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Immunohistochemical Detection of *Coxiella burnetii* in Ruminants: A Case Study of *Q Fever* in Indonesia

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Abstract: Q fever is a zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular bacteria. Diagnosis of Q fever is usually determined by serological test and it should be time consuming. Detection of *C. burnetii* antigen immunohistochemically appears to be one of the best choice method for establishment of Q fever diagnosis in animals. The aim of this study was to detect the present of aetiological agent of Q fever using peroxide base method in Idul 'Ied celebration cattles in Jakarta region, Indonesia. A total of 40 samples were collected during 2012 and examined. Gross lesion of the liver samples revealed multifocus necrotic hepatitis, granulomatous hepatitis, cirrhosis and fatty liver degeneration of parenchymal cells. The result showed positive immunoreactive to *C. burnetii* and occurs in 3 out of 40 liver samples.This is the first report on *C. burnetii* infection case diagnosed immunohistochemicallyin ruminants in Indonesia.

Key words: Antibody · *Coxiella burnetii* · Diagnosis · Immunohistochemistry · Ruminants

INTRODUCTION

Query (Q) fever is a worldwide zoonosis [1] caused by *Coxiella burnetii*, an obligate intracellular and highly infectious bacterium that can lives in the monocyte or machrophage [2]. The most common syndromes observed in acute Q fever are prolonged fever of unexplained origin, granulomatous hepatitis and atypical pneumonia [3, 4]. Liver involvement is common in acute Q fever and percutaneous liver biopsy can help in the diagnosis. On the other hand,cases of chronic Q fever hepatitis are rarely reported in the literature [5] and are frequently associated with endocarditis [6-8]. Diagnosis of Q fever can be established serologically by using fluorescent antibody technique [9-11] and or by polymerase chain reaction to detect genetic material deoxyribo nucleic acid of *C.burnetii* [12]. However, those techniques need more time and technically laborious.Immunohistochemistry (IHC) method had developed and thought to be a diagnostic tool for detecting antigen in the organ or tissue. The aim of this study is diagnose Q fever immunohistochemically in cattle which use for Idul 'Ied celebration in Jakarta region, Indonesia.

MATERIALS AND METHODS

Production of anti-*Coxiella burnetii* Antibody: Production of antibody used immunogen formalin-fixed of *C.burnetii*-Nine Mile Strain according to the standard procedure [13] with minor modification. Briefly, the emulsified immunogen incomplete *freund's adjuvant* (CFA) were applied subcutaneously to New Zealand rabbit and followed with booster intramuscularly 3 weeks later using emulsified immunogen-*incomplete freund's adjuvant*(IFA). Two to three weeks after boosting, the level of anti-*C.burnetii* antibody was checked and the sera considered to be harvested if the antibody titre significantly elevated. The generated antibody was then characterized and kept into some aliquots and store in-20°C until use.

Immunohistochemistry: 1 x 10^6 purified *C.burnetii* cells were applied intraperitoneally to Hamster. After three weeks, Hamster was sacrificed and the spleen and liver were harvested. To evaluate the immunoreactivity of produced anti-*C.burnetii* antibodies toward certain *C.burnetii* antigen in the spleen or liver of Hamster,

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immunohistochemistry technique was done based on peroxidase complex reaction [14].

Sampling of Specimens: Selected cattle liver section were got from Idul 'Ied celebrating in 2012 in Jakarta region, Indonesia. The pathological finding of those 40 samples included multifocus necrotic hepatitis, granulomatous hepatitis and cirrhosis. Fatty liver degeneration and hyperemia were also seen in some samples taken and fixed in *buffer neutral formalin* 10% until use.

Histopathological Analyses: Formalin-fixed paraffin-embedded liver specimens were cut to 4 μm thickness and stained with routine Mayer's Haematoxylin-Eosin [15,19,20]. Serial sections were also obtained to perform special stains and immunohistochemical investigations. Special stains, including periodic acid-Schiff,Giemsa and Gram stains, were used for detection of bacteria and fungi. Immunohistochemical analysis was performed as previously described[14] using polyclonal anti-*C. burnetii* antibody with minor modification.

RESULTS AND DISCUSSION

We have produced polyclonal rabbit anti-*C.burnetii* antibody. The generated antibody was further used for detecting *C. burnetii* antigen immunohistochemically in cattle. The antibody recognized its parent antigen with molecular weight of 27 kDa in Western Blott assay (Fig.1). This is in accordance with those as reported by Zhang *et al.* [16] that high conserved of outer membrane protein of *C.burnetii*is antigenic determinant. In the other hand, the antibody also recognized epitope of *C. burnetii* in spleen and liver of experimentally-infected hamster.

Liver section of Hamster revealed numerous granulomas disseminated in the liver parenchyma outside the portobiliary spaces. These granulomas were composed mainly of macrophages, without neutrophils, central clear space and fibrin ring. With the immunohistochemical analysis, bacteria were seen as coarse granular immunopositive material in the macrophage cytoplasm (Fig. 2). *C. burnetii* could only be visualized within liver granulomas, as small, focal collections of infected mononuclear cells with a macrophage morphology.

