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Molecular and histological detection of *Coxiella burnetii* in ruminants in East Java, Indonesia

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Abstract

Coxiella burnetii is an obligate intracellular bacterium that causes Q fever and belongs to the gamma subdivision of Proteobacteria. Several studies discovered incidences of Q fever in various parts of Indonesia, primarily in ruminants. Identification and detection of Q fever in East Java province was reported in 1976. Since then, there has been no report of the presence of this disease in the East Java region. This research aimed to detect the current presence of *C. burnetii* in ruminants in East Java using samples of the liver, spleen, kidney, lung, and heart of 125 cattle and 156 local goats slaughtered in slaughterhouses in East Java province. Detection of *C. burnetii* was performed by nested PCR method using outer membrane protein (OMP) and 16S rRNA primers, followed by confirmation using the immunohistochemistry method and hematoxylin-eosin staining. The results showed that *C. burnetii* bacteria were only detected in 8 out of 80 (10%) local goat samples from Malang by nested PCR using OMP primer. In comparison, nested PCR with 16SrRNA primer could only detect *C. burnetii* in 3 out of 8 goat's positive samples. Furthermore, immunohistochemistry showed immunoreactivity in the lung, spleen, and kidney. The positive sample of *C. burnetii* was found only in a small part of Malang local goats but neither in cattle in Malang nor other ruminants in other areas of East Java province. The results of this study strengthen the assumption that Q fever has already been endemic in East Java province.

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Introduction

C. burnetii is a Gram-negative and obligate intracellular bacteria that causes Q fever disease or coxiellosis (1,2). *C. burnetii* is the only species of the genus *C.* and belongs to the gamma subdivision *Proteobacteria* alongside *Legionella*, *Francisella*, *Pseudomonas*, and *Escherichia* (2-4). *C. burnetii* earned its name in 1938 due to recharacterization by Cornelius B. Philip on *Rickettsia burnetii*. These organisms are found as the causative agent of sporadic unexplainable fever cases (query) in humans in Brisbane and are also found in mites in Nine Mile (2). These bacteria were previously assumed to be part of the genus *Rickettsia* because they can only live in living cell medium and have the morphology characteristic of *Rickettsia* (5,6). The spread of Q fever has been identified in all countries except New Zealand (7,8). CDC classified Q fever as a zoonotic disease without pathognomonic symptoms in humans and animals (9). Acute infection of *C. burnetii* in humans often causes fever (critical febrile illness) (10,11), flu-like syndrome, malaise, headache, coughing (12), and pneumonia (13). Chronic infection occurs several months after exposure to *C. burnetii*

in the form of chronic endocarditis (2). Endocarditis cases without treatment or proper management may be fatal and end in death (14), with a mortality rate of more than 50% (9). Q fever cases in humans are closely related to ruminants, the primary source of infection for this disease (15). Goats and sheep are known to be the primary source of infection in Q fever outbreak cases in humans in the Netherlands from 2007-2010, causing 4,026 people with acute illness, six deaths, and more than 400,000 infected and at risk of developing chronic cases (16). This disease has high morbidity with low mortality but is very infective. A small number of these bacteria in the environment (1-10 bacteria) can infect the host (17). The primary infection route of this disease is through inhalation of droplets containing bacterial particles (12). Q fever is one of Indonesia's strategic zoonoses (18), but the government has largely ignored it. Indonesia has no official record of human clinical Q fever cases. Kaplan and Bertagna published the first finding of a Q fever case in Indonesia in 1953, where Q fever was detected in cow blood serum samples (19). Rumawas reported Q fever cases in the Surabaya region in 1976 (20). Thirty years later, some researchers attempted to detect *C. burnetii* in Indonesian ruminants. *C. burnetii* was detected in cattle and sheep in Bogor and Bali region (19,20), cattle in Jakarta (21), ex-import cattle in North Sumatra (22), goats in Yogyakarta (23), ex-import cattle in Depok (24) and a cow for religious slaughter in Cimanggu, Bogor which redetected in 2015-2016 (25). Exploration and molecular detection of this bacteria on animals in another part of Indonesia remain unexplored. East Java is one of Indonesia's regions with the highest livestock population. This region contained around 18.5% of the total livestock population in Indonesia, according to animal husbandry statistics data of Indonesia in 2020 (26).

