

Original Article



Molecular Detection of *Coxiella burnetii*, the Causative Agent of Q Fever on Cattle in the Slaughterhouses in Lampung Province, Indonesia

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ABSTRACT

Background: *Coxiella burnetii* is an obligate intracellular bacterium responsible for Q fever, a neglected and rapidly rising zoonotic disease in Indonesia. Domestic ruminants serve as the main reservoir for human cases, and the disease has three forms of infection: Subclinical, acute, and chronic.

Objectives: This study aims to investigate the presence of *C. burnetii* in meat cattle in Lampung Province, Indonesia, using nested polymerase chain reaction (PCR), and to identify the pathogenesis of Q Fever.

Methods: Samples, including lung, heart, spleen, kidney, and liver, were collected from 100 Brahman cross cattle at slaughterhouses in Lampung Province. Subsequently, *C. burnetii* was detected using nested PCR. Hematoxylin-eosin staining was applied to each positive organ sample to observe the morphology of the infection. Confirmation testing was performed using immunohistochemistry (IHC) to determine the antigen localization.

Results: Nested PCR targeting the *com1* gene identified *C. burnetii* DNA in the spleen and heart of 3% of subjects. Phylogenetic analysis showed complete sequence identity among positive samples and significant similarity to previously identified goat isolates from East Java. Histopathological examination of tissues from affected cattle revealed depletion of the white pulp, splenic congestion, and macrophage infiltration, indicative of chronic splenitis. Inflammatory changes in the heart valves suggested the occurrence of endocarditis. Additionally, IHC validated the detection of *C. burnetii* antigens in macrophages and vascular endothelial cells.

Conclusion: This study provides preliminary molecular and pathological evidence of *C. burnetii* infection in cattle from Lampung Province, Indonesia. The results show the often-overlooked presence of the bacteria in the meat supply chain and underscore the need for proactive surveillance to mitigate potential occupational and public health risks.

Keywords: Cattle, *Coxiella burnetii*, Lampung, Q fever, Zoonoses

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Introduction

Q fever is a zoonotic infection of ruminants caused by *Coxiella burnetii*, a gram-negative obligate intracellular bacterium (Maurin & Raoult, 1999). Transmission to humans and animals occurs predominantly through inhalation of contaminated aerosols (Maurin & Raoult, 1999; Rodolakis, 2006; Angelakis & Raoult, 2011). Documented transmission routes include direct contact with infected animals, contaminated materials, exposure to dust particles, entry through skin wounds, and blood transfusions (Baca & Paretsky, 1983; Fournier et al., 1998; Woldehiwet, 2004). Sexual transmission between animals has also been suggested (Porter et al., 2011). The environmental stability and infectivity of *C. burnetii* contribute to the widespread distribution and public health significance. Q fever affects a diverse array of hosts, including mammals, poultry, and arthropods, particularly ticks (Angelakis & Raoult, 2011). Domestic ruminants serve as the primary reservoirs of cases in humans (Astobiza, 2012), including cattle, sheep, and goats (Fournier et al., 1998; Maurin & Raoult, 1999).

According to reports, Q fever is globally distributed, except in New Zealand. This exception is attributed to the country's effective biosecurity system, the low potential of the local tick (*Haemaphysalis longicornis*) as a disease reservoir, and the absence of reported *C. burnetii* infections in both animals and humans (Rodolakis, 2006; Angelakis & Raoult, 2011). In 2009, a major outbreak occurred in the Netherlands, affecting more than 2300 people and resulting in 26 deaths. The outbreak was suspected to have originated from a goat farm infected with *C. burnetii* (Enserink, 2010). Australia, one of the primary cattle exporters to Indonesia, is not free from this disease, as shown by Cooper et al. (2011) in a seroprevalence study of beef cattle in Queensland, Australia, in which 16.8% of serum samples tested seropositive for *C. burnetii* infection.

Despite the rapidly rising incidence in Indonesia (Ministry of Agriculture, 2013), Q fever remains largely overlooked, with no official reports of cases to date. Recently, studies have discovered *C. burnetii* infection in ruminants in Bali Province and Bogor City (Mahatmi et al., 2007), Jakarta (Setiyono & Subangkit, 2015), North Sumatra (Nasution et al., 2015), and Malang City (Untari et al., 2024). Lampung Province plays an essential role in distributing cattle meat to various cities across Indonesia. According to data, the province has 916,460 meat cattle and produces 22,895,178.54 kg of beef (BPS-Statistics of Lampung Province, 2024). There has been

no evidence of Q fever in this region. The results of this study will provide preliminary data on the presence of Q fever in Lampung Province, Indonesia, and the first molecular analysis of *C. burnetii* in the area.

The study on *C. burnetii* identification used the nested polymerase chain reaction (nested-PCR) test, as described by Zhang et al. (1998). Other studies have evaluated nested PCR and have shown it can be used to detect *C. burnetii* (Ogawa et al., 2004). Nested PCR is a molecular detection technology and a modified version of conventional PCR characterized by two different primer sets and a twice-conducted amplification process. It has higher sensitivity and specificity compared to conventional PCR (Carr et al., 2010). Nested PCR has been successfully used to detect animals shedding *C. burnetii* within a flock (Niemczuk et al., 2014).

The *com1* gene is used to detect *C. burnetii* in the nested PCR method. It encodes an outer membrane protein (OMP), a highly conserved, very specific gene found in multiple copies in the *C. burnetii* genome. Detection of *C. burnetii* using the gene has high sensitivity and specificity. According to a previous study, the consistent presence of the *com1* gene makes it an ideal target for molecular detection (Mares-Guia et al., 2019). Although the *IS111* gene is highly sensitive and can be used for *C. burnetii* detection, it is not sufficiently specific for *C. burnetii* due to the presence in *Coxiella*-like endosymbionts (CLEs), which can lead to misidentification (Khademi et al., 2024). The *16S rRNA* gene is highly conserved for broad bacterial classification, not for the precise differentiation of closely related species (Khademi et al., 2024).

