3390/pathogens9110884

Körner, S., Makert, G.R., Mertens-Scholz, K., Henning, K., Pfeffer, M., Starke, A., Nijhof, A.M., & Ulbert, S. (2020). Uptake and fecal excretion of *Coxiella burnetii* by *Ixodes ricinus* and *Dermacentor marginatus* ticks. *Parasit Vec.* (2020) 13:75.doi: 10.1186/s13071-020-3956-z

Klyachko, O.S., Stein, B.D., Grindle, N., Clay, K., & Fuqua, C. (2007). Localization and visualization of a *Coxiella*-type symbiont within the lone star tick, *Amblyomma americanum*. *Applied and Environmental Microbiology*, 73, 6584 - 6594. DOI:10.1128/AEM.00537-07

Almeida, A., Marcili, A., Leite, R.C., Nieri-Bastos, F.A., Domingues, L.N., Martins, J.R., & Labruna, M.B. (2012). *Coxiella* symbiont in the tick *Ornithodoros rostratus* (Acari: Argasidae). *Ticks and Tick-Borne Diseases,* 3(4), 203-6. DOI:10.1016/j.ttbdis.2012.02.003.

Machado-Ferreira, E., Dietrich, G., Hojgaard, A., Levin, M.L., Piesman, J.F., Zeidner, N.S., & Soares, C.A. (2011). *Coxiella* symbionts in the Cayenne tick *Amblyomma cajennense*. *Microb. Ecol.* 62,134–142. DOI:10.1007/s00248-011-9868-x

Zhang NC, Zia SH, Liu FT, Zhang NZ. (1951). Preliminary study on the problem of Q fever in Beijing [in Chinese]. *Chinese Medical Journal*, 37: 235.

Zhai SC, Liu SH. Q fever: report of a case [in Chinese]. *Chinese Journal of International Medicine* 1957; 5: 316.

Yu SR, Zhang SL, Li QJ, Zhang BX (1981). Isolation and identification of Q fever rickettsia in Sichuan In Monograph on *Rickettsiaceae* and *Chlamydiaceae* infections [in Chinese]. Chinese Journal of Epidemiology Press, Beijing; 18.

El-Mahallawy, H.S., Lu, G., Kelly, P.J., Xu, D.H., Li, Y., Fan, W., & Wang, C. (2014). Q fever in China: a systematic review, 1989–2013. *Epidemiology* and *Infection*, 143, 673 - 681. doi:10.1017/S0950268814002593

Jiao, J., Lu, Z., Yu, Y., Ou, Y., Fu, M., Zhao, Y., Wu, N., Zhao, M., Liu, Y., Sun, Y., Wen, B., Zhou, D., Yuan, Q., & Xiong, X. (2021a). Identification of tick-borne pathogens by metagenomic next-generation sequencing in *Dermacentor nuttalli* and *Ixodes persulcatus* in Inner Mongolia, China. *Parasites & Vectors*, 14 :287. 10.1186/s13071-021-04740-3.

Jiao, J., Zhang, J., He, P., Ouyang, X., Yu, Y., Wen, B., Sun, Y., Yuan, Q., & Xiong, X. (2021b). Identification of Tick-Borne Pathogens and Genotyping of *Coxiella burnetii* in *Rhipicephalus microplus* in Yunnan Province, China. *Frontiers in Microbiology,* 12: 736484. 10.3389/fmicb.2021.736484

Li, N., Li, S., Wang, D., Yan, P., Wang, W., Li, M., Yu, Z., & Liu, J. (2019). Characterization of the Rickettsia-like and *Coxiella*-like symbionts in the tick *Dermacentor everestianus* Hirst, 1926 (Acari: Ixodidae) from the Qinghai-Tibet Plateau. *Systematic and Applied Acarology*, 24, 106 - 117. 10.11158/saa.24.1.8