East Java holds an essential role in supplying meat consumed by Indonesian people. Indonesian researchers have yet to identify and detect Q fever disease in this region. The finding of this research will provide initial data on the presence of this disease in East Java and be the first data that provides molecular analysis of *C. burnetii* in East Java.

Materials and methods

An exploratory case study was conducted to determine the presence of *C. burnetii* bacteria in ruminants in several districts of East Java province, Indonesia, from 2018 to 2020. The presence of *C. burnetii* bacteria in sample organs was analyzed using the nested PCR method of Outer Membrane Protein (OMP) primer and 16S rRNA, followed by a confirmation test using the immunohistochemistry method also hematoxylin and eosin staining test. The collected data was then descriptively evaluated.

Samples

Liver, spleen, heart, lung, and kidney samples from 125 cattle and 156 goats were collected from slaughterhouses in Surabaya, Madura, and Malang regions (Table 1). The samples were stored in two different media for further examination. Half of the organ samples were stored in 10% buffer-neutral formalin for immunohistochemistry and hematoxylin-eosin analysis. At the same time, the other half of the samples were stored at -20° C for PCR and sequencing.

Table 1: Source of samples

Regions	Species	Quantity (head)
Surabaya	Cow	60
	Goat	46
Madura	Cow	35
	Goat	30
Malang	Cow	30
	Goat	80

Samples taken from liver, spleen, heart, kidney and lung.

DNA extraction

DNA of *C. burnetii* was extracted using QIAmp DNA Mini Kit Cat. No. 51304 (QIAGEN, Netherland) according to the previous method (27). Crushed organ samples were processed sequentially with cell lysis solution (QIAGEN, Netherland), 300 µl isopropanol, and 70% ethanol. Alcohol evaporation was performed for one hour after the last suspension was centrifuged. The DNA extract from that process was stored in a microtube at 4°C.

First PCR

PCR mixture created from 3 µl DNA samples, 0.3 µl of each first PCR primer (Table 2), 20.25 µl RNA-free water, 3 µl dNTP, 3 µl Taq buffer, and 0,15 µl Taq polymerase (Takara Shizo, Shiga, Japan). The positive control used was *C. burnetii* strain Nine Mile (NM). Afterward, samples were amplified in a thermally cycled GeneAmp PCR System 9700 (Applied Biosystem, US) using the following program (Table 3). The products from the first PCR (Table 4) were then stored at 4°C and used in nested PCR stages.

Nested PCR

0.3 µl of each primer used for nested PCR were mixed with 3 µl dNTP, 22.25 µl RNA-free water, 3 µl Taq buffer, 0.15 µl Taq polymerase (Takara Shizo, Shiga, Japan), and 1 µl DNA sample from the first PCR to obtain PCR mixture. Amplification was performed by thermocycler GeneAmp PCR System 9700 (Applied Biosystem, US) using the following program (Table 4), and the nested PCR product obtained was then visualized by the electrophoresis method.

Amplification product detection

Electrophoresis was performed using agarose gel 1.5% in Tris Acetate EDTA solution added by ethidium bromide at 100 volt and 50 Hz for around 30 minutes. The electrophoresis result was observed and photographed with the help of UV luminescence.

Table 2: Primer used in the nested PCR method

Protein	PCR Stages	Primer sequences	Product size (bp)
OMP (30)	First PCR	OMP1(AGTAGAAGCATCCCAAGCATT) OMP2 (TGCCTGCTAGCTGTAACGATT)	500
	Nested PCR	OMP3 (GAAGCGCAACAAGAAGAACAC) OMP4 (TTGGAAGTTATCACGCAGTTG)	437
16S rRNA (37)	First PCR	Cox16SF1(CGTAGGAATCTACCTTRTAGWGG) Cox16SR2 (GCCTACCCGCTTCTGGTACAATT)	1.321-1.429
	Nested PCR	Cox16SF2 (TGAGAACTAGCTGTTGGRRAGT) Cox16SR2 (GCCTACCCGCTTCTGGTACAATT)	624-627

