

## Oral Presentation (PAT-5)

### Update: Q Fever in Indonesia

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#### INTRODUCTION

Q Fever is zoonotic disease caused by *Coxiella burnetii*, an intracellular obligate and negative Gram bacterium with pleomorphic shape (Kaplan and Bertagna 1955). Centers for Disease Control and Prevention (CDC) classify *Coxiella burnetii* as potential bioterrorism agent within B rank for its ability and characteristics (CDC 2013).

The main reservoir animals for Q fever are ruminants (Maurin and Raoult 1999). Q fever infection whether in animal or human generally occur through inhalation, vector bites, or through oral ingestion—the last two routes are considered as secondary routes (Angelakis and Raoult 2010). Clinical symptoms of Q fever in both animal and human generally asymptomatic. Q fever can cause abortion in the third trimester of pregnancy and pneumonia in ruminants. Whether in human, acute Q fever can cause flu like syndrome and can develop into hepatitis, endocarditis, and for some severe chronic case, it caused death (Fournier et al. 1998).

Office international des epizooties (OIE) classify Q fever into a re-emerging disease group (OIE 2010). Based on OIE data in 2012, the distribution of Q fever in animals occur in almost all country in the world including ASEAN (OIE 2012).

Q fever was first discovered in Australia in 1935 (Kaplan and Bertagna 1955). Q fever disease transmission in animal occurs almost in every country in the world. Based on OIE data, Indonesia was classified as no information region (OIE 2012). World Health Organization (WHO) reported that Q fever was first found in cow serologically in Indonesia in 1953 (Kaplan and Bertagna 1955). Q fever in ruminants was again reported by Indonesian researcher between 2006 to 2015 in Bali, West Java, Jakarta, and Medan (Mahatmi et al. 2007; Setiyono et al. 2008; Nasution et al. 2015).

Positive Q fever result was obtained especially from ex imported cows, thus it was suspected that cows from abroad might play main role of transmission of Q fever in Indonesia. However, there was also report of positive result in local ruminants, such as Bali cattle, goat, and sheep (Mahatmi et al. 2007). Researches in

various country also stated of potential local ruminant as reservoir animal, among them are Tibet sheep in China, Alpine and Saanen goat in Italia, camel (*Camelus dromedaries*) in Saudi Arabia, or Swedish dairy cattle in Swedia (Mohammed et al. 2014). Based on the historical study of Q fever in Indonesia, this research was performed by taken sample from both ex-import cattle and local ruminants in several region in East Java (Malang, Surabaya, Madura), Central Java (Boyolali), and West Java (Bogor, Bandung, Depok) which are known as regions with high population of ruminants in Indonesia. So far, surveillance data of Q fever has not been recorded from these regions.

#### MATERIALS AND METHODS

##### Samples

Samples used in this research were internal organ of ruminant (spleen, lung, kidney, liver, and heart) taken from slaughter house in East Java (Malang, Surabaya, Madura), West Java (Bogor, Bandung, Depok), Central Java (Boyolali), and also spleen organ of Eid al-Adha cattles in Bogor area in 2016. Each sample divided into 2 pieces, one piece fixed in buffered-neutral formalin (BNF) 10% for immunohistochemical analysis, and another piece stored in -20°C for nested PCR examination.

##### Immunohistochemistry

Formalin-fixed-embedded tissue were cut into 5 µm thickness and affixed on object glass coated with 1% poly l-lysine. Immunohistochemical method was applied according to the standard protocol (CDC, 2013) by using polyclonal anti-*Coxiella burnetii* primary antibody with some modifications.

##### Nested PCR

DNA extraction of samples was conducted by using DNA purification Kit (Qiagen). DNA obtained was then stored in 4°C and ready for polymerase chain reaction (PCR).

a. **First PCR**

PCR was conducted by making PCR mixture consisting DNA sample, primer OMP 1 (5'-AGT AGA AGC ATC CCA AGC ATT-G), OMP2 (TGC CTG CTA GCT GTA ACG ATT-G), PCR buffer, dNTP, Taq buffer and Taq polimerase. Positive control used was *C. burnetii* strain Nine Mile (NM). The samples were amplified in thermal cyclor (Perkin-Elmer Gene Amp PCR systems 9600). First PCR product obtained is 500 bp in length.

b. **Nested PCR**

Primer pair used for nested PCR were OMP3 (5'-GAA GCG CAA CAA GAA GAA CAC-3') and OMP4 (5'-TTG GAA GTT ATC ACG CAG TTG-3') designed for *C. burnetii* outer membrane. Suspension consist of DNA samples from first PCR, primer OMP3 and OMP4, PCR buffer, dNTP, DNA free water, Taq buffer (Takara Shizo, Shiga, Japan) and 0,15 µl Taq polymerase (Takara Shizo, Shiga, Japan). Amplification was conducted again in thermal cyclor (Perkin-Elmer Gene Amp PCR systems 9600). The expected nested PCR product is 437 bp in length.

c. **Amplification detection**

Electrophoresis of amplification product used agarose gel 1,5% in Tris Acetate EDTA solution. After electrophoresis process, gel was put inside ethidium bromide (60 µg/ml) for 20 minutes and visualized under UV light (UV luminescence) and captured into photo.