Ni, J., Lin, H., Xu, X., Ren, Q., Aizezi, M., Luo, J., Luo, Y., Ma, Z., Chen, Z., Tan, Y., Guo, J., Liu, W., Qu, Z., Wu, Z., Wang, J., Li, Y., Guan, G., Luo, J., Yin, H., & Liu, G. (2020). *Coxiella burnetii* is widespread in ticks (Ixodidae) in the Xinjiang areas of China. *BMC Veterinary Research*, 16. 10.1186/s12917-020-02538-6

Machado-Ferreira, E., Vizzoni, V.F., Balsemão-Pires, E., Moerbeck, L., Gazêta, G.S., Piesman, J.F., Voloch, C.M., & Soares, C.A. (2016). *Coxiella* symbionts are widespread into hard ticks. *Parasitology Research*, 115, 4691-4699. 10.1007/s00436-016-5230-z

Qi, Y., Ai, L., Zhu, C., Ye, F., Lv, R., Wang, J., Mao, Y., Lu, N., & Tan, W. (2022). Wild Hedgehogs and Their Parasitic Ticks Coinfected with Multiple Tick-Borne Pathogens in Jiangsu Province, Eastern China. *Microbiology Spectrum* e0213822. 10.1128/spectrum.02138-22

Fu, M., He, P., Ouyang, X., Yu, Y., Wen, B., Zhou, D., Xiong, X., Yuan, Q., & Jiao, J. (2022). Novel genotypes of *Coxiella burnetii* circulating in rats in Yunnan Province, China. *BMC Veterinary Research*, 18. DOI 10.1186/s12917-022-03310-8

Lu, X., Ma, H., Liu, J., Sun, N., Yu, J., Yang, Z., Guo, S., Huang, L., & Ye, S. (2022). First case of *Coxiella burnetii* infection in *Palaemonetes sinensis* cultured in Liaoning, China. *Diseases of Aquatic Organisms*, 148, 145-151 . DOI:10.3354/dao03654

Zhuang, L., Du, J., Cui, X., Li, H., Tang, F., Zhang, P., Hu, J., Tong, Y., Feng, Z., & Liu, W. (2018). Identification of tick-borne pathogen diversity by metagenomic analysis in *Haemaphysalis longicornis* from Xinyang, China. *Infectious Diseases of Poverty*, 7, .45. DOI:10.1186/s40249-018-0417-4.

Brenner, A.E., Muñoz-Leal, S., Sachan, M., Labruna, M.B., & Raghavan, R. (2021). *Coxiella burnetii* and related tick endosymbionts evolved from pathogenic ancestors. *Genome Biology and Evolution*, 13(7), 1-15. DOI:10.1093/gbe/evab108

TENG K-F and JIANG Z-J. 1991. Economic insect fauna of China. Fasc 39. Acarina: Ixodidae. Beijing, Science Press, Academia Sinica. 1-359.[in Chinese]

Machida RJ, Knowlton N (2012) PCR Primers for metazoan nuclear 18S and 28S ribosomal DNA sequences. *PLoS ONE* 7(9): e46180. DOI:10.1371/journal.pone.0046180.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, Di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, & Regev A. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat. Biotechnol.* 29, 644-652.

Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M.G., Blood, P.D., Bowden, J.C., Couger, M.B., Eccles, D.A., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J., Pochet, N., Strozzi, F., Weeks, N.T., Westerman, R., William, T., Dewey, C.N., Henschel, R., LeDuc, R.D., Friedman, N., & Regev, A. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nature Protocols*, 8, 1494-1512. DOI:10.1038/nprot.2013.084

Huson DH, Auch AF, Qi J, Schuster SC. MEGAN analysis of metagenomic data. *Genome Res*. 2007;17(3):377-386. DOI:10.1101/GR.5969107.

Krueger F, James FO, Ewels PA, Afyounian E. and Schuster-Boeckler B. 2021. FelixKrueger /TrimGalore: v0.6.7. DOI:10.5281/ZENODO.5127899.

Langmead B. and Salzberg S L 2012. Fast gapped-read alignment with Bowtie 2. *Nat. Methods* 9, 357–359. DOI:10.1038/nmeth.1923.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto J, Hansen T, Paslier DL, Linneberg A, Nielsen HB, Pelletier É, Renault P, Sicheritz-Pontén T, Turner KH, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J., Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD & Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464(7285):59-65. DOI:10.1038/nature08821.