Table 3: Amplification gradient of first PCR

Stages	OMP		16S rRNA	
Pre-denaturation	94˚C, 3 min.	1 cycle	93˚C, 3 min.	1 cycle
Denaturation	94˚C, 1 min.		93˚C, 30 sec.	
Annealing	54˚C, 1 min.	35 cycles	56˚C, 30 sec.	30 cycles
Elongation	72˚C, 2 min.		72˚C, 1 min.	
Post elongation	72˚C, 4 min.	1 cycle	72˚C, 5 min.	1 cycle
Cooling	4˚C, ∞	1 cycle	4˚C, ∞	1 cycle

Table 4: Amplification gradient of nested PCR

Stages	OMP		16S rRNA	
Pre-denaturation	94˚C, 3 min.	1 cycle	93˚C, 3 min.	1 cycle
Denaturation	94˚C, 1 min.		93˚C, 30 sec.	
Annealing	56˚C, 1 min.	35 cycles	56˚C, 30 sec.	30 cycles
Elongation	72˚C, 1,5 min.		72˚C, 1 min.	
Post elongation	72˚C, 4 min.	1 cycle	72˚C, 5 min.	1 cycle
Cooling	4˚C, ∞	1 cycle	4˚C, ∞	1 cycle

Sequencing

Agencourt AMPure XP (Beckman Coulter) purified the best band from nested PCR results and then cloned using pCR4Blunt-TOPO vector (Invitrogen, Thermo Fisher Scientific, US) and sequenced by BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystem, Thermo Fisher Scientific, US). DNA sequence obtained from sequencing was read by BLAST and aligned by MEGA X. Phylogenetic analysis was performed by software MEGA X by comparing DNA sample sequence from the alignment with other 16S RNA sequences taken from GenBank. The stability of the phylogenetic tree is predicted by bootstrap analysis with 1,000 repetitions.

Histopathology slides preparations

Organ slices were put into ethanol 70%, ethanol 70%, ethanol 80%, ethanol 90%, ethanol 95% (dehydration), and xylol I and II (clearing) for 2 hours each. They would then be put into melted paraffin at 56 ºC for 2 hours. The tissues were retrieved by tweezer and followed by embedding with paraffin to obtain tissues in paraffin blocks. Tissues from organ samples in paraffin blocks were cut into five µm and attached to object glass. Additional sections were stained by the immunohistochemistry method.

Immunohistochemistry

The staining process by immunohistochemistry was performed by a combination of the previously used method (22) and instruction from the developer of kit Dako EnVision® + Dual Link System-HRP (DAB+) Code 4065 (22) with modifications. Tissue preparations on object glass coated by poly l lysine were deparaffinized and then reheated in citrate buffer as an antigen retrieval process for 20 minutes. The next step after washing by PBST (Phosphate Buffer Saline + Tween 20) 0,1% was blocked by skim milk 5% in PBST for 40 minutes, followed by drops of primary antibody (Polyclonal antibody, Rabbit anti-*C. burnetii*, Faculty of Veterinary Medicine, IPB University). The tissues were incubated overnight at four °C. The next step was washing with PBST and adding the blocking solution Dual Endogenous Enzyme B ock from Dako EnVision® + Dual Link System-HRP (DAB+) for 30 minutes at room temperature. Secondary antibodies (Labelled Polymer-HRP from Dako EnVision® + Dual Link System-HRP (DAB+))

were added after washing with PBST, followed by incubation for 30 minutes at room temperature. The next step was adding chromogen 3.3-diaminobenzidine (DAB), counterstained by Mayer's hematoxylin, and washing it with distilled water. Dehydration was performed on graduated alcohol, and clearing was done with xylol. Tissues on object glass were mounted and covered by a cover glass. The slides were examined under the microscope in 100-400x magnification to be observed quantitatively and qualitatively with positive reaction indicated by brown color.

Hematoxylin and eosin staining

Slides were submerged in xylol I, II, and III for 3-5 minutes each and rehydrated by graduated alcohol after being washed with running water for 10-15 minutes. For the next step, the preparations were stained by hematoxylin stain for 10-15 seconds and then washed with running water. Afterwards, the trials were stained by eosin stain for 1-2 minutes, then washed again with running water. The stained slides were dehydrated in graduated alcohol and followed by clearing using xylene and examined using a light microscope with xylol (22).

