

# Detection of *Coxiella burnetii* Infection in Various Organs from Beef Cattle in Depok, West Java, Indonesia

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**Abstract**—*Coxiella burnetii* (*C. burnetii*) is negative Gram bacteria that caused zoonotic disease Q fever. The main reservoir of this disease are ruminants such as cow, goat, and sheep. Q fever infection happened to human relatively easily through aerosol, direct contact, and consumption of animal products from infected animal that has not been appropriately handled. This disease spread to almost all corner of the world and is a threat to food security and bioterrorism. Identification of *C. burnetii* infection in beef cattle in Indonesia is still limited. This research aim was to detect *C. burnetii* infection in various organs of beef cattle from Depok, Indonesia, through molecular examination using nested PCR (nPCR). The result of this research showed that the samples are identified to be positive of Q fever through nPCR method in various organs. Distribution of infection detected on heart, spleen, and kidney that mostly found in spleen and heart. This result indicates that *C. burnetii* probably can caused endocarditis in beef cattle like in human. This result also give a warning to more attention to the zoonotic threats for human in slaughter house.

**Keywords**—Q fever, Depok, nested PCR

## I. INTRODUCTION

Query fever or Q fever is a disease caused by *Coxiella burnetii* (*C. burnetii*), which also called coxiellosis. Q fever causative agent was first isolated by Macfarlane Burnet and Mavis Freeman in 1937. At that time, Q fever agent was assumed to come originate from rickettsia [1, 2]. Cox and Gordon Davis in 1938 were able to isolate Q fever causative agent from ticks in Montana, USA. This agent has similarity with *proteobacterium* group, which are *Legionella* and *Francisella*. This agent was then named *Coxiella burnetii* which is included to *Proteobacterium*, Class *Gammaproteobacteria*, ordo *Legionellales*, and family *Coxiellaceae* [1, 3].

Q fever is included as zoonotic disease that can be transmitted from animal to human and vice versa. Livestock such as cow, goat, and sheep are the main reservoir of this disease. Wildlife and pet animal,

especially mammals, can be a source of infection. Presence of ectoparasite such as tick can also serve as potential vector for the spread of the disease. The main route of infection from this disease is through inhalation but can also happen per oral. Direct contact or inhalation of secreta, excreta, and remains of aborted fetus from infected animal can be a source of infection to both animal and human. Milk and meat consumption originating from infected animal that are not properly handled can be another source of infection. This becomes a threat to animal derived food safety [1, 2, 4, 5].

Q fever is a zoonotic disease that has spread in various countries. Based on OIE data in 2017, Q fever distribution happens in all countries in the world. Canada, USA, Australia, and several countries in Europe, Africa, and ASEAN have reported the presence of Q fever infection in animal [6]. Q fever has been an important health problem in various countries such as United States, France, Britain, Italy, Germany, Spain, Canada, Japan, Australia, South Korea, Taiwan, and several other countries in South East Asian. United States of America categorized Q fever as category B bioterrorism agent as this agent is moderately easy to spread [7].

Indonesia has not yet to provide information on Q fever cases reported officially. This is because the clinical symptom of this disease is asymptomatic and thus often mistaken by other disease and ended up undiagnosed [8]. Q fever was first found in Indonesia on 1953. Kaplan and Bertagna in 1955 reported 188 cow serum were positive of Q fever [9]. Identification and study on Q fever disease after 1953 in Indonesia has never been reported again. New researches began again from 2006 to 2015 [8, 10]. This research has the aim to detection of *C. burnetii* infection in various organs of beef cattle from Depok, Indonesia, through molecular examination using nested nPCR.

## II. MATERIALS AND METHODS

### A. Sample

Samples used in this research are heart, lung, liver, spleen, and kidney tissue from beef cattle that slaughtered in slaughterhouses in Depok region in 2017. Each organ were trimmed into 3 cm thick and it was coated by aluminium foil and put inside cool box filled with ice pack and stored in -30 °C freezer for molecular examination. Samples were taken by purposive random sampling with 50 samples of beef cattle.