Raoult, D., Marrie, T.J., & Mege, J.L. (2005). Natural history and pathophysiology of Q fever. *The Lancet. Infectious Diseases*, 5 4, 219-226. DOI:10.1016/S1473-3099(05)70052-9

Marmion, B.P., Sukocheva, O.A., Storm, P.A., Lockhart, M., Turra, M., Kok, T., Ayres, J.G., Routledge, H., & Graves, S.E. (2009). Q fever: persistence of antigenic non-viable cell residues of *Coxiella burnetii* in the host--implications for post Q fever infection fatigue syndrome and other chronic sequelae. *QJM : Monthly Journal of the Association of Physicians*, 102 10, 673-84. DOI:10.1093/qjmed/hcp077

Williams-Newkirk, A.J., Rowe, L., Mixson-Hayden, T., & Dasch, G.A. (2014). Characterization of the bacterial communities of life stages of free living lone star ticks (*Amblyomma americanum*). *PLoS ONE* 9, e102130; DOI:10.1371/journal.pone.0102130

Qiu, Y., Nakao, R., Ohnuma, A., Kawamori, F., & Sugimoto, C. (2014). Microbial population analysis of the salivary glands of ticks; a possible strategy for the surveillance of bacterial pathogens. *PLoS ONE* 9, e103961. DOI:10.1371/journal.pone.0103961

Andreotti, R., Pérez de León, A.A., Dowd, S.E., Guerrero, F.D., Bendele, K.G., & Scoles, G.A. (2010). Assessment of bacterial diversity in the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* through tag encoded pyrosequencing. *BMC Microbiol*. 11, 6. DOI:10.1186/1471-2180-11-6.

Glazunova, O.I., Roux, V., Freylikman, O., Sekeyová, Z., Fournous, G., Tyczka, J., Tokarevich, N.K., Kováčová, E., Marrie, T., & Raoult, D. (2005). *Coxiella burnetii* genotyping. *Emerging Infectious Diseases*, 11, 1211 - 1217. DOI:10.3201/eid1108.041354

Arricau-Bouvery, N., Hauck, Y., Béjaoui, A., Frangoulidis, D., Bodier, C.C., Souriau, A., Meyer, H., Neubauer, H.K., Rodolakis, A., & Vergnaud, G. (2005). Molecular characterization of Coxiella burnetii isolates by infrequent restriction site-PCR and MLVA typing. *BMC Microbiology*, 6, 38. DOI:10.1186/1471-2180-6-38.

Svraka, S., Toman, R., Škultéty, Ľ., Slabá, K., & Homan, W.L. (2006). Establishment of a genotyping scheme for *Coxiella burnetii*. *FEMS Microbiol. Lett.* 254, 268–274.

DOI:10.1111/J.1574-6968.2005.00036.X

Huijsmans, C.J., Schellekens, J.J., Wever, P.C., Toman, R., Savelkoul, P.H., Janse, I., & Hermans, M.H. (2011). Single-nucleotide polymorphism genotyping of *Coxiella burnetii* during a Q fever outbreak in The Netherlands. *Appl. Environ. Microbiol.* 77, 2051–2057 DOI:10.1128/AEM.02293-10.

Pacheco, R.D., Echaide, I.E., Alves, R.N., Beletti, M.E., Nava, S., & Labruna, M.B. (2013). *Coxiella burnetii* in ticks, Argentina. *Emerging Infectious Diseases*, 19, 344 - 346. DOI: 10.3201/ eid1902. 120362.

