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PATHOGENITY TEST OF LOCALLY ISOLATED BACTERIA (*Pasteurella multocida*) USING KOCH POSTULATES

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ABSTRACT

The study aimed to find out whether bacteria (*Pasteurella multocida*) that is isolated from buffalo in HSU (Hulu Sungai Utara) is the cause of SE (*Septicema epizootica*) disease in the swamp buffaloes using Koch Postulates. Total of 10 Balb-C mice of two weeks-aged were infected with 100 µl cultured containing 4×10^8 CFU (1.5 McFarland Scale) of *P. multocida* subcutaneously in the neck, and observed every 4 hours until the animal died. Samples were taken from the spleen, lungs, and heart with different times of death within 15, 35, and 59 h with sterile swab cotton. Samples were cultured on a nutrient broth medium (NB), inoculated on a soy trypticase agar (TSA), and incubated for 24 h at 37 °C. Separate colonies were stained with Gram and spore staining. The colonies were tested by catalase, biochemical, indol motility (SIM) sulfite, confectionary, and planted on Mac Conkey Agar media. *P. multocida* was identified following Carter's method of showing lung, spleen, and positively infected *P. multocida* samples. It was concluded that *P. multocida* bacteria isolated from buffalo in HSU are pathogenic and can cause SE disease.

Keywords: *Pasteurella multocida*, Koch postulates, SE (*Septicemia epizootica)* and *Subcutaneous*

INTRODUCTION

Swamp buffaloes have been facing some diseases, and pasteurellosis caused *Pasteurella multocida* is one of important infectious diseases in the field. *P. multocida* can infect many domestic or wild animals, and also caused of highly contagious diseases such as cholera in poultry, hemoragical septicemia in buffaloes, and atrophical rhinitis in swine. Hemoragical septicemia has short incubation time ranged of 2-5 days. In the acute case, the disease causes mortality and sometimes without significant clinical symptoms. It is caused of the bacteria are multiplying very rapidly when entering the body of victim. Preventing and controlling of the disease can be optimized by good management of the animals, giving antibiotics and vaccination. Researches about HS vaccines have been carried out continuously but not any vaccine was effective in long time. The matters of the vaccine used is not good protective due to the differences of antigenic properties between isolate used the vaccine and isolate that infected animals in the field.

Before one isolate become a vaccine, it will be done some tests to indicate whether the isolate has antigenic properties or has not using Koch Postulate test. Koch Postulate test criteria can determine one organism as a disease cause if fit with some prerequisites. Firstly, it has to be found in all observed cases. Secondly, it has be compounded and cultivated in pure culture. Thirdly, it has ability to cause original infection in spite of some cultured generations. Fourthly, it could be founded from inoculated animal and can be cultured.

Based on those matters, the research was carried out in order to test the pathogenisity and the ability to cause SE (*Septicemia epizootica*) of *P. multocida* that isolated from buffaloes of HSU (Hulu Sungai Utara). The study aimed to find out whether *P. multocida* bacteria that is isolated from buffalo in HSU is the cause of SE disease in swamp buffaloes.

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MATERIALS AND METHODS

Experiment was carried out in the Microbiology Laboratory of Faculty of Agriculture ULM from April to June 2015. *P. multocida* bacteria were isolated from the buffaloes in HSU South Kalimantan and then stored in refrigerator at a certain time used as sample in the experiment. Ten Balb-C mices of two weeks-aged were infected by 100 μl (0.1 ml) cultured containing 4 x 10^8 CFU (1.5 McFarland Scale) of *P. multocida* at subcutaneous necks of mices. The mices were observed for every 4 hour until died. The dead mices were necropsied and diagnosed with the changes of pathologies on the bodies. At postmortem diagnosis, sample organs such as lungs, *lymfoglandula prescapularis* and *submandibularis, oedema liquid*, spleen, bone-marrow, limp, *pericardium liquid*, visceral liquid, and heart-blood. After post-mortem diagnosis, the mice dead bodies were stored at room temperature. The other samples of lungs, *lymfoglandula prescapularis* and *submandibularis, oedema liquid*, tonsil, bone-marrow, limp, *pericardium liquid*, viseral liquid, and heart-blood were taken at 17, 35 and 39 h after died.

