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Cellular Pathogenesis of Query Fever in Cattle

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Abstract: Query (Q) fever is a zoonosis caused by *Coxiella burnetii* and it has been almost widespread around the world. Ruminants are the animals that most at risk of becoming infected by Q fever. The aim of this research was to study the cellular pathogenesis of Q fever in cattle. Samples used were spleen, heart, lungs, kidney and liver from 100 random cattle slaughtered at Bogor Slaughterhouses, Indonesia. The result showed that *C. burnetii* antigen detected immunohistochemically in all organs except in heart by using anti-*C. burnetii* antibody. These finding provoked speculation that *C.burnetii* infection maybe had reached other organs or tissues facilitated by infected macrophages through lymph vessels. The macrophage leads to play a pivotal role in the spreading of *C.burnetii* to other internal organs and tissues of ruminants.

Key words: Cattle · *Coxiella burnetii* · Pathogenesis · Q Fever

INTRODUCTION

Query fever *(Q fever)* is a contagious zoonotic disease caused by an obligate intracellular microbe, *Coxiella burnetii* [1]. Animals contract Q fever particularly ruminants and in same cases of the wild animals. Transmission of Q fever often occurs through direct contact to infected animals or contaminated food by *C.burnetii* such as meat, milk and other animal products [2,3]. *C. burnetii* resists extreme temperature, dry air, some antiseptics and possess spore-like form, so possibly existing for weeks to months in the environment [4]. Clinical signs of Q fever in human showed flu-like symptoms in acute type, while in chronic type revealed pneumonia and endocarditis which can be fatal [5]. Q fever in animals initiated with no pathognomonic symptom such as pneumonia, abortion and other non specific signs [6]. In ruminant, infection risk factors of Q fever include age, reproduction status, lactation and milk production level [7].

Q fever cases in Indonesia firstly found serologically in 1937 in 188 cattle [8] and no one after that serologically investigated it. In the other hand, material genetic of *C. burnetii* had been detected by polymerase chain reaction (PCR) as many as 5.12% in ruminant in the previous study [24]. Several methods had been developed for detection of causal agent of Q fever in animals. Lepidi *et al.* [9] had developed autoimmunohistochemistry to detect *C.burnetii* at cardiac valve using monoclonal antibody with haematoxylin counterstain. Spleen is a biggest secondary lymphoid organ consist of lymphocytes and erythrocytes which functionally construct the red and white pulp [10-13]. Study of Q fever in cattle immunohistochemically believe would be useful for understanding the disease properly and appropriate for the confirmation test as the previous describe [14]. The aim of this study was to know the pathogenesis of Q fever in cattles through histopathological analyses.

MATERIALS AND METHODS

Collecting Samples: A total of 100 pack samples with each pack consist of spleen, heart, lungs, kidney and liver of cattle were collected from Cibinong and Bogor Slaughterhouses, West Java Province, Indonesia. Due to Indonesia's Government regulation prohibited to slaughter the productive female cattle at slaughterhouses, thus no organ of reproductive system was collected [27]. The samples were then kept into plastic pouch contain 10% Buffer Neutral Formalin (BNF) for further histological process. Samples were cut for 3 mm, put into *tissue* cassette then enroll in dehydration process with immerse samples consecutively into ethanol 80%, 90%, ethanol absolut I and ethanol absolut II for each 2 hours.

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Samples Preparation: Organ samples were washed with phosphate buffered saline (PBS) and then fixed within BNF 10%. After dehydration process, then clearing use xylene twice, each 60 minutes. Next process is infiltering use soft paraffin for 60 minutes at 48°C then blocked using appropriate mold and incubates at room temperature overnight. Block paraffin contain samples was then attached at holder of microtome and cutting 4–6 µm using rotary microtome. Samples were put on warm water 45°C in water bath, take it and put onto object glass, dried within incubator at 60°C.

Histopathological Process: Glass slide contain samples was immersed into xylene twice for 5 minutes each [25]. Afterwards, doing rehydration process using absolute alcohol I-III, alcohol 96%, 80% for 5 minutes each and rinse use H_2O for 5 minutes then dried it in the air. The slide is ready used for histological staining.

Haematoxylin-Eosin Staining: Sample slide stained with Haematoxylin-Eosin (HE) intended to look at tissue changes or lesion due to harmful substances or pathogenic microbs. The samples slide were processed with immersed it into Mayer's Haematoxylin staining for 8 minutes, rinse in tap water, pour lithium carbonate 15 times and again rinse with tap water [15]. Afterwards, samples were dying within Eosin for 2 minutes, in 90% alcohol 10 times, absolute alcohol I- 10 times, absolute alcohol II- 2 minutes and xylene I and II -1 minute, respectively. The sample slide was then dropped with Permount[T.M.] and covered with cover glass and ready for examine under light microscope.

Immunohistochemical Analysis: Analyses of tissue organ was performed according to the previous protocol [16-17] with minor modification using immunohistochemical (IHC) Kit (Cat No. MS-1378-PO, LabVision). Briefly, samples in the glass slide was washed with PBS pH 7.2, then incubated in 3% H_2O_2 (Cat No. K0679, Dako LSAB-system HRP) for 10 minutes. Again, the slide was washed with PBS pH 7.2 for 3 times 5 minutes each. Non specific reaction arise might be due to endogenous peroxidase was blocking with 1% bovine serum albumin (BSA) (Sigma USA) for 60 minutes. Slides were then incubated with primary antibody (1:500) rabbit anti-*C. burnetii* antibody [23] at 4°C for 24 hours, washed 3 times with PBS pH 7.2 and then incubated with secondary antibody, biotin labeled-anti rabbit antibody for 1 hour. Afterwards, the slide was washed 3 times for 5 minutes each with PBS pH 7.2, then was dropped with streptavidin horse radish peroxidase for 40 minutes. Furthermore, the slide was washed with PBS pH 7.2 and applied chromogen diamino benzidine (DAB) for 5 seconds and rinse using H_2O followed by PBS pH 7.2. Counterstain was done using Mayer's Haematoxylin (Cat No. 115938, Merck) for 10 minutes, then again the slide was washed with tap water, mounted use Permount[T.M.] and ready for examine under light microscope.

