Detection of Haemophilus Parasuis from The Respiratory Tract of Pigs

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Abstract. Study in order to detect the presence of Haemophilus parasuis in pig farms in Tabanan, Badung, and Gianyar Bali have been undertaken. A total of 197samples including nasal and tracheal swabs, organs and synovial fluids were collected from 69, 28 and 100 animals from pig farms in Tabanan, Badung and Gianyar regency, respectively. Samples were collected mainly from animals which showing clinical signs of coughing, asphyxiate, swollen joints and death animals. Isolation and identification of Haemophilus spp was performed following the methods described by Priadi et.al. (2004) and Nedbalkova et al. (2006) with slightly modification. Antimicrobial sensitivity test was performed using the disc diffusion method by Kirby-Bauer.Haemophilus parasuis was detected from one sample which shown resistant to streptomycin only and sensitive to ampicillin, doxycycline and kanamycin, respectively.

Keywords: **Hamemophilus spp, pigs, respiratory tract, sensitivity test**

I. INTRODUCTION

Besides cows, buffaloes, sheep, and goats, pig is one of meat production animal. Majority of the pig farming in Bali is raised for meat consumption and also for ceremonies purposes. Apart from Bali pig farming can be found in other areas such as North Sumatra, Papua, East Nusa Tenggara and Moluccas. Some obstacles in pig farming including reduction in body weight, and infectious diseases. Infection due to Haemophilus spp particularly H. parasuis rarely reported in Indonesia,especially in Bali no report of disease due to this bacteria. This bacteria inhabits in the upper respiratory tract of pig. Several strains of Haemophilus have been reported to cause diseases which typically produce edematous of the serous such as fibrous polyserositis, polyarthritis, meningitis, and pneumonia [1][2][3]. Disease caused by Haemophilus spp. can affect pigs at any age but mostly early post weaning animals with clinical signs of fever up 40-41°C, difficulties in breathing, coughing, swollen joints, lameness and paralysis. The animals died within 2-5 days whereas recover animals suffer arthritis chronically, coughing and bronchitis [4][5]. Upon pathological examination meningitis, serositis, pleuritic, bronchopneumonia, pericarditis and arthritis can be observed as sole or multiple manifestation [6].There are numbers of cases in the pig farms in Bali in which the animals showed clinical signs such as fever, respiratory distress (coughing, abdominal breathing), swollen joints and lameness of hind legs similar to the clinical signs of the disease caused by Haemophilus parasuis. This study aimed to observe the prevalence of Haemophilus spp in pig farms and its sensitivity to the antibiotics normally used in the pig farms in Bali.

II. RESEARCH METHOD

Samples

Samples including nasal swabs, tracheal swabs, organ and synovial fluids were collected from 197 pigs which showed signs such as coughing, asphyxiate, and swollen joints from the three regency namely: Tabanan, Badung and Gianyar, respectively. All samples were collected aseptically and kept in Stuart transport media.

Isolation and Identification of Bacteria

Isolation of Haemophilus spp was performed by culturing samples on to Sheep Blood Agar (SBA) close to Staphylococcus aureusstreak culture as a source of NAD

(feeder culture). Plate culture were then kept in a plastic container with AnaerogenTM (OxoidR) for CO2 7-15% aerated, then incubated at 37○C for 24-48 hours following the methods by Priadi et al. (2004) and Nedbalkova et al. (2006) [7][8]. Identification was performed by the conventional methods which is Gram staining, oxidase and catalase test, and biochemical test using API® 20E™(Biomerieux). Characteristics of Haemophilus sp isolate were tiny colonies on blood agar close to the Staph. aureus streak culture, Gram negative rods, hemolytic, where hemolytic, catalase and oxidase varied. Isolates showing those characters were further identified by indole test, urease and fermentation of carbohydrate (glucose, arabinose, lactose, mannitol, xylose, galactose, mannose, raffinose, sorbitol and inositol [9]. Further confirmation was performed using the API® 20E™ (Biomerieux).

Antimicrobial Sensitivity Test

Sensitivity test was performed following the disc diffusion method by Kirby-Bauer with slightly modification [10]. The antibiotic paper disc used were ampicillin, kanamycin, streptomycin, doxycycline and trimethoprim sulfamethoxazole. Escherichia coli ATCC 8739 was used as a control. One to two colonies of bacteria were put into 3 ml bouillon, incubated at 37°C for 2-6 hours then the culture turbidity was adjusted to McFarland 0.5. Following this, the culture were spread on to the surface of Mueller Hinton Agar plate and antibiotic paper discs were put on the surface of it and incubated at 35°C for 18-24 hours. The diameter of inhibition zone of each antibiotic was then recorded.