### B. DNA extraction and amplification by nPCR

Frozen fresh tissue of each organ were weighted to 3-5 mg each and grind for extraction. DNA extraction was performed by using DNA purification kit *puregene/puregene DNA purification kit; solid tissue protocol* (Qiagen®). DNA extraction procedure used was according to the provided guide book. The examination were by *pooling* 10 cows per sample.

DNA amplification was performed by conventional PCR using two-step method or nPCR. Primer used in PCR test can be seen in Table 1. Sample DNA amplification was performed by using *thermal cycler* (Perkin-Elmer Gene Amp PCR systems 9600). *First* PCR was programme for 35 cycles. Denaturation process were done in 94 °C for 1 minute, annealing in 54 °C for 1 minute, and extension in 72 °C for 2 minutes. Expected PCR first product is DNA with 501 bp length [11].

*2<sup>nd</sup> round (nested PCR)* was also programmed to be done in 35 cycles. It begins with heating in 94°C for 3 minutes, followed by denaturation in 94°C for 1 minute, *annealing* in 56°C for 1 minute, extension in 72°C for 1 minute 30 second, and final extension in 72°C for 4 minutes, and finally cooling in 4°C. Expected *nested* PCR product is 437 bp length [11]. Primers that used in nPCR shown in Table 1 that previously described in [12].

The result of DNA amplification through PCR then read by gel electrophoresis. Electrophoresis reading were visualized as DNA fragments in bands. Visualisation of electrophoresis result were done by ultraviolet illuminator and captured by camera.

TABLE I PRIMERS USED IN NESTED PCR

PCR Step	Primer	Base sequence
1 <sup>st</sup> round	OMP1	5'-AGT AGA AGC ATC CCA AGC ATT-3'
	OMP2	5'-TGC CTG CTA GCT GTA ACG ATT-3'
2 <sup>nd</sup> round (nPCR)	OMP3	5'-GAA GCG CAA CAA GAA GAA CAC-3'
	OMP4	5'-TTG GAA GTT ATC ACG CAG TTG-3'

## III. RESULTS

There are three positive samples from nPCR examination. The positive result is found on heart, lung, kidney, and spleen that shown on Table 2 and the electrophoresis result from the positive samples shown in Fig. 1.

TABLE II. RESULT OF NPCR IN VARIOUS ORGAN

Pooling	Positive Organs				
	Heart	Lung	Liver	Kidney	Spleen
1	-	-	-	-	-
2	-	+	-	+	+
3	-	-	-	-	+
4	-	-	-	-	-
5	+	-	-	+	-

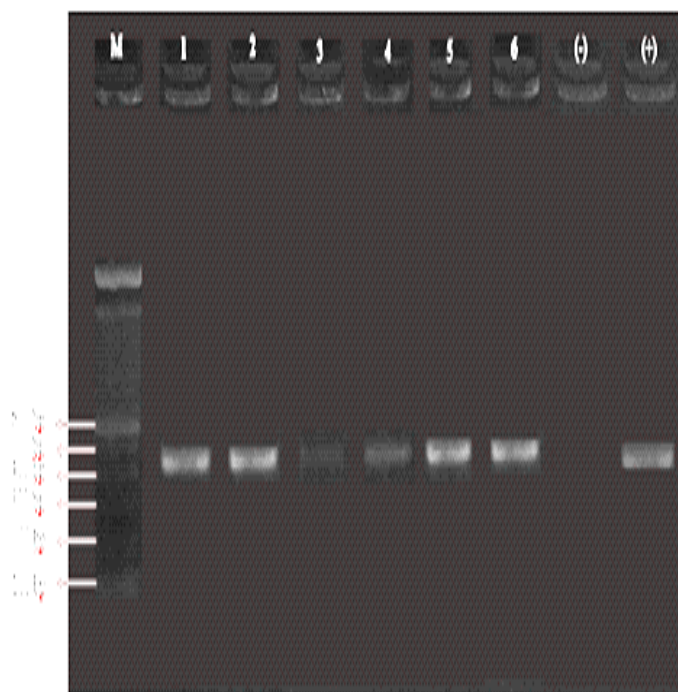


Fig. 1. Electrophoresis result of nPCR from various organs. M (marker); 1 (lung, pooling 2); 2 (kidney, pooling 2); 3 (spleen, pooling 2); 4 (spleen, pooling 3); 5 (heart, pooling 5); 6 (kidney, pooling 5); (-) (negative control); (+) (positive control)

The infection of *C. burnetii* apparently distributed to various organs. Kidney and spleen are the organs that most effecting of infection. Heart and lung also infected of *C. burnetii*, but in liver not detect of infection. Distribution of infection of *C. burnetii* shown in Fig. 2.