Results

Data in Table 3 show that *C. burnetii* bacteria were only detected in goat organs from Malang, but neither from samples of cattle organs in Malang nor other ruminants from other areas of East Java. *C. burnetii* bacteria were only detected in 8 out of 80 head (10%) local goat samples from Malang (Figure 1; Table 5). *C. burnetii* was found in the lungs of six goats, one goat's spleen, and one goat's kidney. A positive result was obtained by nested PCR method using OMP primer. Sequencing and phylogenetic analysis of gen *com-1* with OMP primer was performed on the eight positive samples (Figures 2 and 3). Positive sample sequence analysis from goat organs showed that the eight sequences were 100% identical (Figure 4). All positive sample sequences have been registered in GenBank under accession numbers MW41908, MW816117, and MW848690 - MW848695.

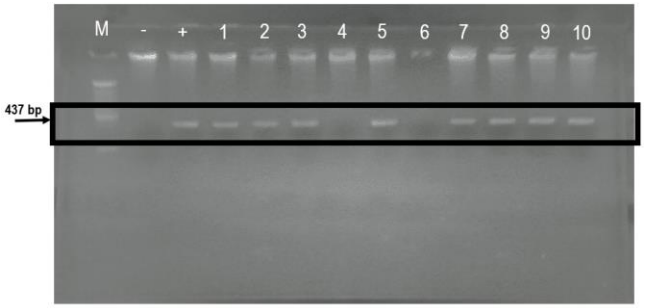

Figure 1: PCR results with OMP's specific bands (437 bp) showed 8 positive samples. Note: (M) marker, (-) negative control, (+) positive control, (No.1-10) samples.

Table 5: Detection of *C. burnetii* bacteria in ruminant sample organs in East Java using nested PCR OMP primer test

Region	Animal	Number of samples (head)	Nested PCR OMP Primer Test	
			Negative	Positive
Surabaya	Cow	60	60	0
	Goat	46	46	0
Malang	Cow	30	30	0
	Goat	80	72	8
Madura	Cow	35	35	0
	Goat	30	30	0

OMP nucleotide sequence phylogenetic analysis showed the relationship between positive samples sequences with *C. burnetii* isolate from cattle in the Philippines (LC534651), KZQ1 strain from rats in South Korea (KM115542), 216 and 214 strain from rats in China (JX522484, JX522485), *C. burnetii* isolate from porcupine in China (MK388245), *C. burnetii* isolates from *Hyalomma asiaticum* mites in China (MF045790), RGGG isolates from cattle in India (MF142256), Xin Qiao strain from mites in China (AF317647), even with *C. cheraxi* TO-98 strain, a *C.* species found in crayfish (EF413062) obtained from GenBank (Figure 2). These sequences have high similarity with various identity percentages from 99.4% to 100% (Figure 3). Phylogenetic analysis with OMP nucleotide sequence also showed that genetically, positive samples from Malang local goats were different from endosymbiont *C.* obtained from *Argas reflexus* mite (MF359024), often called *C.*-like-bacteria (CLB). *C. burnetii* is especially detected in a goat's lungs (6/8). This is assumed to be connected to the pathogenesis of this disease.

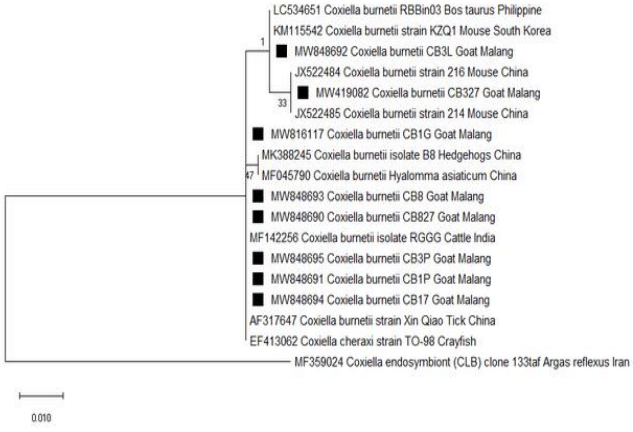

Figure 2: Phylogenetic tree constructed using Tamura-Nei/Maximum Likelihood methods based on com1 gene (OMP) sequences of *C. burnetii*. Sequences with black square labels are Malang sample sequences.