Given that most animals infected with Q fever do not show characteristic symptoms or are subclinical, detection efforts for the agent are highly needed. One effective method for detecting antigens is immunohistochemistry (IHC). It has been widely used to detect *C. burnetii*. A study using histopathology and IHC showed that *C. burnetii* antigen was found in the placentas of infected goats (Sanchez et al., 2006). Hansen et al. (2011) conducted a similar study to examine the distribution of *C. burnetii* antigen in cattle placentas. Nasution et al. (2015) conducted a retrospective study using IHC in organs from cattle infected with *C. burnetii* and detected the antigen in the liver, spleen, and lungs.

Agent detection using PCR and IHC can help identify the pathogenicity caused by *C. burnetii* through morphopathological manifestation in several organs. Although most Q fever cases are asymptomatic, infection also

causes acute or chronic clinical symptoms. Q fever is an acute condition that usually presents in three main manifestations: Flu-like syndrome, atypical pneumonia, and hepatitis. However, certain acute infections may cause lymphadenitis. Endocarditis and vasculitis are clinical manifestations of a chronic Q fever condition (Eldin et al., 2017). Therefore, this study aims to detect the presence of *C. burnetii*, the causative agent of Q fever in ruminant livestock, using PCR testing, and to identify the pathogenesis in Lampung Province, Indonesia.

Materials and Methods

Time and place

This study was carried out between July and September 2024 at the Pathology Research Laboratory, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia.

Sample gathering

Samples were collected from organs of 100 Brahman cross cattle at the Rumah Potong Hewan (RPH) slaughterhouses in Lampung Province, Indonesia. Collected organ samples included liver, spleen, heart, lungs, and kidneys, with up to 100 of each measuring approximately ± 3 cm in thickness. These samples were divided into two parts: one placed in an empty tube and the other in a tube containing 10% buffered neutral formalin (BNF).

DNA extraction

DNA extraction from the organ samples was performed using a Qiagen® DNeasy Blood & Tissue Kit (Qiagen N.V., Netherlands). The organ sample observation was conducted using a pooling method, which means pooling several sample specimens into a single container, such as a tube. Organ pooling was performed by combining samples from five different cattle into a single tube. This approach was used as a preliminary screening method to detect potential positive cases. When a pooled sample tested positive, individual organs from the corresponding cattle were re-extracted and tested separately, with each organ placed into a separate tube. All samples, a mix of liver, spleen, heart, lungs, and kidney, were collected in amounts up to 0.05 g, then aseptically ground and placed in microtubes. About 180 μ L of ATK buffer and 20 μ L of proteinase K were added to the specimen-containing tubes, and the mixture was homogenized with a vortex until a suspension formed. The specimens were incubated at 56 °C for 2-3 h. About 200 μ L of AL buffer was further added to the suspen-

sions, and the mixture was homogenized in a vortex for 15 s. Subsequently, 200 μ L of ethanol was added to the suspensions, which were vortexed for 15 seconds. The suspension was pipetted into spin columns in collection tubes, centrifuged at 6000 g (8000 rpm) for 1 min, and the bottom liquid was discarded. Using new collection tubes, the suspensions were added with 500 μ L of buffer AW1 before being centrifuged again at 8000 rpm for 1 min. About 500 μ L of buffer AW2 was further added, and the mixture was centrifuged at 14000 rpm for 3 min. The filtered liquid was discarded again, and the suspensions were transferred into 1.5 mL microtubes and added with 100 μ L of buffer AE. The mixture obtained was incubated at room temperature for 1 min, then centrifuged at 8000 rpm for 1 min.

First-round PCR

The first round of PCR tests was performed by mixing 3 μ L of extracted DNA from the test sample, primers OMP 1 (5'-AGT AGAAGC ATC CCA AGC ATT-3') (IDT®, Integrated DNA Technologies, USA) 100 μ M and OMP 2 (5'-TGC CTG CTA GCT GTA ACG ATT-3') (IDT®, Integrated DNA Technologies, USA) 100 μ M for 0.75 μ L each, 12.5 μ L of Toyobo® KOD one PCR master mix (Toyobo Co., Ltd., Japan), and 7 μ L of DNA-free distilled water. The positive control used was the DNA of the *C. burnetii* strain Nine Mile 2 (ATCC), and amplification was performed in a SensoQuest® Labcycler (SensoQuest GmbH, Germany) programmed for 35 cycles. The denaturation process was conducted at 94 °C for 1 min, annealing at 54 °C for 1 min, and extension at 72 °C for 2 min. The product of the first PCR round was a 500 bp DNA fragment. The primer sequence for the first round of PCR was based on Zhang et al. (1998) and Ogawa et al. (2004).

Nested PCR

Nested-PCR was performed with a pair of primers OMP 3 (5'-GAA GCG CAA CAA GAA GAA CAC-3') and OMP 4 (5'-TTG GAA GTT ATC ACG CAG TTG-3') (IDT®, Integrated DNA Technologies, USA). These primers are from the *C. burnetii* outer membrane, weighing 29 kDa, and are part of a conserved gene region. A nested-PCR reaction mixture was made by combining 1 μ L of the first-round PCR product, 0.75 μ L of each primer, 12.5 μ L of 10 $\times$ Toyobo® KOD one PCR master mix (Toyobo Co., Ltd., Japan), and 10 μ L of DNA-free distilled water. Amplification was carried out over 35 cycles using the same thermal cycler, SensoQuest® Labcycler (SensoQuest GmbH, Germany), as in the first round. The initial denaturation occurred at 94 °C for 3 min, followed

by denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, extension at 72 °C for 1 min and 30 s, final extension at 72 °C for 4 min, and a cooling phase at 4 °C. The expected amplicon size for the nested PCR was 438 base pairs. The primers used in this reaction targeted a conserved region of the *C. burnetii* OMP gene, as described by Zhang et al. (1998) and Ogawa et al. (2004).

