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First Molecular Detection of *Coxiella burnetii* in Beef Cattle in West Java, Indonesia

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Summary

*Coxiella burnetii* (*C. burnetii*) is a bacterial agent causing Q fever which is widespread all over the world. Livestock such as cattle, goat, and sheep are the main sources of infection for this disease. Infection of *C. burnetii* causes abortion of livestock, resulting in economic damage. Q fever is zoonotic disease and potential public health hazard. To date, little is known about the infection of *C. burnetii* in livestock in Indonesia. The objective of this research is to screen the genome of *C. burnetii* bacteria in beef cattle in West Java, Indonesia. Organ tissue samples were collected from cattle slaughtered in slaughterhouses, West Java. *C. burnetii* genome was detected from cattle samples in all three samplings area by nested PCR (nPCR) targeting *com1* gene of *C. burnetii*. Sequencing analysis of 16S rRNA gene revealed the amplicons showed 99.9% nucleotide identity to *C. burnetii* strains Heizberg, 1843, 2574, 701CbB1, and 14160-001. Our results indicate that the infection of *C. burnetii* occurs in Indonesian beef cattles and highlight the risk of exposure to *C. burnetii* infection in human.
Coxiella burnetii which causes zoonotic disease Q fever is a Gram-negative obligate intracellular bacterium. Livestock such as cattle, goat and sheep are the main reservoir hosts of C. burnetii. C. burnetii infection in ruminants causes abortion and reproductive disorder that resulting in economic loss of agricultural field (1).

C. burnetii infection is widespread in the world and a crucial health problem for both animals and humans in some areas. However, little is known about C. burnetii infection in Indonesian animals. In 1955, serosurveillance study of C. burnetii in Indonesian cattle by WHO revealed that 188 bovine sera were positive among 205 specimens (2). Based on world animal health information database released by world organization for animal health (OIE), Indonesia was included in no information category in 2009-2014 period. In first half of 2015 Indonesia was categorized as infection; however on the second half of the year up to 2018, Indonesia went back to no information category (3). Molecular studies of C. burnetii in Indonesia is limited on its genome detection on animals in two regions (4). The objective of this research is to identify molecular evidence of C. burnetii infection in beef cattle in West Java, Indonesia.

Samples used in this research were lung, heart, liver, kidney and spleen tissues of 50 beef cattle each from slaughterhouses in West Java Province, with Bogor region conducted in 2016-2017, Depok region in 2017 and Bandung in 2017. Sampling sites location was represented as Figure 1. The samples were kept at −30 °C to be further processed for DNA extraction. DNA extraction was performed by using Puregene Core Kit A (QIAGEN, Valencia, CA, USA) following the manufactures’ instructions.

Extracted DNA was then screened by nPCR with pooling method. There were fifteen pools that consisted of five organs each from ten animals. For nPCR screening, we followed the method of Ogawa et al. (5). nPCR targeting coml gene of Coxiella was performed with Takara Ex Taq DNA polymerase (Takara Bio, Shiga, Japan), using OMP1-OMP2 and OMP3-
OMP4 primers set (6). nPCR screening was conducted in the integrated laboratory, Faculty of Veterinary Medicine, IPB University, Indonesia.

Positive samples selected by nPCR screening with OMP primers were then subjected to PCR targeting 16S rRNA gene of the genus Coxiella following the method of Seo et al. (7). The PCR was conducted with Tks Gflex DNA polymerase (Takara Bio) using Cox16SF1-Cox16SR2 primers (7). The amplicons with Agencourt AMPure XP (Beckman Coulter, Brea, CA), cloned into pCR4Blunt-TOPO vector (Invitrogen; Thermo Fisher Scientific, Waltham, MA) and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems; Thermo Fisher Scientific). PCR and DNA sequencing were performed in the Research Center for Zoonosis Control, Hokkaido University, Japan.

The obtained sequence data was analyzed by NCBI BLAST analysis tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). For phylogenetic analysis, multiple sequence alignment including C. burnetti and Coxiella-like bacteria (CLB) was built with CLUSTAL W and phylogenetic tree was constructed by MEGA X Program under maximum likelihood with 1000 bootstrap replicates (8).

We initially screened C. burnetti by nPCR targeting com1 gene encoding a 27-kDa outer membrane protein (OMP). The OMP primer sets were proved to show high specificity and sensitivity for identifying C. burnetii genome (6). The PCR products of approximate 440 bp in size were amplified organs from nine out of 15 pooled samples. Lung, heart, liver, kidney and spleen were identified positively from all three samplings area.