## RESULT AND DISCUSSION

Immunohistochemical analysis showed that 1 of 19 Eid al-Adha cattle's spleen samples from Bogor in 2016 have an immunoreactive positive to anti-*Coxiella burnetii* primary antibody (Fig.1). This positive result indicated by the existence of brownish colour in cell's cytoplasm in spleen.

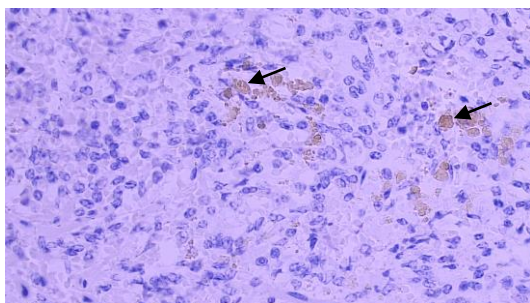


Figure 1. Immunoreactive positive in spleen samples (black arrow).

PCR detection results of *Coxiella burnetii* from 7 region showed that there are some positive result from ruminant samples in Bogor, Bandung, Depok, Malang, and Boyolali. While in Surabaya and Madura there is no positive results from 40 ruminant's sample organs. This is valuable finding

since there is lack of research which focused on Q Fever in Indonesia. This finding expand the distribution area of Q fever from Bali, West Java, Medan and Jakarta up to Central Java and East Java. Combine with the previous research, it should indicate that there was a potency of wide distribution of Q Fever in Indonesia.

Based on OIE data, Indonesia was classified as no information region exposed to Q fever (11). World Health Organization (WHO) report stated that Q fever as first found in cow serum in Indonesia in 1953 (4). Q fever positive result in ruminants was again reported by Indonesian researcher between 2006 to 2015 in Bali, West Java, Jakarta, and Medan (5;8;13). Q Fever distribution pattern in animals very diverse, not only restricted in ex-import cattle, but also in local ruminant.

PCR positive result of *Coxiella burnetii* in local ruminant indicated that there is possibility of local isolat also could be found in Indonesia. Local animal as potential reservoir for Q Fever had been reported before. Researches in various country stated the potential local ruminant as reservoir animal such as Tibet sheep in China (14), Alpine and Saanen goat in Italia (12), camel (*Camelus dromedaries*) in Saudi Arabia (7), and also Swedish dairy cattle in Swedia (9).

Broadness distribution pattern of Q Fever makes transmission from animal to animal or animal to human become more possible. This disease can spread through many ways including inhalation method. Through this method, people or animals that contact frequently with infected animal has high risk to infected by *Coxiella burnetii*. Abbatoir workers, farmer, veterinarian, and animal keeper are some high risk profession to be infected with Q Fever.

Broad distribution of Q Fever is also influenced by the presence of vector since Q Fever is one of vector borne-disease. Vector like ticks or mosquitos were strongly suspected as potential transmission of *Coxiella burnetii* from animal to other animals. In Indonesia, local ruminant reared in people's farm with lack of hygiene which enable increasing risk of Q Fever from animal to animal and/or animal to human infection.

Another risk factor that support disease transmission from import cow to local ruminant is lack of farm maintenance system which enable frequent interaction between local and import ruminant. This interaction could be managed by the application of standard animal quarantine system. Occasionally, this disease is often ignored in differential diagnosis and thus managed to survive within a certain group of livestock and causes long term financial loss.

The presence of Q Fever disease in Indonesia has not received proper attention from Indonesia's government. The distribution pattern

and picture of Q Fever disease that shown in this research would be expected to provide an awareness of the disease from government and Indonesia's citizens. In fact, Q Fever also found in local ruminants, thus by this finding showed a possibility for abortion case in productive local ruminant in Indonesia might be caused by *Coxiella burnetii*, and not only restricted by *Brucella abortus*.

## CONCLUSION

1. Q fever positive in ruminants in Bogor, Bandung, Depok, Malang, and Boyolali confirmed by using PCR and immunohistochemistry technique.
2. Q fever was not found in Surabaya and Madura

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