IDENTIFICATION OF COXIELLA BURNETII IN RUMINANTS BY NESTED-POLYMERASE CHAIN REACTION IN INDONESIA

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ABSTRACT

Nested-polymerase chain reaction (Nested-PCR) was used to identify Coxiella burnetii, a causal agent of Q fever, a zoonotic disease during 2004 to 2006 in Indonesia. A total of 410 sample organs from slaughterhouses such as liver and lung of cattle (245 samples), sheep (105 samples) and goat (60 samples) from Bogor area and Bali province were investigated. The result showed that 5.12% of ruminant samples tested were positive C. burnetii DNA that consist of 15 cattle samples (3.66%) and 6 sheep samples (1.46%), and no positive result from goat. Interestingly, 3 of 15 positive cattle samples were Bali’s cattle. The evidence showed that C. burnetii maybe widespread in Indonesia. This is the first report of identification of C. burnetii from livestock in Indonesia.

Key words: Q fever, Coxiella burnetii, Zoonosis, Nested-PCR, Ruminant

INTRODUCTION

Q fever is a zoonotic disease caused by rickettsial organism, Coxiella burnetii. The organism is an obligate intracellular bacterium, gram-negative and highly pathogenic for both humans and livestock (Baca and Parettsky, 1983; Maurin and Raoult, 1999). C. burnetii firstly described in 1935 by Derrick in Australia’s abattoir worker. Afterwards, Q fever has been found throughout the world (Fournier et al., 1998).

In Indonesia, based on sero-epidemiology survey in 1937 as reported firstly by World Health Organization (WHO) revealed that 188 bovine sera were positive Q fever (Kaplan and Bertagna, 1955). Furthermore, Van Peenen et al. (1978) had been reported seroepidemiological evidence for occupational exposure to Q fever in Indonesia. In 2001, a tourist from Japan who temporarily lived in Indonesia was suffering from flu and C. burnetii DNA had also been detected from his serum (Miyashita et al., 2001). Recently, Richard et al. (2003) reported serologic evidence of rickettsia agents that were present among inhabitants of Gag Island, East region of Indonesia. Although a few serological evidence of anti-C. burnetii antibody was revealed, however, intensive study on Q fever has not be done yet in Indonesia.
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In Indonesia, based on sero-epidemiology survey in 1937 as reported firstly by World Health Organization (WHO) revealed that 18% bovine sera were positive Q fever (Kaplan and Bertagna, 1955). Furthermore, Van Peenen et al. (1978) had been reported seroepidemiological evidence for occupational exposure to Q fever in Indonesia. In 2001, a tourist from Japan who temporarily lived in Indonesia was suffering from flu and C. burnetii DNA had also been detected from his serum (Miyashita et al., 2001). Recently, Richard et al. (2003) reported serologic evidence of rickettsia agents that were present among inhabitants of Gag Island, East region of Indonesia. Although a few serological evidence of anti-C. burnetii antibody was revealed, however, intensive study on Q fever has not been done yet in Indonesia.

Acute Q fever manifestation in humans resembles other diseases such as flu-like illness with headache and cyclic fever (Harris et al., 2000; Mantie, 2003). So, maybe part of clinicians and public health officials tendency to regardess it as an important disease. On the other hand, Q fever is categorized in B list bioterrorism agent by many countries such as USA, UK and many European countries (CDC, 2003). Chronic form of the disease are characterized by endocarditis, generally involving the aortic heart valves, and those with chronic kidney disease or granulomatous hepatitis (Raoult, 2002; Seshadri et al., 2003).

In livestock, Q fever revealed non specific clinical sign and sporadically caused abortion in cattle, sheep and goats (Hwe et al., 1992; Ho et al., 1999; Berri et al., 2001; Scrimgeour et al., 2003). Diagnosis of Q fever is usually based on serological test, since isolation of C. burnetii is time consuming, hazardous and technically necessary special equipment (Maurin and Raoult, 2000). Recently, Setyono et al. (2005) has developed new criteria for immunofluorescence assay for diagnosis of Q fever. Moreover, polymerase chain reaction (PCR) assay for detection of C. burnetii has also been evaluated (Ogawa et al., 2004).

The objective of this study was to detect C. burnetii from ruminants; cattle, sheep and goats that origin from Bogor area and Bali province by Nested-PCR. This report describes the result of Nested-PCR assay for direct identification of C. burnetii in ruminants in Indonesia.

MATERIALS AND METHODS

Samples
Four hundred and ten samples from slaughterhouses in Bali and Bogor were examined. The specimen were liver and lung that consist of 245 from cattle (175 Brahmman cross, 70 Bali’s cattle), 105 sheep and 60 goats.

DNA extraction from organs
DNA from liver and lung samples were extracted by PUREGENE DNA Purification Kit (Gentra systems, Minneapolis, Minnesota, USA) according to the instruction manual.

PCR assay
First PCR was performed according to the previous study (Ogawa et al. 2004). A pair of primer used (OMP 1: 5'-AGT AGC ATG CCC AGC ATT G-3') and (OMP 2: 5'-TGC TCT GTA GCC TGA ACC CAG T-3') was designed for C. burnetii 29 kDa outer membrane protein gene. C. burnetii strain Nine Mile was used as positive control. A total of 30 μl reaction mixture contained 3 μl DNA sample, 30 pmol of each primer, 3 μl of 10x PCR buffer, 3 μl of 10x dNTP mixture, 18.25 μl D2O and 0.15 μl of Takara EX Taq (Takara Shuzo, Shiga, Japan). The mixture was prepared for use in DNA thermal cycler (Perkin-Elmer Gene-Amp PCR systems 9600) with 35 cycles. The program of amplification was initially in 94°C for 3 min (taq activation), denaturation at 94°C for 1 min, annealing at 54°C for 1 min, extension at 72°C for 2 min, and finally extension at 72°C for 4 min. The PCR products were then subjected to electrophoresis in 1.5% agarose gels and visualized by ethidium bromide staining. The product of PCR is 500 bp.