To further verify the detection of C. burnetti, out of the nine positive pooled samples, two (CB25 and CB30) were subjected to sequencing analysis of 16S rRNA gene of the genus Coxiella. Large DNA fragments of 1,322 bp in size were amplified from CB25 and CB30 by PCR using Cox16S primer sets. Both amplicons were fully sequenced and showed 100% nucleotide sequence identity with each other. The sequences were deposited in GenBank with
accession numbers LC464973 and LC464975. BLAST search revealed both amplicons shared 99.9% nucleotide sequence identity with *C. burnetii* strains including Heizberg (CP014561), 1843 (CP014557), 2574 (CP014555), 701CbB1 (CP014553) and 14160-001 (CP014836). The sequence alignment analysis identified three single nucleotide polymorphisms at position number 514, 716, and 1286 in DNA fragment of 16S rRNA of Indonesian *C. burnetii* (Table 1).

Phylogenetic tree of the 16S rRNA gene sequences of *Coxiella* spp. formed four main clades (Figure 2). Clade 1, 2, and 3 consisted of CLBs related to *C. burnetti* isolated from tick (9) (10). The *C. burnetti* identified in this study (CB25 and CB30) formed clade 4 with other *C. burnetii* from human and ruminants. These results demonstrated that *C. burnetii* was identified from the cattle samples.

Nine samples were positive for *C. burnetii* by nPCR with OMP primers, which were widely used for detection of *C. burnetii*. Further, large-size DNA fragments from 16S rRNA gene of *C. burnetii* were obtained from two samples. BLAST search confirmed the 16S rRNA amplicons were arose from *C. burnetii*. Our study demonstrates that *C. burnetii* exist in beef cattle in West Java, Indonesia.

The 16S rRNA gene is present in every bacterium with 1,500 bp sequence length and highly conserved. Identification of bacteria by the 16S rRNA gene is often conducted for bacteria that are hard or slow-growing to culture (11). Identification of *Coxiella* spp. by using 16S rRNA gene has been performed on horse, cattle and mites in South Korea (7) (12). However, it has not been used for Indonesian *C. burnetii*. In this study, we demonstrated that 16S rRNA gene sequencing is useful to identify *C. burnetii* in Indonesia.

There were three single nucleotide polymorphisms in 16S rRNA gene of Indonesian *C. burnetii*, while this gene is highly conserved. To infer deep genetic phylogenetic relationship,
DNA sequencing analysis of other genes of Indonesian *C. burnetii* and multilocus variable-number tandem-repeat analysis (13) are required in future.

*C. burnetii* can survive in host body in subclinical state. Animals infected in subclinical phase can still shed *C. burnetii* bacteria to the environment and become a source of infection to healthy animal (14). In addition, *C. burnetii* can survive in the environment for a long period of time. Dust contaminated by air borne bacterium and carried by the wind is the main source of infection (15). Increasing number of samples, target animals and sampling area are needed to understand current endemicity of *C. burnetii* in Indonesia. In conclusion, cattle in Bogor, Depok and Bandung in West Java, Indonesia were infected by *C. burnetii* that was validated by sequencing of 16S rRNA gene of *C. burnetii*.

**Conflict of Interest**

This work was supported by the Joint Usage/Research Center, Research Center for Zoonosis Control, Hokkaido University and funded by Ministry of Research, Technology and Higher Education of Indonesia in PMDSU program.

**References**


**Figure 1.** Map of three sampling locations in West Java, Indonesia

**Figure 2.** Phylogenetic tree based on 16S rRNA gene partial sequences of *Coxiella* spp. Indonesian *C. burnetii* identified in this study was marked with closed circles (●). Accession numbers were described in the taxa. The bootstrap values after 1000 replicates are shown in each branch. The scale bar showed the evolutionary distance of each sequence. CLB, *Coxiella*-like bacteria.
Table 1 Nucleotide sequence polymorphisms in 16S rRNA of *C. burnetii*

<table>
<thead>
<tr>
<th>C. burnetii strain</th>
<th>Position in the amplicon</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>514</td>
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<tr>
<td>CB25</td>
<td>C</td>
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<tr>
<td>CB30</td>
<td>C</td>
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<tr>
<td>Heizberg</td>
<td>T</td>
</tr>
<tr>
<td>701CbB1</td>
<td>C</td>
</tr>
<tr>
<td>18430</td>
<td>C</td>
</tr>
<tr>
<td>2574</td>
<td>C</td>
</tr>
<tr>
<td>14160-001</td>
<td>C</td>
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Figure 2