III. RESULTS AND ANALYSIS

Isolation and Primary Test of Haemophilus sp isolate

Five (2.5%) of the 197 samples were suspected Haemophilus sp based on their morphological characteristics on SBA. The five samples comes from 3 pigs from Tabanan (2 tracheal swabs, BIIT50 and BIIT53 and one lung sample BIIT1) and 2 pigs (two nasal swabs, BIBd1 and BIBd2) from Badung, respectively. Characteristics of the isolate were transparent tiny non-hemolytic satellite colonies growing near the Staph. aureus streak as shown on Figure 1. All five isolates were Gram positive rods, catalase positive, however the isolates from Tabanan (BIIT50, BIIT53, and BIIT1) were oxidase negative whereas those two (BIBd1 and BIBd2) from Badung were oxidase positive.

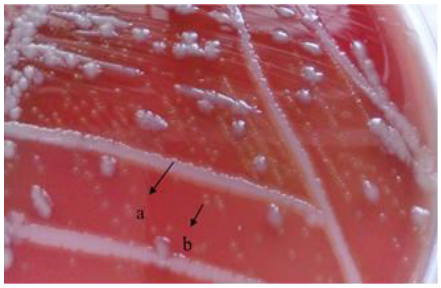

Fig. 1. Suspected colonies of Haemophilus sp (b) growing close to (a) Staphylococcus aureus streak colonies.

Biochemically Identification of Haemophilus sp.

Based on the biochemical confirmation test using API®20E™ two isolates BIIT1 and BIBd2 were identified as Enterobacter cloacae, BIIT53 as Proteus mirabilis, whereas BIIT50 and BIBd1 did not belong to a specific bacterial species. However, when comparing these two isolates physical and biochemical characteristics with H. parasuis, H. pleuropneumoniae, H. haemolyticus and H. influenza, one isolate, BIIT50 showed close characteristic to H. parasuis (see Table 1).Generally the occurrence of Glasser's disease in pig farms with characteristics syndrome is low. One case of the disease was reported by Priadi et al., (2006) occurred in pig farm in Pulau Bulan where it affected 10 weeks old post weaning pigs. The animals had pneumonia and died, lung and synovial fluids were sampled and Haemophilus parasuis serotype 12 was isolated.

TABLE 1.
PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF TWO ISOLATES (BIIT50 AND BIBD1) IN COMPARISON TO HAEMOPHILUS PARASUIS, HAEMOPHILUS PLEUROPNEUMONIA, HAEMOPHILUS HAEMOLYTICUS, ANDHAEMOPHILUS INFLUENZA.

Characteristics criteria	*H.parasuis**	*H.pleuropneumonia**	*H.haemolyticus**	*H.influenzae**	BIBd1	BIIT50
Growth factor	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Oxidase	-	-	+	+	-	-
CO_2(7-15%)	+	+	+	+	+	+
Indole	-	-	d	d	-	-
Hemolytic	-	-	+	-	-	-
Growth onMacConkeyAgar	-	-	-	-	+	-
Sucrose	+	+	-	-	-	+
Lactose	d	d			+	+
ONPG	d	+	-	-	-	-
ADH	-	-	-	-	-	-
LDC	-	-	-	d	-	-
ODC	-	-	?	?	+	-
H_2S	d	+		d	+	+
Urease	-	-		d	+	-
Glucose	-	-	+	+	-	-
MAN	+	+	-	-	+	+
Rhamnose	-	-	-	-	-	-
Melibiose	d	-	-	-	-	+
Arabinose	d	-	-	-	+	+
Mannitol	-	+	-	-	-	-
Sorbitol	-	-	-	-	-	-
Inositol	d	-	-	-	-	-
Sucrose	+	+	-	-	-	-
Maltose	+	+	+	+	+	+

*Adapted fromMacedoet al., 2014; d = >90% negative

Similarly, in other country the prevalence of Glasser's disease in pig farms appeared to be low. Smart et al., (1989) reported a prevalence of 1.0% in Southern Ontario following 17,332 pigs reported sick [11]. Apparently Haemophilus sp is a fastidious fragile organism which is very sensitive to changing in humidity and temperature.Therefore it is very hard to isolate the bacteria. Moreover often the disease is in combination with other diseases in the respiratory tract of the pig [12].