RESULTS AND DISCUSSION

A total of 14 out of 100 samples were immunohistochemically positive to Q fever indicated by brown color appeared within the samples. All the positive results were found in macrophage and lymphocytes within lungs, kidney, white and red pulp of spleen and sinusoid of the liver. The representative result of those peroxidase-based analyses for spleen of cattle to *C.burnetii* are shown in Figure 1. An aggregate brown color is produced which is a complex reaction between rabbit anti-*C.burnetii* antibody and *C. burnetii* antigen which presence in the organ.

The obtained results showed that *C.burnetii* infection had spread to lungs, kidney, spleen and liver and might be up to other organs. This can be thought possible due to the macrophages as cell tropism of *C.burnetii* bring it to the lymphoid tissue and other organs through lymph vessels [18]. Another putative hematological route of *C.burnetii* infection possibly through the mechanism of agent accumulates in the spleen, activate the monocytes to be macrophages and

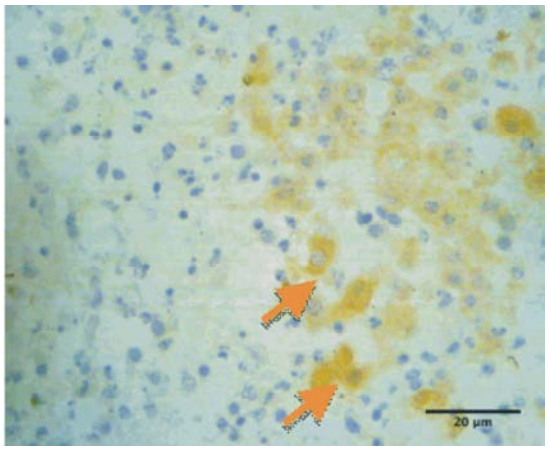

Fig. 1: Peroxidase-based analyses for spleen of cattle to *C.burnetii* was revealed by positive immunoreactivity of spleen to anti-*C. burnetii* antibody which signed with brown color in the cytoplasm of macrophages. x1000 magnification.

Table 1: Description of histopathological lesions of heart, spleen, lungs and liver samples correlated with Q fever cases at Bogor Slaughterhouses, Bogor, Indonesia.

Organ	Lesion found in samples with or without positive Q Fever
Heart	Myocardial Degeneration
	Inflammatory cells
	Neutrophil
	Lymphocyte
	Fibrosis
Spleen	White pulp depletion
	Red pulp congestion
	Inflammatory cells
	Neutrophil
	Lymphocyte
Lungs	Parenchym Hemorrhage
	Interstitial Pneumonia
	Pleuritis
	Vascular Congestion
Liver	Spotted Inflammatory
	Fibrosis
	Vascular Congestion
	Hemorrhage
	Cloudy Swelling
	Fatty Degeneration
	Necrosis

then engulf the microbs. Although infection of *C.burnetii* generally occured by aerosol route, however, pathological lesion of infection can found not only in the lung but also expressed in the spleen and liver [19] even in the kidney.

In this study, most of heart samples assayed showed less-changes. By using HE staining we found 84 samples were normal, 10 samples showed myocardial degeneration, one sample suffered from acute endocarditis indicated by accumulation of neutrophil cells, 2 samples chronic type of endocarditis and one sample with chronic fibrosis. Meanwhile, 2 positive Q fever cases solely showed myocardial degeneration. The occurrence of endocarditis might be caused by other pathogens which infect the cattle. On the other hand, poor diet particularly vitamin E and Selenium during in the farm potent to be cause myocardial degeneration as mentioned previously [20, 26] either in positive or negative Q fever cases. Myocardial degeneration was signed with misleading of muscle structure and the myocardium would be paleness. Histopathological analyses of heart, spleen, lungs and liver were briefly summarized in Table 1.

Lymphocytosis in ruminant generally caused by process related to response to chronic diseases [21]. Acute type of splenitis signed by accumulation of neutrophil cells, whereas chronic splenitis often characterized by proliferation of lymphocyte cells in red pulp of spleen. Active chronic infection commonly found in the case with accumulation of lymphocytic and neutrophil cells [28]. Lymphocytosis and fibrosis found here were cattle samples with negative Q fever immunohistochemically. We assumed these pathological lesion might caused by other pathogens and had been occured in a long time before. Histopathological changes of heart, spleen, lungs and liver were not pathognomonic for *C. burnetii* infection. Although chronic type of Q fever in ruminant potential to produce non specific lesion during pathogenesis of the disease, however, typically histopathological changes of Q fever in heart, spleen, lungs and liver might be able detected in tissue specimens during acute infection as describe previously [22].

In conclusion, *C. burnetii* antigen detected immunohistochemically in liver, spleen, lungs and kidney samples arise speculation that the infection has been spread to other organs and tissues which is facilitated by lymphoid cells. The macrophages suspected to play a pivotal role in the cellular pathogenesis of Q fever in ruminants.

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