DNA electrophoresis

PCR products were electrophoresed (Mupid ex-U®, ADVANCE, Japan) in agarose Biotechnology Grade® (First Base Biochemicals Sdn Bhd, Malaysia) gel 1.5% diluted in 1x Tris-Acetate-EDTA (TAE) Ultrapure Grade® (First Base Biochemicals Sdn Bhd, Malaysia) by using 50 V and 50 Hz settings for 50 min. Each nested-PCR sample product was placed inside the agarose gel up to 5 µL in volume. The first well was filled with 5 µL of DNA marker BenchTop® (Promega Corporation, USA), while the remaining wells were filled with the nested-PCR products. The agarose gel was electrophoresed with ethidium bromide, and the amplicon bands were observed under a UV illuminator.

Sequencing and phylogenetic analysis

The PCR products for each primer combination were purified and sequenced using the Sanger method at First BASE Sdn Bhd in Malaysia. The sequence was subsequently edited in MEGA 12 and analyzed with the Basic Local Alignment Search Tool (BLAST) in GenBank™ to identify and compare it with previously reported sequences. The analysis was carried out by reconstructing the phylogenetic tree using neighbor-joining, performing 1000 bootstrap replicates, and using the maximum composite likelihood substitution model, with reference sequences representing subtypes for comparison. Phylogenetic tree reconstruction was conducted using MEGA 12 software (Felsenstein, 1985; Saitou & Nei, 1987; Takamura et al., 2004).

Histopathological slides processing

Histopathological preparations using hematoxylin-eosin (H&E) and immunohistochemical (IHC) staining were performed on organs individually identified as PCR-positive. Each collected organ was placed in cassettes and immersed in 10% BNF for 24 hours. Dehydration was performed with various ethanol concentrations: 70% ethanol, 2 applications of 80% ethanol, 3 applications of 95% ethanol, and 2 applications of absolute ethanol. The organ underwent an initial immersion in 70% ethanol for 6 h, followed by 2-h immersion in

tervals in 80% ethanol until achieving absolute ethanol 2. The organ samples were then immersed in xylol 1 and xylol 2. The organ was then embedded in paraffin to form tissue blocks. The tissue block was trimmed and sectioned on a microtome for H&E staining. A routine H&E staining procedure was performed as described by Ma et al. (2024).

Immunohistochemical staining

Immunohistochemical (IHC) staining of organ samples was performed using the Starr Trek Universal HRP Detection System Kit (Biocare® Medical, LLC, USA) and a protocol based on Ozkaraca et al. (2016). Tissue sections were mounted onto glass slides coated with 1% poly-L-lysine (Biogear®, Netherlands). Deparaffinization was performed with xylol, followed by rehydration through a graded ethanol series and rinsing with distilled water. Antigen retrieval was performed by heating the slides in citrate buffer (Sigma-Aldrich®, USA) using a microwave for 20 min, followed by 3 washes in phosphate-buffered saline (PBS) (Sigma-Aldrich®, USA), each for 5 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 30 min, followed by 3 rinses with PBS. Non-specific binding was blocked using 1% fetal bovine serum (FBS) (Biogear®, Netherlands) for 30 min, followed by three additional PBS washes. A primary antibody (rabbit polyclonal anti-*C. burnetii* FKH-IPB) was applied, and the slides were incubated overnight at 4 °C.

Rabbit polyclonal anti-*C. burnetii* FKH-IPB antibody in a previous study (Herlina et al., 2019) has been used for the detection of *C. burnetii* in meat cattle. After incubation, the slides were washed 3 times in PBS, then incubated with a secondary antibody (biotinylated universal link) for 30 min. Streptavidin-HRP was then applied for 30 min, followed by washing. Chromogen 3,3'-diaminobenzidine (DAB) was applied for 10 seconds, followed by immersion in distilled water. Counterstaining was performed with Mayer hematoxylin for 10 s. The slides were rinsed, dehydrated through graded ethanol, cleared with xylol, mounted with permount under cover slips, and the stained slides were examined under a light microscope.

Results

Molecular and phylogenetic analysis

C. burnetii was found in 3 out of 100 male Brahman cross cattle (3%) when tested using the nested-PCR method with OMP primer, and 4 organs tested positive for the bacteria, including the heart and the spleen (Table 1).

Table 1. The positive results of *C. burnetii* detection by nested-PCR

Eartag No.	Visceral Organ				
	Lungs	Heart	Spleen	Liver	Kidney
5137	-	-	+	-	-
0828	-	-	+	-	-
0694	-	+	+	-	-