No.	Sequence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	**MW848695 *Coxiella burnetii* CB3P Goat Malang**		100	100	100	100	100	100	100	100	100	100	100	99,75	100	100	100	100	89
2	**MW848694 *Coxiella burnetii* CB17 Goat Malang**	0		100	100	100	100	100	100	100	100	100	100	99,75	100	100	100	100	89,4
3	**MW848693 *Coxiella burnetii* CB8 Goat Malang**	0	0		100	100	100	100	100	100	100	100	100	99,75	100	100	100	100	89,4
4	**MW848692 *Coxiella burnetii* CB3L Goat Malang**	0	0	0		100	100	100	100	100	100	100	100	99,75	100	100	100	100	89,5
5	**MW848691 *Coxiella burnetii* CB1P Goat Malang**	0	0	0	0		100	100	100	100	100	100	100	99,75	100	100	100	100	89
6	**MW848690 *Coxiella burnetii* CB827 Goat Malang**	0	0	0	0	0		100	100	100	100	100	100	99,75	100	100	100	100	89,4
7	**MW816117 *Coxiella burnetii* CB1G Goat Malang**	0	0	0	0	0	0		100	100	100	100	99,72	99,75	100	100	100	100	89
8	**MW419082 *Coxiella burnetii* CB327 Goat Malang**	0	0	0	0	0	0	0		100	100	100	100	99,73	100	100	100	100	89,9
9	LC534651 *Coxiella burnetii* RBBin03 *Bos taurus* Philippines	0	0	0	0	0	0	0	0		100	100	99,46	99,73	100	100	100	100	90,1
10	KM115542 *Coxiella burnetii* strain KZQ1 Mouse South Korea	0	0	0	0	0	0	0	0	0		100	100	99,71	100	100	100	100	88,8
11	JX522484 *Coxiella burnetii* strain 216 Mouse China	0	0	0	0	0	0	0	0	0	0		100	99,71	100	100	100	100	89,7
12	JX522485 *Coxiella burnetii* strain 214 Mouse China	0	0	0	0	0	0	1	0	1	0	0		99,71	100	99,45	99,46	99,40	89,7
13	MK388245 *Coxiella burnetii* isolate B8 Hedgehogs China	1	1	1	1	1	1	1	2	1	1	1	1		100	99,71	99,8	99,68	89,5
14	MF045790 *Coxiella burnetii* from *Hyalomma asiaticum* China	0	0	0	0	0	0	0	0	0	0	0	0	0		100	100	100	89,2
15	MF142256 *Coxiella burnetii* isolate RGGG Cattle India	0	0	0	0	0	0	0	0	0	0	0	3	1	0		100	100	89,6
16	AF317647 *Coxiella burnetii* strain Xin Qiao Tick China	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0		100	89,4
17	EF413062 *Coxiella cheraxi* strain TO-98 Crayfish Australia	0	0	0	0	0	0	0	0	0	0	0	3	1	0	0	0		90,5
18	MF359024 CLB clone 133taf of *Argas reflexus* Iran	37	37	35	35	37	37	37	33	30	53	29	28	35	43	28	57	310	

Figure 3: The comparison of *C. burnetii* nucleotide sequences by OMP primer. The numbers above the diagonal line showed the percentage of identity. The numbers below the d agonal lines show the nucleotide base differences.

Nested PCR method examination by 16SrRNA primer was only able to amplify 3 out of 8 positive samples tested with nested PCR using OMP primer. This is presumably because the DNA sample used is no longer stable, so DNA damage is possible, which causes fragmentation of the DNA samples so that some samples are not amplified with 16S rRNA. Sequencing and phylogenetic analysis of the three samples showed high similarity with a 99,86-100% percentage. These three sequences have been registered in GenBank under accession numbers MW450479, MW450480, and MW450598 (Figure 4). Sequences amplified by 16S rRNA were sequences of samples obtained from the lung and kidney.

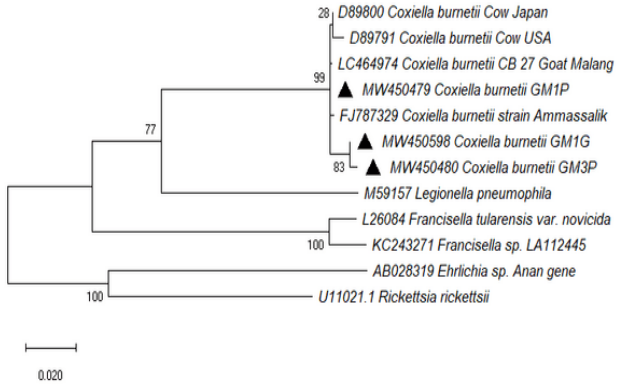

Figure 4: The phylogenetic tree was constructed using Tamura-Nei/Maximum Likelihood methods based on the 16S rRNA sequences of *C. burnetii.* Sequences with black triangle labels are Malang sample sequences.