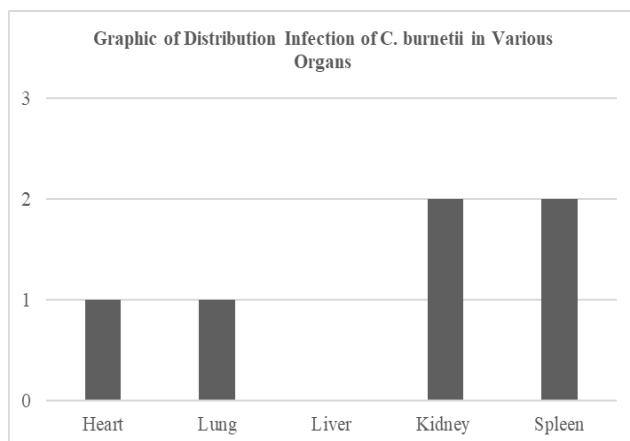


Fig2. Distribution Infection of *C. burnetii* in various organs

#### IV. DISCUSSION

*Coxiella burnetii* infection widely spreads across the globe and causes health issues either to human and animals. Indonesia is considered by OIE as “no information” country group from 2016 to 2019. Nonetheless, Indonesia was ever on “infection/infestation” group on 2015 [13]. Identification and research of this disease has not been done a lot in Indonesia therefore the lack of official data is occurred. According to this study, beef cattle that slaughtered in slaughterhouse in Depok positively infected of *C. burnetii* by PCR examination (Table 2). This result gives addition information of data about which region in Indonesia that got positively infected by Q fever. Q fever identification on ruminants in Indonesia had been done before in Bogor, Bali, Medan, and several others region [8, 10]. This result also can give a picture of that Q fever already spread over some regions in Indonesia. However, Q fever has not yet been known by people in Indonesia, thus quite often misunderstood as another illness with same symptoms [8]. Q fever in ruminants is linked with the incidence of abortus and reproduction disorder such as metritis. *C. burnetii* may survive in host for several years with subclinical symptoms. Animals suffering from subclinical Q fever may still excrete *C. burnetii* to the environment. Q fever can cause abortus in the last third trimester of gestation and pneumonia in cattle. Reproductive abnormalities and abortion can cause economical loss to the farmers.

Positive result of Q fever on cattle in slaughterhouse from this study give zoonosis and community health threat for slaughterhouse workers, veterinarian, animal keeper, and to the people who directly contact with that animals. Main infection route from animal to human is airborne. Route of infection to human by airborne has 50% infection rate by 1.18 bacteria (infD50%) and 50% illness rate by 5.58 bacteria (IllD50%) [15]. Flu-like syndrome, pneumonia, hepatitis, and nephritis are acutely signs of Q fever on human. This signs respond definitively differ to each individual from mild to severe. However, most of this acute signs are self-limiting yet can develop to chronic and

cause endocarditis. It has high morbidity rate yet low mortality rate.

In Fig. 2 showed that infection widely spread through almost all over the body organs, that means infection systemically occurred. Infection most likely occurred on kidney and spleen. Infection is also found on heart and lung. Lung and kidney infection to be thought related to acute Q fever that distribute to circulation system therefore heart is infected as well. Spleen is the most important organ on lymphatic system so that systemic infection of *C. burnetii* cause infection to spleen too. More research is needed for advance diagnostic using immunohistochemistry and histopathological examination to determined infection pattern in various organs and to investigate the pathogenesis.

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