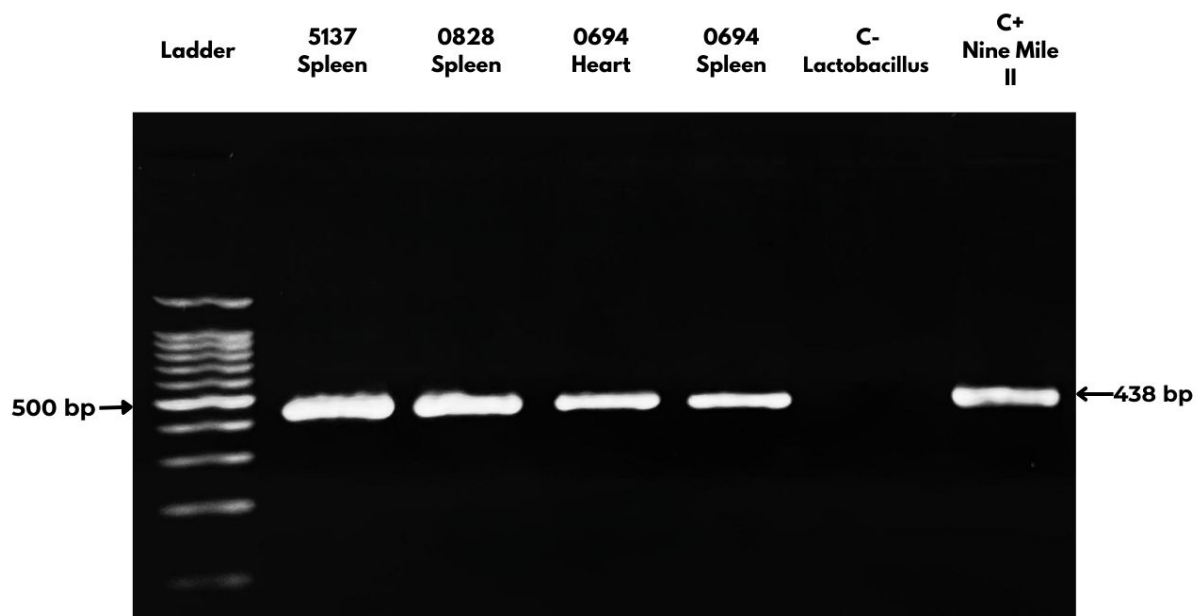
Note: +: Positive for *C. Burnetii*, -: Negative for *C. Burnetii*.

C. burnetii bacterium was identified in the heart of one cattle and in the spleen of three cattle. The clinical examination conducted before slaughter revealed no clinical signs in the cattle. Four organs tested positive using the nested-PCR method, showing amplicon bands of 438 bp, which aligned with the positive control (*C. burnetii* Nine Mile Phase II RSA493 is derived from Nine Mile Phase I [ATCC VR 615]) (accession numbers NC_002971 and NC_004704; Montana, USA) (Figure 1). Nine Mile II is a standard strain of *C. burnetii* that is avirulent and derived from phase 2. The negative control was derived from the DNA extraction product of *Lactobacillus* sp. bacteria.

Sequencing and phylogenetic analysis of the *com1* gene using the OMP primer were performed on the four *C. burnetii*-positive samples. These samples are already registered in the NCBI with accession numbers PV033359, PV033360, PV033361, and PV033362. The phylogenetic analysis of

the nucleotide sequences showed a high relationship with *C. burnetii* isolate CB 3P from the lung of a local goat (*Capra aegagrus*) from Malang, Indonesia (MW848695) (Figure 2), originating from previously conducted similar studies. The positive meat cattle samples from Lampung Province and the local goat isolate (CB 3P) both originate from ruminants. The high correlation is presumably due to the samples being obtained in the same country. The phylogenetic analysis (Figure 2) showed genetic differences among four *Rickettsia* sp. sequences. Strain TCM1 from Thailand (AB359457) as the outgroup species.

An analysis of sequence data (Figure 3) from *C. burnetii*-positive samples collected from the spleen and heart tissues of meat cattle showed 100% identity using the pairwise distance method. This analysis used values shown above and below the diagonal lines. The values above show the percentage of identity, while those below represent the number of

**Figure 1.** The *C. burnetii* detection result via nested-PCR

Note: C-: Negative control., C+: Positive control.

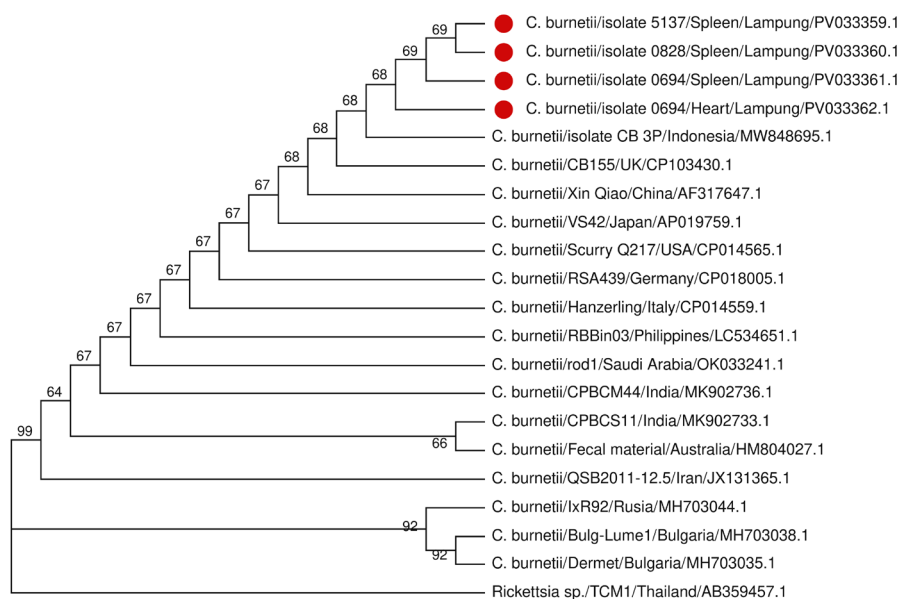


Figure 2. The phylogenetic tree generated using the neighbor-joining method based on the *com1* gene OMP (partial sequence) of *C. burnetii*, with 1000 bootstrap replicates

Note: The sequence of *C. burnetii* originating from Lampung Province, Indonesia, is shown by red dots (•). *Rickettsia* sp. was used as the outgroup species in the phylogenetic tree construction.

nucleotide base differences. The identity percentage between the four Lampung Province, Indonesia, isolates and the *C. burnetii* isolate in raw milk from Iran (JX131365.1) was 99%. The four Lampung Province isolates had a single nucleotide difference from the Iranian isolate (JX131365.1). Three other isolates that show significant differences from the 4 Lampung Province isolates include *C. burnetii* isolates from *Ixodes ricinus* in Russia (MH703044.1), *C. burnetii* isolates from *Luscinia megarhynchos* in Bulgaria (MH703038.1), and *C. burnetii* isolates from *Dermacentor reticulatus* in

Bulgaria (MH703035.1). These three sequences share 45% identity with the 4 Lampung Province isolates. The nitrogen base sequence differences between these three sequences and the Lampung Province isolates amount to 3 nucleotide bases. However, compared with the 3 outgroup species, a 55% similarity was observed (Figure 3). Base nucleotide sequence differences exist between the four positive sample sequences and the outgroup species sequences. The difference value of the base nucleotide can be observed in the numbers below the diagonal lines in Figure 3.