Phylogenetic analysis from amplification by 16S rRNA showed similarities between Malang goat samples and previous research (LC464974), especially MW450479, with a 100% identity percentage (Figure 5). Comparison analysis between the nucleotide sequence of Malang local goat sample with isolate *C. burnetii* from other countries obtained from GenBank (D89800, D89791, FJ787329) showed a close relationship with a percentage 98.54% - 99.85% (Figure 5) and different from *Legionella pneumophila* (M59157), *Francisella tularensis var. novicida* (L26084), or *Francisella sp. LA112445* (KC243271), although the last three species are from the same subdivision, gammaproteobacteria (Figure 5).

No.	Sequence	1	2	3	4	5	6	7	8	9	10	11	12
1	D89800 *Coxiella burnetii* Cow Japan		99.52	99.47	99.55	98.85	99.31	99.84	86.90	84.05	84.09	80.32	80.60
2	D89791 *Coxiella burnetii* Cow USA	7		99.17	99.24	98.54	98.76	99.22	86.76	83.83	83.84	80.21	80.47
3	FJ787329 *Coxiella burnetii* strain Ammassalik	7	11		99.76	99.03	99.31	99.84	86.47	84.15	84.17	79.95	80.36
4	LC464974 *Coxiella burnetii* CB 27 Goat Malang	6	10	3		99.16	99.86	100	87.27	84.52	85.86	80.64	82.50
5	**MW450598 *Coxiella burnetii* GM1G**	15	20	13	12		99.86	100	87.39	84.59	85.73	79.94	82.19
6	**MW450480 *Coxiella burnetii* GM3P**	71	75	68	53	45		100	86.59	84.42	86.79	82.46	84.96
7	**MW450479 *Coxiella burnetii* GM1P**	57	61	45	53	61	105		86.38	85.21	86.37	82.08	84.79
8	M59157 *Legionella pneumophila*	198	200	184	176	70	229	208		83.11	84,31	80.40	79.72
9	L26084 *Francisella tularensis var. Novicida*	255	258	227	220	214	275	246	256		96.93	79.14	77.91
10	KC243271 *Francisella sp.* LA112445	204	207	203	199	202	256	235	201	37		79.77	79.02
11	AB028319 *Ehrlichia sp.* Anan gene	334	333	308	298	297	359	314	361	379	285		83.23
12	U11021 *Rickettsia rickettsii*	311	309	282	274	276	334	297	362	357	281	255	

Figure 5: The comparison of *C. burnetii* nucleotide sequences using 16S rRNA primer. The numbers above diagonal lines showed identity percentage. The numbers below the diagonal showed the number of nucleotide base differences.

All eight samples that confirmed positive in nested PCR examination were then further determinate and analyzed for the pathological change using immunohistochemistry and HE staining method. The examination by immunohistochemistry method used polyclonal antibody *rabbit anti-C. burnetii*, and the result showed positive immunoreaction on the cytoplasm. The positive outcome was found in the kidney, spleen and lungs. The infected kidney showed tubular necrosis, tubular epithelial cell desquamation, enlarged glomerulus, hemorrhage, congestion, and granulomatous inflammation. Histopathological changes in the infected spleen revealed depletion of white pulps, secondary follicle formation, neutrophil inflammatory cell infiltration, neutrophil and macrophage of red pulps, hemosiderin deposit, and accumulation of connective tissue on red pulp. Infected lungs showed granulomatous inflammation, congestion, inflammatory cell infiltration, atelectasis, and fibrosis (Figure 6).