Parker, R.R. and Kohls, G.M. (1943) American Q fever: the occurrence of *Rickettsia diaporica* in *Amblyomma americanum* in eastern Texas. *Public Health Rep*. 58, 1510–1511. DOI:10.2307/4584634

Philip, C.B. and White, J.S. (1955) Disease agents recovered incidental to a tick survey of the Mississippi Gulf coast. *J. Econ. Entomol.* 48, 396–400. DOI:10.1093/JEE/48.4.396

Smith, T.A., Driscoll, T.P., Gillespie, J.J., & Raghavan, R. (2015). A *Coxiella*-like endosymbiont is a potential vitamin source for the lone star tick. *Genome Biol. Evol*. 7, 831–838. DOI:10.1093/gbe/evv016.

Clay, K., Klyachko, O.S., Grindle, N., Civitello, D.J., Oleske, D.A., & Fuqua, C. (2008). Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. *Mol. Ecol.* 17,4371–4381. DOI:10.1111/j.1365-294X.2008.03914.x

LePage, D. and Bordenstein, S.R. (2013) Wolbachia: can we save lives with a great pandemic? *Trends Parasitol.* 29,385–393 DOI:10.1016/j.pt.2013.06.003

Moreira, L.A., Iturbe-Ormaetxe, I., Jeffery, J.A., Lu, G., Pyke, A.T., Hedges, L.M., Rocha, B.C., Hall-Mendelin, S., Day, A., Riegler, M., Hugo, L.E., Johnson, K.N., Kay, B.H., McGraw, E.A., Hurk, A.F., Ryan, P.A., & O'Neill, S.L. (2009). A Wolbachia symbiont in *Aedes aegypti* limits infection with Dengue, Chikungunya, and Plasmodium. *Cell* 139, 1268–1278. DOI:10.1016/j.cell.2009. 11.042

Hamilton, P.T. and Perlman, S.J. (2013). Host defense via symbiosis in *Drosophila*. *PLoS Pathog*. 9, e1003808. DOI:10.1371/journal.ppat.1003808.

Brownlie, J.C. and Johnson, K.N. (2009). Symbiont-mediated protection in insect hosts. *Trends Microbiol.* 17, 348–354 DOI:10.1016/j.tim.2009.05.005.

Ahantarig, A., Trinachartvanit, W., Baimai, V., & Grubhoffer, L. (2013). Hard ticks and their bacterial endosymbionts (or would be pathogens). *Folia Microbiol.* (Praha) 58,419–428. DOI:10.1007/s12223-013-0222-1

Mediannikov, O. (2003) Molecular evidence of *Coxiella*-like microorganism harbored by *Haemaphysalis concinnae* ticks in the Russian far-east. In Rickettsiology: Present and Future Directions (Hechemy, K.E. et al., eds), pp. 226–228, New York Academy of Sciences. DOI:10.1111/j.1749-6632.2003.tb07367.x

Lalzar, I., Friedmann, Y., & Gottlieb, Y. (2014). Tissue tropism and vertical transmission of *Coxiella* in *Rhipicephalus sanguineus* and *Rhipicephalus turanicus* ticks. *Environ. Microbiol.* 16, 3657–3668. DOI:10.1111/1462-2920.12455

DOI: 10.55415/deep-2022-0057.v1

Legends for Figures

Figure 1. **Phylogenetic analysis of Coxiella-like endosymbionts and *C. burnetii* reference strains based on sequences of 16S rRNA in length of 1545 base pairs of nucleotides.**

Panel A. **General view of *Coxiella* bacteria.** Panel B. **Enhanced region for Clade C of Coxiella-like-endosymbionts.**

Candidatus Coxiella mudrowiae harvested in the present study were marked with red ball.

Maximum likelihood tree inferred using the best-fit model of amino acid substitution (LG + I + Γ + F for all alignments) with 1000 bootstrap replicates.

Figure 2 **Scheme for genomic information of *Candidatus* Coxiella mudrowiae detected from *Haemaphysalis concinna*.**

The 3 contigs of transcripts harvested from *H. concinna* in the present study were listed under the reference strain CRt (CP011126.1). Three target contigs were marked with brown color on the similar region of *Candidatus* C. mudrowiae genome (CP011126.1), the intervals failed to achieve between the 3 contigs were marked with bright yellow. Contig 1 to 3 were enhanced to show the annotated genes with yellow blocks.