[illegible]

Figure 3. The *C. burnetii* nucleotide sequence differences based on the OMP primer computed using pairwise distances

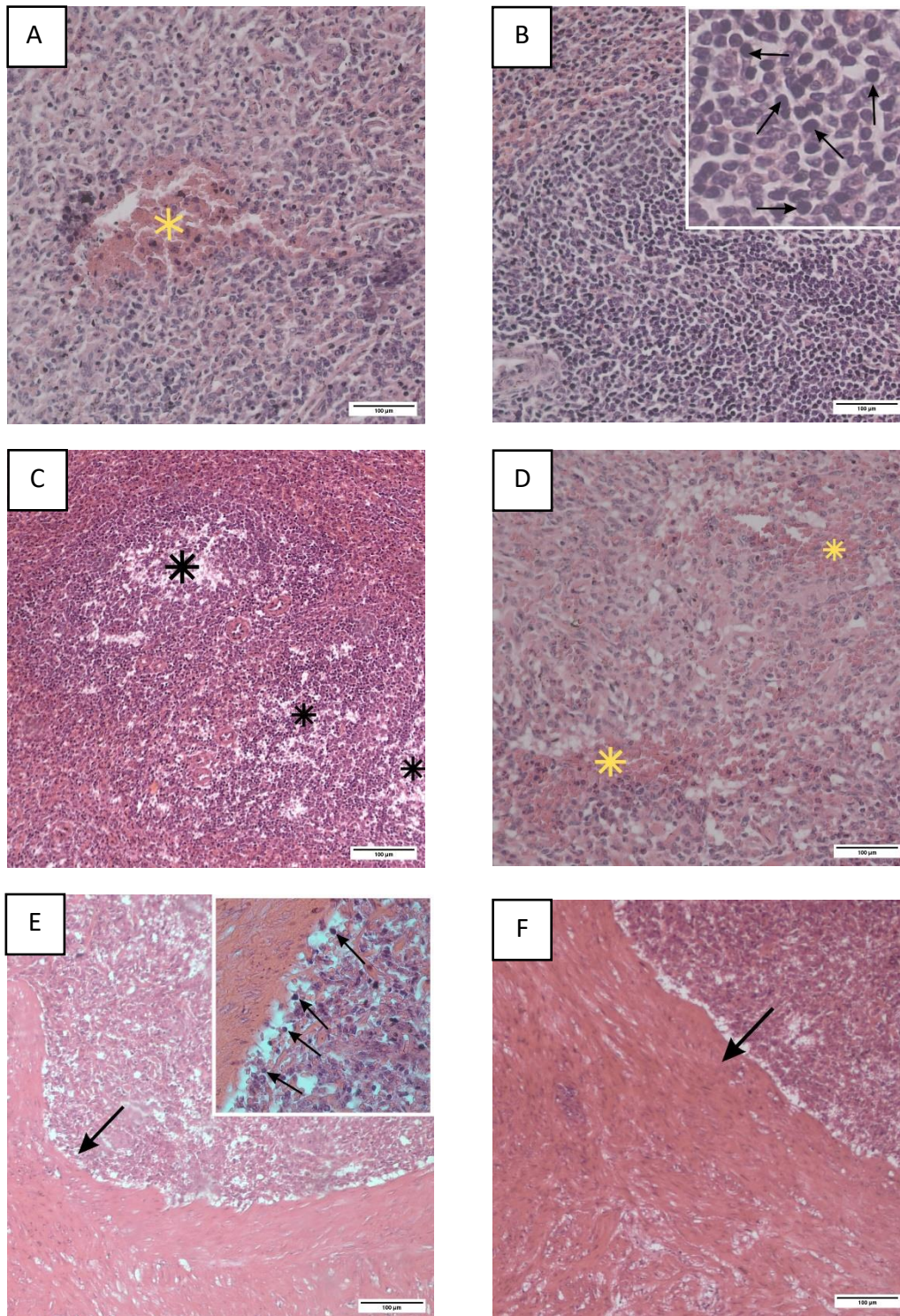


Figure 4. The HE-stained histopathological features of spleens positive for *C. burnetii*

Note: The histopathology of spleen (code 5137) showed vein congestion (asterix), inflammatory cell infiltrations (A), and macrophages (arrows) (code 5137) (B). White pulp depletion (asterix) in the spleen (code 0828) (C) and hemorrhage around the tissue (D). The spleen (code 0694) showed macrophage inflammatory cell infiltration into the trabecular sinus (arrows) (E), and thickening of the trabecula (arrow) (F). 1 bar=100 µm.