Pathogenity Test on Mices

The isolate which was identified then was incubated at 37 $^{\circ}$C for 12 h (a night). By toucabled ose colony, the bacteria were inoculated in 10 ml solution of *brain hearth infusion serum* (BHI) that incubated at 37 $^{\circ}$C for 1 hour. After that, 10 ml was taken and mixed with new BHI solution, then incubated for 3 h before injected intramuscular on the breast of mices. Temperature was observed in every 4 h until mice died.

RESULTS AND DISCUSSION

After infected, body temperature of the mices was increase, and was the high (39 $^{\circ}$C) at 8 h after infected. Symptoms such as red-eyes and running- noses were detected, and then the mices was dead at 24 hours after infected. The clinical symptoms as the results of infected mices can be seen on Table 1.

Table 1. The observed clinical symptoms after infected of mices

Time after infected (h)	Body temperature				clinical symptoms			
	M1	M2	M3	M4 (control)	M1	M2	M3	M4 (control)
0	35^{0}C	36^{0}C	36^{0}C	35^{0}C	N	N	N	N
4	35^{0}C	37^{0}C	37^{0}C	35^{0}C	N	N	N	N
8	37^{0}C	38^{0}C	37^{0}C	36^{0}C	N	N	N	N
12	37^{0}C	38^{0}C	37^{0}C	35^{0}C	N	N	N	N
16	37^{0}C	38^{0}C	37^{0}C	36^{0}C	N	N	N	N
20	37^{0}C	38^{0}C	38^{0}C	36^{0}C	N	N	N	N
24	37^{0}C	38^{0}C	38^{0}C	36^{0}C	Red-eyes & running-nouses	Red-eyes & running-nouses	Red-eyes & running-nouses	N
28	37^{0}C	38^{0}C	38^{0}C	36^{0}C	Worsen Red-eyes & running-nouses & dead of mice	Worsen Red-eyes & running-nouses & dead of mice	Worsen Red-eyes & running-nouses & dead of mice mencit	N

Data were collected at the microbiology laboratory of ULM on 27 April 2015. N = Normal

The pathological changes of mice organs such as lung, spleen, and heart could be seen from the color changes of the organs from the normal colors. Normal red color changed to black color and damages organs while taken. Results of bacteria re-isolation by Postulate Koch Test can be seen at Table 2.

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Table 2. Results of bacteria re-isolation by Koch Postulate Test

Organs	After the death		
	15 h Isolation	35 h Isolation	59 h Isolation
Lungs	+	+	+
Heart	+	+	+
Spleen	+	+	+

Data were collected at the Microbiology Laboratory of ULM on 27 Mei 2015

Based on testing of samples of lungs, spleen and heart those isolated, there were found positive of *P. multocida* bacteria. Results of coloring and microscopic testing showed that bacteria were coccobacillus (short-stick), negative-gram, short-chain, and singular-collony with convex surface.

The finding is meet with the statement of Kuhnert et al. (2000) that *P. multocida* is negative-gram bacteria, short-stick, and living normally at nasopharing. The samples were hardly to grow in the media of SIM, Simmon's Citrat, lactosa, manitol, and Mac Conkey, fermenting glucose, positip on catalase. Rimler dan Rhoades (1989) stated that *P. multocida* do not grow Mac Conkey, fermenting glucose, positip on catalase test, occidase, and indole. *P. multocida* bacteria usually do not ferment lactose. The results of Gram coloring of organs can be seen on Figure 1 (lungs), Figure 2 (heart) and Figure 3 (spleen) respectively.

