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 Paretsky, 1983; Maurin and Raoult, 1999). *C.burnetii* firstly discribed 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.

Acute Q fever manifestation in humans resembles other diseases such as flu-like illness with headache and cyclic fever (Harris *et al.*, 2000; Marrie, 2003). So, maybe part of clinicians and public health officials tendency to regardless it as an important disease. On the other hand, Q fever is catagoried 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 (Htwe *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, Setiyono *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 specimens were liver and lung that consist of 245 from cattle (175 Brahmann cross, 70 Bali`s cattle), 105 sheeps 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 AGA AGC ATC CCA AGC ATT G-3`) and (OMP 2 : 5`- TGC CTG CTA GCTGTAACGATT G-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 ppmol of each primer, 3 µl of 10x PCR buffer, 3 µl of 10x dNTP mixture, 18.25 µl D_2W and 0.15 µl of Takara EX Taq (Takara Shizo, 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 94^0 C for 3 min (taq activation), denaturation at 94^0 C for 1 min, annealing at 54^0 C for 1 min, extension at 72^0 C for 2 min, and finally extension at 72^0 C for 4 min. The PCR products were then subjected to electrophoresis in 1,5 % agarose gels and visualized by ethydium bromide staining. The product of PCR is 500 bp.

Nested -PCR
A pair of primer (OMP3 : 5`-GAAGCGCAACAAGAAGAACAC-3`) and OMP4 : 5`-TTGGAAGTTATCACGCAGTTG-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 ppmol of each primer, 3 µl of 10x PCR buffer, 3 µl of dNTP mixture, 18.25 µl D_2W

and 0.15 µl of Takara EX Taq (Takara Shizo, Shiga, Japan). In the second PCR, after heating in 94^0 C for 3 min, 35 cycles of amplification was performed with denaturation at 94^0 C for 1 min, annealing at 56^0 C for 1 min, and extension at 72^0 C for 1 min 30 sec, and final sequence extension at 72^0 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 spesific bands. All bands were determined to be non-spesific because the sizes were different from spesific band of the *C.burnetii* strain Nine Mile control. The result of first PCR is shown in Figure1.

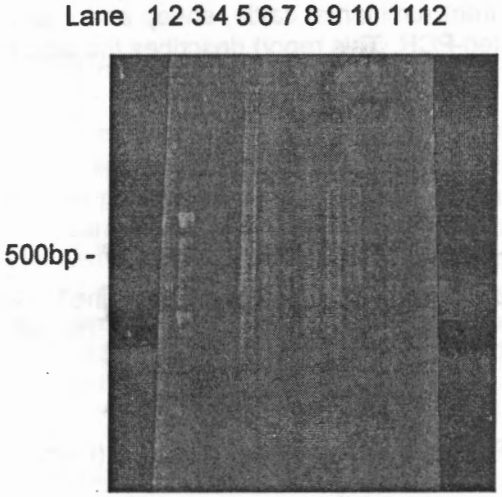

Fig. 1. Identification of *C.burnetii* by first PCR using primers OMP 1 and OMP 2. An agarose gel electrophoresis of 500 bp amplification products after ethydium bromide staining is shown; lane 1, molecular size marker (100 bp DNA ladder); lane 2, positive control; lane 3, negative control; and lanes 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 spesific and same with those spesific band of the *C.burnetii* strain Nine Mile (Figure 2).

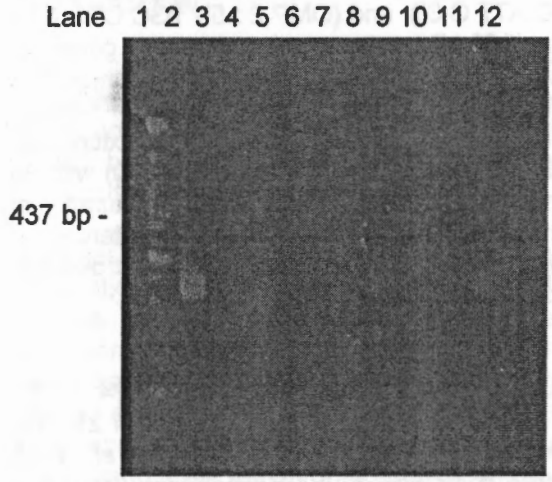

Fig. 2. Identification of *C. burnetii* by nested PCR with primers COM3 and COM4. An agarose gel electrophoresis of the 437 bp amplification products after the Nested-PCR and ethydium bromide staining is shown. Lane 1, molecular size markers (100-bp DNA ladder); lane 2, reference strain *C.burnetii* Nine Mile; lane 3, negative control; and lane 4 to12, 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 1. Identification of *C.burnetii* by Nested -PCR from Ruminants.

Geographical origin	Source of samples	Number of samples	Nested-PCR (+)	Nested-PCR (%)
Bali	Bali's cattle	70	3	0.73
Bali	Goats	60	0	0
Bogor	Brahman	175	12	2.93
Bogor	Sheep	105	6	1.46
Total		410	21	5.12

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 spesific 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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