Discussion

Q fever is a neglected disease in many countries (28), including Indonesia. This research showed that *C. burnetii* was only detected in local goat organs in Malang, East Java. The phylogenetic analysis of positive samples' com1 gene amplification product showed similarities with *C. burnetii* species found in other countries and *C. cheraxi*, a new *Coxiella* species found in crayfish (29). OMP primer was used to amplify *the com1* gene, a primer developed based on the conserved region *com1* gene from 21 strains of *C.*

burnetii, and has been proven to show high specificity and sensitivity, especially for the nested PCR method (30). Phylogenetic analysis from 16S ribosomal RNA gene amplification strengthened *C. burnetii* bacteria from Malang local goat samples as a member of the gammaproteobacteria subdivision along with *Legionella*, *Francisella*, *Pseudomonas*, and *Escherichia* (2-4).

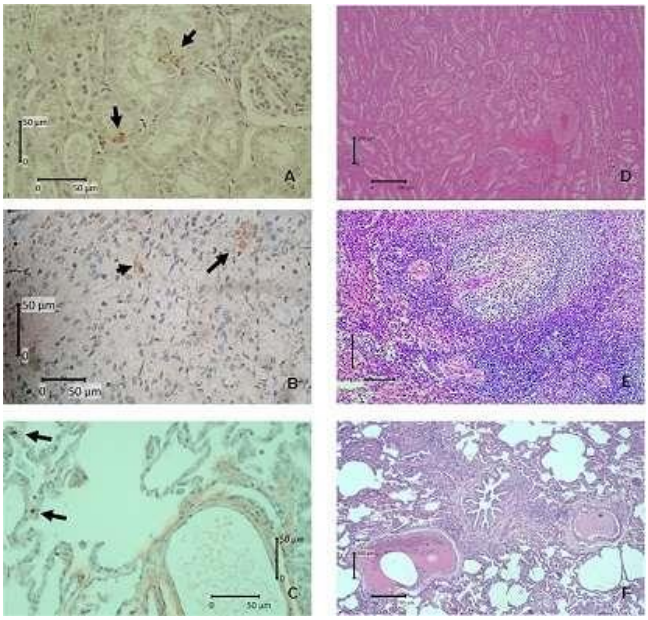

Figure 6: The result of immunohistochemistry and HE staining of positive samples for *C. burnetii*. Immunohistochemistry in the kidney (A), spleen (B), and lung (C) showed positive immunoreactivities (arrows). Histopathology images of the kidney (D) showed inflammatory cells in the interstitium, congestion, and glomerulonephritis. Spleen (E) showed white pulp depletion, soft tissue accumulation, macrophage infiltration, and secondary follicle formation, while lung (F) showed granulomatous inflammatory reactions, congestion, and atelectasis.

The goat is known as the primary source of infection, and in recent research, there are opinions that the camel could also be one of the primary sources of *C. burnetii* infection in humans (31). Goats are infected through inhaling particles or dust contaminated by the *C. burnetii* SCV phase, which is ±30 µm in size (32). Bacterial attachment followed by bacterial penetration capture by lysosome is followed by the formation of intracellular phagolysosome (12). This is shown in immunohistochemistry, which showed immunoreactivity to *C. burnetii* on the cytoplasm of macrophage cells. Still, after bacteria begin replicating in the phagolysosome, the inflammatory response is rapidly reduced and inhibits the clearance process of bacteria from the macrophage by the host immune system (33). *C. burnetii* can breed in an acidic environment of phagolysosome with division speed almost akin to cell division speed (2), which causes cell and tissue destruction from this infection to appear minimal and rarely show clinical symptoms. This explains the research findings, which showed that the bacteria were primarily discovered in the lungs (6 out of 8 samples) despite the animal exhibiting no clinical signs.

Cytokine and lymphokine stimulation due to *C. burnetii* insertion can cause heightened activities from macrophages, leukocytes, and fibrin, initiating local inflammation up to granuloma formation in the tissue (2). This appears in the pathological changes of positive organs, from inflammatory cell infiltration and connective tissue accumulation to the early stage of granulomatous inflammation. This is similar to the finding in a retrospective study on Q fever in Malaysia, where granulomatous inflammation was found in the lung and kidney of a goat positive for Q fever (34). However, tissue damage that occurred did not characterize pathological lesions due to *C. burnetii* infection. Lesions in various organs of goats positive for Q fever are nonspecific, which aligns with the statement that there is no pathognomonic lesion in *C. burnetii* infection in animals (21).