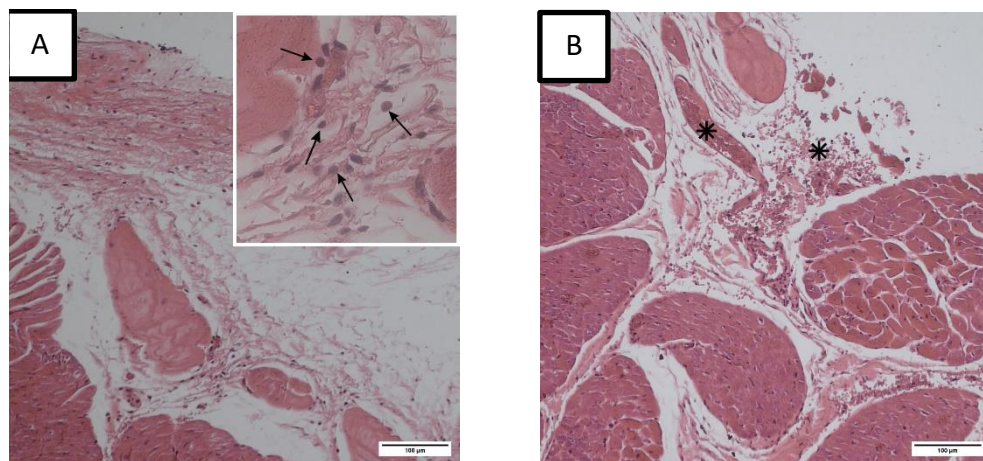


Figure 5. The HE-stained histopathological feature of the heart sample positive for *C. burnetii*

A) Infiltration of mononuclear inflammatory cells into the valve tissue of the heart (code 0694), B) hemorrhage and congestion (asterix) (1 bar=100 µm)

Histopathological analysis

The positive organ samples made into histopathology slides were stained with H&E. Histopathology slides stained with H&E were examined for changes in tissue structure due to *C. burnetii* infection. The organ samples stained with H&E include the spleen (codes 5137, 0828, 0694) and the heart (code 0694), with results shown in Figures 4 and 5. Histopathological analysis of the three spleens positive for *C. burnetii* showed that inflammatory cells, including lymphocytes and macrophages, infiltrated the red pulp, white pulp, trabeculae, and blood vessels. Mononuclear cell infiltrations filled all tissues of the 3 spleens. Macrophages accumulated in large numbers in the tissue, as shown in Figure 4B. Another lesion found in the infected spleens was lymphoid follicle depletion (Figure 4C). Congestion was also found in large numbers in the vein of the three spleens (Figure 4A). Hemorrhage lesions were found in the perivascular space of the red pulp, the trabecula, and the tissues around the red pulp (Figure 4D). Infiltration of macrophage and polymorphonuclear cells was observed in large numbers in the sinus trabeculae (Figure 4E). The trabecular tissue in the spleen also suffered hypertrophy (Figure 4F).

Histopathological analysis of the heart (code 0694) revealed lymphocyte and macrophage (mononuclear) inflammatory cell infiltration of the heart valve tissue (Figure 5A). Hemorrhage was found in several areas of the heart valve (Figure 5B). Congestion occurred in several veins (Figure 4B), but no significant change was observed in the heart muscle cell. The nucleus and cytoplasm of the heart muscle appeared normal with no sign of degeneration, and no vegetation was found in the valve tissue.

Four organs positive for *C. burnetii* from the nested PCR screening were further made into IHC slides to detect the antigen location. IHC staining showed positive immunoreactivity in all 4 organs. The immunoreactive result is shown by brown color in the cell cytoplasm (Figures 6).

An immunopositive reaction in the spleen was significantly detected in the cytoplasm of macrophages and lymphocytes (Figures 6A, 6B, and 6C). Brown color appeared frequently in the white pulp region of the spleen. Syncytium cells were also often found in the white pulp of the spleen, as shown with the arrowhead (Figures 6A, 6B, and 6C). An immunopositive reaction in the heart was observed in the blood vessel wall and in several macrophages (arrow) near the blood vessels (Figure 6D).

Discussion

Molecular and phylogenetic analysis

This study represents the first discovery of the bacteria in Lampung Province, Indonesia, after it had been previously reported in slaughterhouses across several regions, including Bali (4.29%), Bogor City (6.68%) (Mahatmi et al., 2007), Jakarta (7.5%) (Setiyono dan Subangkit, 2015), North Sumatra Province (38.3%) (Nasution et al., 2015), and Malang (10%) (Untari et al., 2024). The detection rate in Lampung Province (3%) was the lowest among the others, because all samples examined were Brahman Cross ex-import cattle originating from Australia. Since the 1970s, Australia has developed and implemented extensive Q fever surveillance and prevention strategies. The disease was officially designated as

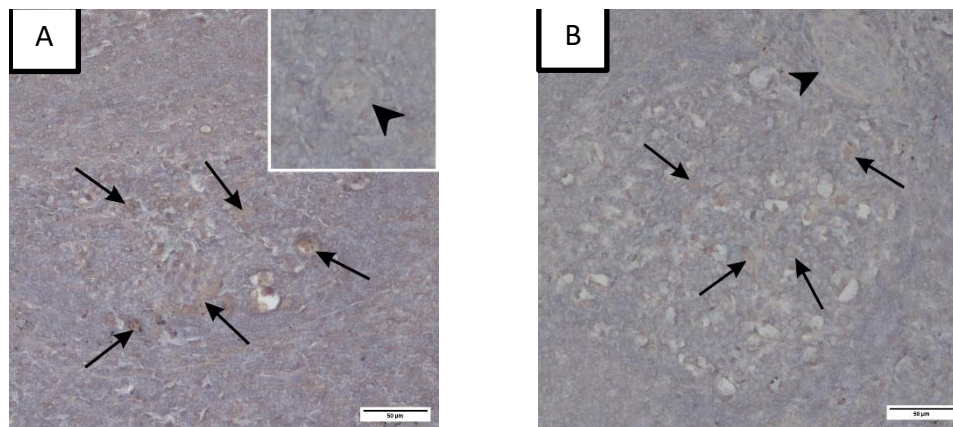


Figure 6. The IHC feature of organ samples positive for *C. Burnetii*

Note: The IHC results for the spleens (code 5137 (A), code 0828 (B), code 0694 (C)) and the heart (code 0694 (D)) showed a positive immunoreaction (black arrow). 1 bar=50 µm.

nationally notifiable in 1977, and Australia remains the only country with a licensed Q fever vaccine (Morissey et al., 2014). Enhanced surveillance conducted in New South Wales between 2005 and 2015 showed that 52.3% of reported cases were associated with high-risk occupations. Among these cases, 53.8% were related to direct contact with livestock, while 17.3% were by exposure to native or feral animals (Clutterbuck et al., 2018). The efforts underscore Australia's strong national capacity for Q fever monitoring and control. More importantly, not all cattle examined in other provinces were imported; some local breeds were also included in the detection. The animals examined by Untari et al. (2024) were local goats from Malang, Indonesia. Q fever surveillance in Indonesia remains very limited, and there is currently no government-implemented vaccination program for local cattle and goats.

