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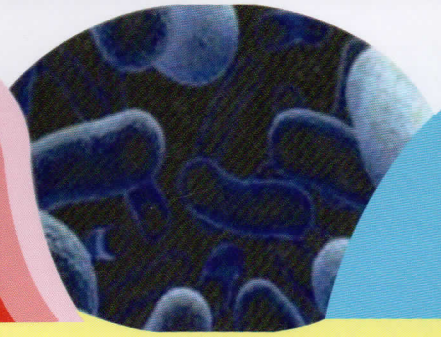

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ISOLATION AND IDENTIFICATION OF *BRUCELLA SUIS* IN PIGS AS ZOONOTIC DISEASE IN ENDEMIC AREAS OF EAST JAVA, INDONESIA

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Abstract

Background: Brucellosis in pigs at East Java Indonesia has not only cause great economic losses due to a decrease in productivity of livestock but also are zoonotic. Infection on free brucelosis pigs were initially begun with the infected pigs both male and female, or the use of superior male pigs together. The elimination of the disease either on a group or population is considered as the most effective way to prevent the spread of the disease in pigs. Prevention efforts mainly addressed to vaccination, sanitary maintenace and government policy. The purpose of this study was to isolated and identified *Brucella suis* as the causative agent.
Material and Methods: The survey area were the pig farm owned by breeder farmers in the area of East Java Indonesia, at Kediri, Malang, Blitar and Probolinggo district. Blood samples obtained were tested with RBT. Pigs are suspected of being infected with Brucella if the RBT was positive that characterized with agglutination in the test results. If RBT was positive, bacteriological examination will be performed, with samples of visceral foetus organ, ie liver, spleen, placenta and amniotic fluid. Isolation and identification of *Brucella suis* were used Brucella Broth and Brucella Agar, and if the bacteri growthwill be continued with biochemical test ie H2S, urease, citrate, catalase and oxidase test. The positive results of *Brucella suis* showed positive urease, catalase andoxidase,but negative for citrate and H2S.
Results: RBT and bacteriolgical examination showed that 1 sample was positive *Brucella suis*, and 19 negative. The positive results showed positive urease, catalase and oxidase,but negative for citrate and H2S
Conclusion: Based on RBT test and bacteriological examination, there was 1 positive sample of brucellla suis, that is sample coming from Kediri district.

Key words: *Brucela suis*, pig, isolation,identification, zoonotic

Introduction

Brucellosis is an infectious disease that can affect humans and animals (Alton et al., 1991). The cause of this disease is the bacterial genus *Brucella* which is an intracellular microorganism and can cause abortions and infertility (*orchitis* and *epididymitis*) in sheep, cattle, goats and pigs (Christi et al., 1968). This disease in humans is characterized with faint, fever, chills, sweating, pain in the joints, headaches and pain in the whole body (Priadi et al., 1992). Reservoirs of brucellosis due to *Brucella. suis* are wild animals and pigs (Corbel, 1985; Sudibyo, 1997). Infection on free brucelosis pigs were initially begun with the infected pigs both male or female, or the use of superior male pigs together. The spread of *Brucella suis* in pigs that bred by artificial insemination are also common (Corbel, 1997; Madkour, 1989). Piglets usually get the infection from their sow. The infection occurred at birth or fed up to infectious sow (Enright, F.M. 1990).

Screening tests or rapid test performed today in East Java, Indonesia still using *B. abortus* isolates of cattle species, that is S19, thus often occurs inaccurate results for the diagnosis of Brucellosis in pigs (Nicoletti, 1990).

Materials and Methods

The entire research was conducted appropriately following the ethics in using experimental animals and has been approved by the ethics commission of the Faculty of Veterinary Medicine, Universitas Airlangga.

The survey area were the pig farm owned by breeder farmers in the area of East Java Indonesia, at Kediri, Malang, Blitar and Probolinggo district. Blood serum samples and fetus originated from pigs who have abortus were collected. Blood samples were taken from pig that have experienced abortion, through the sow's ear veins to obtained the serum.

Serum samples were tested by RBT method (Corner and Alton, 1982) using artificial antigen commercial RBT made by Pusvetma Surabaya. The serum sample was mixed with antigen RBT. Both solutions placed on the glass object then stirred by rotating clockwise and then anti-clockwise direction gently for 2 minutes. The results obtained was negative : no agglutination occurred, a mixture of antigen antiserum looked pink homogeneous colored, (+1): seen a smooth agglutination and in perimeter seen as dotted line. (++): seen a clear smooth agglutination with wide margins in perimeter and a little bit clear of fluid around, (+++): seen a coarse / large agglutination and clear fluid around, dubious: seen agglutination in pink homogeneous colored. Pigs stated infected if RBT result was positive.