C. burnetii in local goats shows the high infection potency from goat to human. The distance between farms and slaughterhouses is less than 1 km, and between animal pens and farmers' houses is less than 1 km. The risk of pneumonia and Q fever in humans increases in people living near goat farms, especially within 500-1,000 meters of goat farms (35). The relation between positive cases in animals and humans still needs to be proven in this research because there is still no abortus sample material from a goat or sample from a human. The presence of these bacteria in the farm, if not managed well, can cause an outbreak in animals and humans one day because these bacteria can survive for a long time in the environment.

The poor hygiene condition of the farm is assumed to be one of the causes of infection in male goats. There is no report related to the abortion rate in goats in this region, which makes it hard to detect the source of infection. The clinical manifestation of this disease is unspecific and may influence goat performance and, in turn, affect the economy of the owner. Biosecurity must be implemented to prevent exposure and minimize transmission of *C. burnetii* to humans or animals (36). An effort from the government and the people are needed to prevent the outbreak of this disease in human in the future (37).

Conclusion

This result showed that *C. burnetii* was only detected in local goats from the Malang region, with no specific histopathological changes in each positive organ. *C. burnetii* was neither detected in local cattle from the Malang region nor other ruminants in other areas of Malang. A positive sample of *C. burnetii* found only in Malang local goats

strengthens the assumption that Q fever is already endemic in East Java, just like in West Java. Further research with a broader coverage area accompanied by an increase in the variety of detected ion methods and sample types is needed to better understand the presence of Q fever in East Java.

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Conflict of interest

The authors declare that they have no conflict of interest between the authors.

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الكشف الجزيئي والنسيجي للكوكسيلية البورنيتية في المجترات في جاوة الشرقية، إندونيسيا

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الخلاصة

الكوكسيلية البورنيتية هي بكتيريا إلزامية داخل الخلايا تسبب حمى كيو وتنتمي إلى قسم غاما الفرعي للبكتيريا البروتينية. اكتشفت العديد من الدراسات حدوث حمى كيو في أجزاء مختلفة من إندونيسيا، خاصة في الحيوانات المجترة. تم الإبلاغ عن تحديد واكتشاف حمى كيو في مقاطعة جاوة الشرقية في عام ١٩٧٦ . ومنذ ذلك الحين، لم يتم الإبلاغ عن وجود هذا المرض في منطقة جاوة الشرقية. يهدف هذا البحث إلى الكشف عن الوجود الحالي لبكتيريا الكوكسيلية البورنيتية في الحيوانات المجترة في جاوة الشرقية باستخدام عينات من الكبد والطحال والكلى والرئة والقلب لـ ١٢٥ رأسا من الماشية و١٥٦ ماعزا محليا مذبوحة في مسالخ في مقاطعة جاوة الشرقية. تم إجراء الكشف عن الكوكسيلية البورنيتية بواسطة تفاعل السلسلة المتبلمرة المتداخلة باستخدام بروتين الغشاء الخارجي و١٦S rRNA الإشعال، تليها التأكيد باستخدام طريقة الكيمياء المناعية وتلطيخ الهيماتوكسيلين يوزين. أظهرت النتائج أن بكتيريا الكوكسيلية البورنيتية تم اكتشافها فقط في ٨ من أصل ٨٠ (١٠٪) من عينات الماعز المحلية من مالانج بواسطة تفاعل السلسلة المتبلمرة المتداخل باستخدام بروتين الغشاء الخارجي التمهيدي. بالمقارنة، يمكن لتفاعل السلسلة المتبلمرة المتداخل مع التمهيدي ١٦SrRNA اكتشاف الكوكسيلية البورنيتية فقط في ٣ من أصل ٨ عينات إيجابية للماعز. علاوة على ذلك، أظهرت الكيمياء المناعية نشاطا مناعيا في الرئة والطحال والكلى. تم العثور على العينة الإيجابية لمرض الكوكسيلية البورنيتية فقط في جزء صغير من الماعز المحلي في مالانج ولكن لم يتم العثور عليها في الماشية في مالانج ولا غيرها من الحيوانات المجترة في مناطق أخرى من مقاطعة جاوة الشرقية. تعزز نتائج هذه الدراسة الافتراض بأن حمى كيو كانت مستوطنة بالفعل في مقاطعة جاوة الشرقية.

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