C. burnetii is most frequently identified in the spleen, particularly due to its role as a defense organ, consisting of red and white pulp. The spleen also filters foreign materials and microorganisms. The *C. burnetii* bacteria in the bloodstream are filtered and engulfed by macrophages in the white pulp of the spleen (Boes & Durham, 2017). The bacteria preferentially replicate in macrophages, which are abundant in the white pulp tissue of the spleen (Porter et al., 2011) and have been identified in the cardiac tissue of a bovine specimen. The cattle identified by ear tag number 0694 are concurrently affected by two bacterial infections in the spleen and heart. The *C. burnetii* infection in macrophages within the spleen white pulp may disseminate through the bloodstream, reaching the heart, which circulates blood throughout the body and returns it to the lungs (Buckberg et al., 2018). Macrophages are abundant in the heart during chronic

infection. *C. burnetii* present in the bloodstream can replicate within macrophages found in the valve tissue of the heart (Roult et al., 2005; Meghari et al., 2008).

The phylogenetic analysis compared to 16 *C. burnetii* isolates from various countries based on the *com1* (partial sequence) gene showed 45%-100% identity. The OMP (*com1* gene) primers selected to detect *C. burnetii* exhibit high sensitivity and specificity because they are highly conserved and designed from the OMP of the bacteria (Zhang et al., 1998; Ogawa et al., 2004; Mahatmi et al., 2007; Kargar et al., 2015). The results of phylogenetic analysis show a close relationship with local goat isolates from East Java Province, Indonesia. The percentage of identity between the sequences of isolates from Lampung Province and East Java Province, Indonesia, was 100%, with no differences in the nucleotide base sequence. The genetic similarity may suggest both isolates originated from a common ancestral strain that has spread across regions through livestock movement, trade, or shared environmental reservoirs (Domenico et al., 2018). In Indonesia, inter-island or inter-provincial movement of animals is relatively common, and it could facilitate the dissemination of the same strain among different livestock populations. The high degree of similarity might reflect low genetic diversity among *C. burnetii* strains currently circulating in Indonesia. This situation is common in regions where epidemiological surveillance is limited, and only a few dominant strains have been established and maintained over time.

Isolates originating from Lampung Province have a low identity percentage of 45% with *C. burnetii* isolates from *Ixodes ricinus* in Russia (MH703044.1), *L. megarhynchus* from Bulgaria (MH703038.1), and *D.*

reticulatus from Bulgaria (MH703035.1). This level of divergence strongly suggests that the isolates may belong to different strains or genotypes that have evolved separately over time. *C. burnetii* isolates from Lampung Province, Indonesia, and those from Russia and Bulgaria probably evolved in different ecological and environmental conditions, contributing to significant genomic divergence. Genetic diversity may arise from differences in adaptation to specific hosts and vectors across geographic areas (Joulié et al., 2017). The four isolates from Lampung Province, Indonesia, were derived from meat cattle, which are the host of *C. burnetii*. In contrast, the Russian isolate originated from the tick *Ixodes ricinus*, while one Bulgarian isolate was obtained from the tick *D. reticulatus*, which acts as a vector. Another Bulgarian isolate was recovered from the wild bird *L. megarhynchos*, a reservoir host.

Q fever is a zoonotic disease transmitted from animals to humans (Porter et al., 2011). In cattle, Q fever is generally subclinical and asymptomatic, with the principal mode of transmission to humans via inhalation. Infection can occur through direct contact with infected animals or related products, specifically during animal slaughter (Eldin et al., 2017). Furthermore, Q fever is classified as an occupational disease, with those frequently in contact with animals at increased risk of infection (Njeru et al., 2016). Workers in slaughterhouses, specifically veterinarians and those engaged in slaughtering, skinning, meat cutting, and organ separation, constitute the highest-risk group. Individuals tasked with transporting meat to the market are also at risk of spreading the disease. The identification of *C. burnetii* in imported cattle meat in Lampung Province, Indonesia, raises significant concerns for the government, prompting the need for animal quarantine to establish early detection protocols before distribution.

Histopathological analysis

The accumulation of inflammatory cells, neutrophils, and macrophages showed the presence of chronic infection in the spleen (chronic splenitis) (Figure 4B) (Lefkowitz, 2001). *C. burnetii* is an obligate intracellular bacterium whose tropism is in the macrophage, using it as a replication site, and can be found during chronic conditions (Russell-Lodrigue et al., 2009). The characteristic pathological lesion is a doughnut-shaped granuloma, resembling an empty central zone surrounded by fibrin, polymorphonuclear leucocytes, macrophages, neutrophils, and giant cells forming a ring (Eldin et al., 2017). This lesion is usually found in humans with acute infection and rarely occurs in animal samples (Galache

et al., 2004). The doughnut granuloma lesion was not found in the three spleen samples analyzed.