In 2012, we collected liver samples from cattle which use for Idul 'Ied celebration in several regions in Jakarta, Indonesia. We found 3 out of 40 samples positive immunoreactive to anti-*C.burnetii* antibody produced with cases of necrotic hepatitis associated with assumed acute Q fever. This produce speculate that may be cases of *C. burnetii* infection in ruminant may be occured in Jakarta region, but may be also already spread outside of Jakarta. The reasoning might be due to the cattle for celebration actually came from elsewehere even from other provinces in Java Island. This result also confirmed that the antibody is useful for detection of *C.burnetii* antigen using peroxidase-based methods. Although sensitivity and specificity of the antibody are further remain to be assayed, however, the result of this study is quite promising in pathologically diagnosis of Q fever in Indonesia. The representative illustration for hematoxylin-eosin staining and immunohistochemical analysis of *C. burnetii* infection in cattle were shown in Figure 3a and Figure3b, respectively.

So far, Q fever cases in sheep and goat in Indonesia serologically had reported by Setiyono *et al.* [17] with around 20% seropositive to *C.burnetii*.

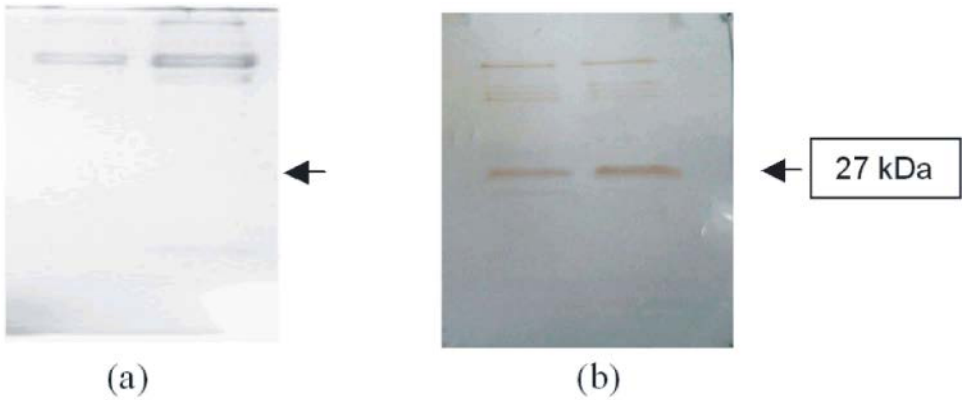


Fig. 1: (a) SDSPAGE of *C.burnetii* antigen and (b) Western Blotting Assay of *C.burnetii* protein by using rabbit polyclonal anti-*C.burnetii* antibody. The antibody recognized a protein of 27 kDa that conserved in outer membrane protein of *C.burnetii*.

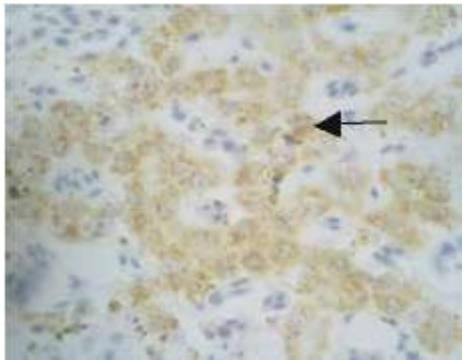
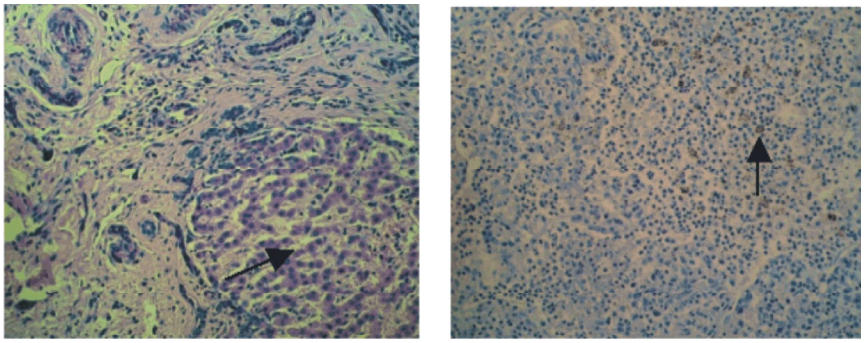


Fig. 2: Liver of Hamster as a positive control showed positive immunoreactive (arrow) to anti-*C.burnetii* antibody with brown color appeared in the cytoplasm of macrophage. IHC.x 100.



(a) (b)

Fig. 3: Histopathological change of cattle liver stained with hematoxylin-eosinand Immunoperoxidase-based methods. It was seen macrophage and fibrin ring with HE (a) and positive immunohistochemical reaction to *C.burnetii* antigen with IHC (b). x 200 magnification.

Moreover, with similar methods it also reported 3% of Bali cattle in Bali island had been infected by *C.burnetii* [18]. This concise report will be completing data for Q fever particularly diagnosed immunohistochemically in ruminants in Indonesia.

In conclusion, 3 out of 40 (7.5%) cattles were positive Q fever which use for Idul 'Ied celebrating in Jakarta, Indonesia in 2012. This report is the first immunohistochemically detection of *C.burnetii* in a case of necrotic hepatitis associated with assumed acute Q fever in Idul 'Ied cattle in Indonesia.

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