Nested-PCR
A pair of primer (OMP3: 5'-GAGGCGAACGAGGAGAACACC-3') and OMP4: 5'-TTGGAAATTCACCAGGTG-3') for second PCR was designed from C.burnetii 29 kDa outer membrane protein gene and used according to the previous report (Zhang et al., 1998 and Ogawa et al., 2004). A total of 30 μl mixture contained 3 μl DNA samples (from first PCR), 30 pmol of each primer, 3 μl of 10x PCR buffer, 3 μl of dNTP mixture, 18.25 μl D2O
and 0.15 µl of Takara EX Taq (Takara Shizo, Shiga, Japan). In the second PCR, after heating in 94°C for 3 min, 35 cycles of amplification was performed with denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min 30 sec, and final sequence extension at 72°C for 4 min. Electrophoresis and visualization of PCR products were performed as the first PCR. The product of second PCR is 437 bp.

RESULTS

Four hundred and ten samples were examined for C. burnetii using first PCR assay. The result of the first PCR with OMP 1 and OMP 2 primers showed some non-specific bands. All bands were determined to be non-specific because the sizes were different from specific band of the C. burnetii strain Nine Mile control. The result of first PCR is shown in Figure 1.

![Image of gel electrophoresis with bands](image)

**Fig. 1. Identification of C. burnetii by first PCR using primers OMP 1 and OMP 2.** An agarose gel electrophoresis of 550 bp amplification products after ethidium bromide staining is shown. Lane 1, molecular size marker (100 bp DNA ladder); lane 2, positive control; lane 3, negative control; and lanes 4 to 12, samples.

Nested-PCR assay with OMP 3 and OMP 4 primers detected positive results from 3 of 70 Bali’s cattle, 12 of 175 Brahman cross, 6 of 105 sheep and no positive result in 60 goats from Bali. The positive bands were determined to be specific and same with those specific band of the C. burnetii strain Nine Mile (Figure 2).

![Image of gel electrophoresis with bands](image)

**Fig. 2. Identification of C. burnetii by nested PCR with primers COM3 and COM4.** An agarose gel electrophoresis of the 437 bp amplification products after the Nested-PCR and ethidium bromide staining is shown. Lane 1, molecular size markers (100-bp DNA ladder); lane 2, reference strain C. burnetii Nine Mile; lane 3, negative control; and lane 4 to 12, samples.

The results of this study indicate that Nested-PCR assay is useful and more sensitive than first PCR in detection of C. burnetii DNA in the same samples examined. In addition, Nested-PCR assay is also useful for direct typing of C. burnetii in solid tissue samples.

Detection of C. burnetii in Ruminants

All samples either liver or lung from ruminants were tested by Nested-PCR assay and shown in Table 1. C. burnetii DNA had detected in samples as follows: In cattle from Bogor area was 2.93%, in sheep from Bogor was 1.46%, in Bali’s cattle was 0.73%, and none of the goat from Bali (0%). Overall, 21 of 410 samples from ruminants (5.12%) showed positive result.

<table>
<thead>
<tr>
<th>Geographical Origin</th>
<th>Source of Samples</th>
<th>Number of Samples</th>
<th>Nested-PCR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bali</td>
<td>Ball’s cattle</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>Bali</td>
<td>Goats</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Bogor</td>
<td>Brahman</td>
<td>175</td>
<td>12</td>
</tr>
<tr>
<td>Bogor</td>
<td>Sheep</td>
<td>105</td>
<td>6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>410</strong></td>
<td><strong>21</strong></td>
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</table>

DISCUSSION

We detected C. burnetii in ruminants in Indonesia during 2004 to 2006 using PCR assay. In our study, 21 of 410 (5.12%) samples from cattle, sheep and goats were positive (Table 1). Nested-PCR assay demonstrated to be highly specific and useful for direct identification of C. burnetii from ruminants organ such as liver and lung.

The highest prevalence of C. burnetii was detected in cattle from Bogor area (2.93%) that might be associated with the origin of these livestock. Most of them were imported from Australia and USA whereas Q fever still to be serious problem in those countries. On the other hand, we also detected C. burnetii DNA in 3 of 70 Bali’s cattle. This is interesting due to livestock in Bali province maybe kept outdoors throughout the year and high human interaction, so high risk to expose to various pathogen microorganisms. The prevalence of C. burnetii in sheep from Bogor area (1.46%) maybe associated with farming management type of these livestock. It would be early warning system for both humans and livestock health issue, because of zoonotic aspect of the disease. The absence of C. burnetii in goats from Bali might be due to the geographical isolation. Generally, these livestock kept housing so far from village.

The results of this study indicated that Nested-PCR assay is useful for detection C. burnetii in ruminant organs. This is the first report of identification of C. burnetii in livestock in Indonesia.

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<td>0</td>
</tr>
<tr>
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<td>Brahman</td>
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<td>12</td>
<td>2.93</td>
</tr>
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