As shown in Figure 4D, hemorrhage lesions were found in the tissues around the red pulp. Generally, hemorrhage is the leak of blood from the blood vessels (extravasation) due to the destruction of the wall (Melenotte et al., 2018). Damage to the blood vessels may also have been caused by *C. burnetii* infection (Leahey et al., 2015). The bacteria or endotoxin may cause a leak in the blood vessels by inducing blood clotting through interactions with the body's immune system (Yao et al., 1995). Congestion occurred in the vein of the 3 spleens as shown in Figure 4A. This condition, in which blood pools within blood vessels, is commonly caused by disorders of systemic and portal circulation. Microscopically, the sinus was dilated and filled with red blood cells (Valli, 2007).

Infiltration of inflammatory macrophages and polymorphonuclear cells was observed in the sinus trabeculae (Figure 4E). The trabecular sinus is the pathway for blood circulation, playing an important role in blood filtration and processing. The trabecular sinus facilitates blood to reach the venous sinuses, which will then join into trabecular veins and finally out of the spleen through the splenic vein (Klei, 2017). Macrophage infiltration into the trabecular sinus indicates chronic infection (Jiao et al., 2024). The trabecular tissue in the spleen also suffered hypertrophy (Figure 4F). The thickening of trabecular tissue indicates a chronic infection in the spleen (Tasca et al., 2009; Hermida et al., 2018). Chronic infection activates fibroblasts in the perivascular area to respond to inflammation by increasing the production of extracellular matrix components (Antar et al., 2024).

Lymphoid follicle depletion occurred when there was a decrease in cell density in the white pulp, primarily due to the lymphoid cells experiencing cytolysis (Figure 4C). *C. burnetii* is one of the microbiological agents that can cause lymphoid follicle depletion. White pulp depletion may occur due to infection with a foreign agent or exposure to chemical agents. During bacteremia, the spleen enlarges and becomes acutely inflamed, and lymphoid follicles degenerate (Valli, 2007).

The histopathological features of the heart sample (Figures 5A and 5B), positive for *C. burnetii*, indicate that the infection is in the initiation stage (Lepidi et al., 2003). Endocarditis is usually histologically identified by the presence of vegetations and inflammatory reaction in the valve tissue (Lepidi et al., 2002).

IHC staining showed positive immunoreactivity in the cytoplasm of the macrophage (Figures 6A, 6B, and 6C). Macrophages are often found in the white pulp region and play a role in phagocytosis of *C. burnetii*. As an obligate intracellular bacterium infecting macrophages and other phagocytic cells, *C. burnetii* can replicate in the cytoplasm of phagocytic cells (Dragan & Voth, 2020). It survives in the acidic macrophage cytoplasm, eluding phagocytosis, due to three proteins: Superoxide dismutase, catalase, and macrophage infectivity potentiator (Cbmip), which help it survive in the intracellular environment (Akporiaye & Baca, 1983). Furthermore, *C. burnetii* is usually found in the heart valve tissue, as shown in Figure 6D. Immunopositive reactions are often found in macrophages, neutrophils, and endothelial cells of blood vessels (Atzpodien et al., 1994).

Syncytium cells are often found in the white pulp of the spleen, as shown in (Figures 6A, 6B, and 6C). A syncytium is a multinuclear cell formed by the fusion of several cells (Tang et al., 2021). In an infection caused by *C. burnetii*, syncytia are not typically formed, and the bacteria mainly replicate within macrophage vacuoles. The multinuclear cell formation may have resulted from a macrophage response to a chronic bacterial infection (Ku et al., 2020).

The discovery of significant lesions such as chronic splenitis and endocarditis through histopathological examination, even though the animals did not show clinical symptoms before slaughter, most probably indicates the presence of subclinical *C. burnetii* infection at high levels. The pathogen demonstrates remarkable persistence, with infections in animals often persisting and remaining asymptomatic (Kazar, 2005; Roest et al., 2013). The bacteria's ability to cause chronic infections is facilitated by the intracellular, large-cell variant, which survives harsh phagolysosomal conditions, enabling long-term persistence in macrophages (Kazar, 2005). Subclinical infections can interfere with weight gain, feed efficiency, and other metabolic functions. Although not immediately apparent, these losses contribute to a decline in the economic value of meat cattle. Meat cattle that appear healthy but are infected with *C. burnetii* can be a source of zoonotic transmission through the air in slaughterhouses, or through contact with organs and body fluids (Baca & Paretsky, 1983; Fournier et al., 1998; Woldehiwet, 2004).

Conclusion

In conclusion, this study confirmed the presence of *C. burnetii*, the causative agent of Q fever, in slaughtered meat cattle from Lampung Province, Indonesia. Molecular detection using nested PCR identified the pathogen

in the spleen and heart of 3% of examined animals. Phylogenetic analysis showed high similarity to previously reported goat isolates from East Java. Histopathological results showed chronic infection, particularly in the spleen, while IHC confirmed the presence of *C. burnetii* antigens within macrophages and endothelial cells. Despite the absence of clinical symptoms, the infection was clearly established. These results represent the first molecular and pathological evidence of Q fever in cattle in Lampung Province, Indonesia, suggesting the need for surveillance to protect public and occupational health in the meat production sector. Periodic ELISA or PCR testing should be implemented for slaughtered cattle, particularly from high-density farms or regions with prior detections. Q fever awareness and screening programs are also recommended for at-risk personnel, including abattoir workers, farm staff, and veterinarians. Integrated one health surveillance systems that combine veterinary and human health data are crucial for detecting new threats. Further studies can examine genetic diversity using the multi-spacer sequence typing method.

Ethical Considerations

Compliance with ethical guidelines

The Animal Ethics Committee of the IPB University provided ethical approval and oversight under certificate number 225/KEH/SKE/VII/2024.

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Authors' contributions

Conceptualization and supervision: Agus Setiyono, Vetrizah Juniantito; Software, data curation, and data analysis: Asyifa Gisya Alayina; Data collection and investigation: Asyifa Gisya Alayina; Methodology, project administration, formal analysis, validation, visualization, and writing: All Authors.

Conflict of interest

All authors declared no conflict of interest.

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