Isolation and identification of *Brucella suis* used Brucella Broth and Brucella Agar (Oxoid, England) with the addition of supplements. When the bacteria growth, it will be followed by a biochemical test namely H2S, urease, citrate, catalase and oxidase test. The positive results of *Brucella suis* showed positive urease, catalase and oxidase but negative for H2S and citrate.

Results

The survey showed that pig farms in Kediri, Malang, Blitar and Probolinggo showed the incidence of abortion. Twenty 20 sows showing symptoms of abortion. From Kediri, Malang Probolinggo and Blitar were obtained 10, 3, 5 and 2 respectively. The RBT showed that 1 sample from Kediri was positive and 19 other were negative (Table1)

Table 1: Rose Bengal Test Results

No.	Area	Samples Amount	Result	
			Positive	Negative
1	Kediri	10	1	9
2	Malang	3	0	3
3	Probolinggo	5	0	5
4	Blitar	2	0	2

Table 2: Bacteriological Test

Area	Number of Sample	Brucella agar media	H2S	Urease	Citrate	Catalase	Oxidase
Kediri	1	negative	0	0	0	0	0
	2	negative	0	0	0	0	0
	3	growth	negative	Positive	negative	positive	positive
	4	negative	0	0	0	0	0
	5	negative	0	0	0	0	0
	6	negative	0	0	0	0	0
	7	negative	0	0	0	0	0
	8	negative	0	0	0	0	0
	9	negative	0	0	0	0	0
	10	negative	0	0	0	0	0
Malang	1	negative	0	0	0	0	0
	2	negative	0	0	0	0	0
	3	negative	0	0	0	0	0
Probolinggo	1	negative	0	0	0	0	0
	2	negative	0	0	0	0	0

One sample from Kediri showed growth in Brucella agar media, thus further processed for purification, and incubated for 3 days before the biochemical tests were done. Biochemical test showed positive urease, catalase and oxidase test but negative for H2S and citrate test. The results of this test ensure that isolated bacteria was *Brucella suis.*

Discussion

The test results using *Rose Bengal Test* (RBT) towards blood serum of pigs showed that there was a positive reaction in 1 sample. Positive results of RBT were characterized by agglutination, means that the antigen and antibody was homolog and eventually agglutinated. Negative results of RBT proved that antigen and antibody was not homolog thus agglutination not occurred (McCughey, 1972; Mylrea, 1972, Rolfe and Sykes, 1987).

Negative H_2S tests mean that bacteria did not break sulfides to H_2S, where it was characterized by the absence of black color on TSIA media. The negative urease means that the bacteria did not own an urease enzyme that have capability hydrolyzing urea which changed yellow-colored alkaline become pink acid by using methyl red indicator. The negative citrate means that bacteria could not break the citrate so that there was no carbon element for cell metabolism which may changed green color to blue with bromine thymol blue as an indicator.

Catalase is produced by certain bacteria, which acts as a catalyst in breakdown of hydrogen peroxide into water and oxygen. If bubbles are produced, the organism is catalase positive and if bubbles are not produced, the organism is catalase negative.

The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain. When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine**) to** (indophenols) **purple** color end product. When the enzyme is not present, the reagent remains reduced and is colorless (Sulaiman, et al., 1993; Sapardi, et al., 2004).

Brucella suis affected gestation pigs aged 2-3 months. *Brucella suis* affected both male and female pigs. In gestation female pigs would cause miscarriage, while in male pigs would cause orchitis. The Brucella's germs outside the sow body could survive in a variety of environmental conditions within a certain time. The ability of Brucella bacteria living on dry land is four days outside the room temperature, in the moist soil can survive for 66 days and on a muddy soil to survive for 151-185 days (Gray and Martin,1980). According to Sudibyo (1998), *Brucella* bacteria can survive for 2 days in dirt or cages waste with relatively high temperatures. In the livestock drinking water germs can survive for 5-144 days and in the waste water for 30-150 days (Heck et al., 1980). This disease still remains a problem in many countries in the world because of its economic impact as well as the health impacts of its veterineries (Darwesh and Benkirane, 2001; Lumb, 2003).

Serological diagnosis accuracy is a very important factor for the success of Brucellosis control and eradication program. Serological examination is the most widely used for the diagnosis of Brucellosis, because of the simple serology test, fast and has a high accuracy (Oliver and Cooper, 1981). The Rose Bengal Test (RBT) method is widely used in many countries as a screening test against Brucellosis. After the screening tests, confirmation of the diagnosis by bacteriological test for bacterial isolates were used for screening tests (Herr et al., 1982; Manickam and Mohan, 1987, Martin, et al., 1987, Wrathall, et al., 1993, Paulo, et al., 2000).

Conclusion

Based on RBT test and bacteriological examination, there was 1 positive sample of *Brucellla suis*, that sample coming from Kediri district..

Conflict of interest: The authors declare that they have no conflict interest.

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