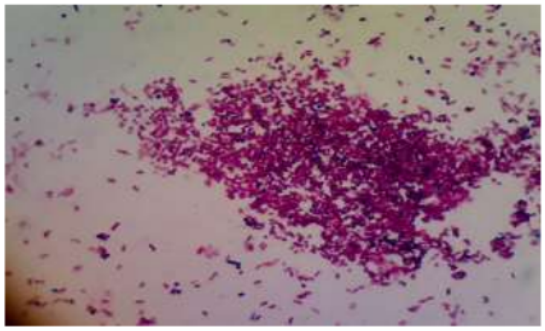

Figure 1. Gram Coloring of *P. multocida* bacteria at Lungs under Microscope (100×)

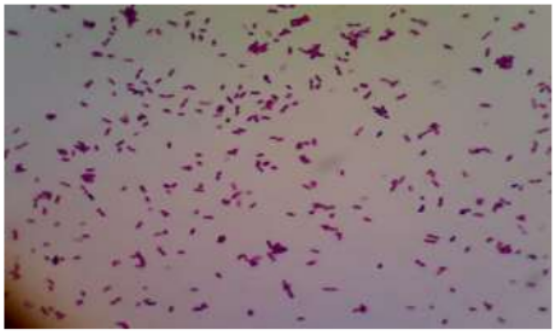

Figure 2. Gram Coloring of *P. multocida* bacteria at Heart under Microscope (100×)

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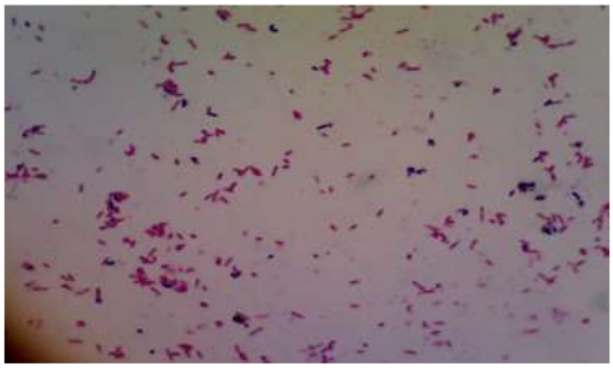

Figure 3. Gram Coloring of *P. multocida* bacteria at Spleen under Microscope (100×)

The coloring results above show that bacteria colony has variety shapes. This confirms the state of Priadi and Natalia (2000) that the *P. multocida* colony is not always similar, depending on some factors media used, age of bacteria in store, frequency of bacteria moved, and so on. The fresh bacteria colony that isolated from infected-animal or experimental-animal is usually mucoid and then by the time change to smooth or rough. Additionally, *P. multocida* bacteria produce smelly gas or vapor.

After gram coloring was done, the spora coloring was carry out from divided colony known as negative gram of *P. multocida* that has not spores. Spores grow in cell that called endospore; it was only one spore in bacteria cell. The endospore was hardly inserted coloring material; therefore it cannot be colored with regular method.

The spora coloring was needed warming-up process to make coloring material inserting inside the spore. The first coloring material contains malachite green would colored the endospore become green, and the second coloring material contains saphranin would colored vegetative cell become red. The coloring material was not tied-binded with membrane cell and cytoplasm; hence it was easy loosen when cleaning by water. In other hand, water cannot introduce endospore wall (endospore membrane), hence the spore was still green when cleaning by water (Lay, 1994). The result of sporan coloring of the research can be seen on Figure 4 (Lungs).

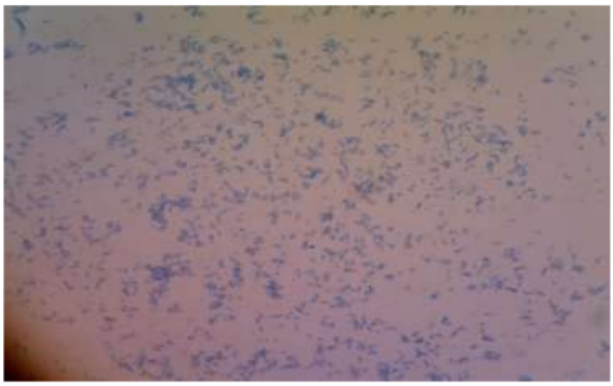

Figure 4. Spora Coloring of *P. multocida* Bacteria at Lungs under Microscope (100×)

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CONCLUSION

Based on the results of the experiment testing the pathogenity of locally isolated bacteria *P. multocida* using Koch Postulate shows that bacteria *P. multocida* isolated from buffalo in HSU are pathogenic and can cause SE disease.

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