Antimicrobial Sensitivity Test

Results of the sensitivity of the isolate towards several antibiotics tested is shown in Table 2 and Figure 2. As shown in Table 2 isolate BIIT50 which is suspected as H. parasuis appeared to be resistant to streptomycin only, whereas H. parasuis reported by Priadi et al. (2004) Nedbalcova et al. (2006) showed resistance to streptomycin, ampicillin and doxycycline. The differences in antibiotic sensitivity pattern is influenced by the improper and long term use of antibiotics which resulted in resistance strain [13]. Our isolate showed sensitive to ampicillin, kanamycin and doxycycline, respectively. There are two ways of bacterial resistance to antibiotics; by chromosomal and extra-chromosomal. Chromosomal resistance is genetically, occurs spontaneous and very fast, whereas extra-chromosomal resistance occurs by the role of R-factor of bacterial plasmid [14][15].

TABLE 2.
DIAMETER OF INHIBITION ZONE OF ESCHERICHIA COLI ATCC 8739, BIIT50 AND BIBD1 TOWARDS AMPICILLIN, KANAMYCIN, DOXYCYCLINE, STREPTOMYCIN, AND TRIMETHOPRIM-SULFAMETHOXAZOLE.

Isolates	Diameter of Inhibition Zone (mm)				
	Ampicillin (Amp)	Kanamycin (K)	Doxycycline (D)	Streptomycin (St)	Trimethoprim-Sulfamethoxazole (Sr)
Escherichia coli ATCC8739	0(R)	21(S)	16(S)	16(S)	24(S)
BIIT50	20(S)	24(S)	30(S)	10(R)	15(I)
BIBd1	8(R)	19(S)	25(S)	13(I)	0(R)

R = Resistant; I = Intermediate; S =Sensitive;

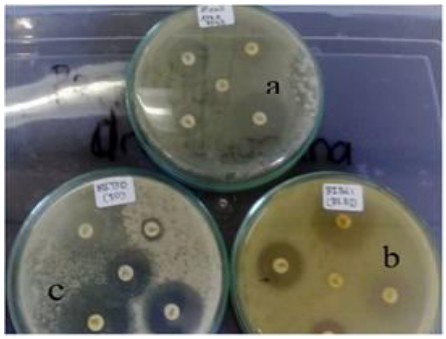

Fig. 2. Antibiotic Sensitivity Test Plates of Escherichia coli ATCC 8739 (a); Isolate BIBd1 (b); and Isolate BIIT50 (c), Showing Diameter of Zone Inhibition on Mueller Hinton Agar.

IV. CONCLUSION

In conclusion we managed to isolate H. parasuis (one isolate) from pigs, where the isolate is resistant to streptomycin only and sensitive to ampicillin, kanamycin and doxycycline, respectively.

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REFERENCES

[1] Lawrence, P., Russell Bey. (2015). Map-Based Comparative Genomic Analysis of Virulent Haemophilus parasuis Serovar 4 and 5. Genom. 3: 59-71.

[2] Bello-Ortí, B., Deslandes, V., Tremblay, Y.D., Labrie, J., Howell, K.J., Tucker, A.W., Maskell, D.J., Aragon, V., Jacques, M. (2014). Biofilm Formation by Virulent And Non-Virulent Strains of Haemophilus parasuis. Vete. Res. 45:104

[3] Oliveira, P.S, Pérez-Simó, Aragón, V., Segalés J., Bensaid, A. (2011). Immunogenicity and Protection Against Haemophilus parasuis Infection after Vaccination with Recombinant Virulence Associated Trimetric Auto-transporters (VtaA). J. Vet. Sci. 14 (1): 111-116.

[4] Macedo, N., Rovira, N., Torremorell, M., (2015). Haemophilus parasuis: Infection, Immunity and Enrofloxacin. Vet. Res. 46(128): 1-8.

[5] Oliveira, S., (2015). Haemophilus Parasuis. Extention. http:// articles.extension. org/pages /27270 /haemophilus-parasuis.

[6] Bochev, I. (2007). Porcine Respiratory Disease Complex (PRDC): A Review. I. Etiology, Epidemiology, Clinical Forms and Pathoanatomical Features. Bulg. J. Vet. Med. 10(3) :131-146.

[7] Priadi, A., Natalia, L., Poernomo, S. (2004). PenyakitGlasser's pada Babi di Pulau Batam, Propinsi Riau. BalaiPenelitianVeteriner, PO Box 151, Bogor 16114. JITV. 9(4).

[8] Nedbalcova, K., Stran,P., Jaglic, Z., Ondriasova, R., Kucerova, Z. (2006). Haemophilusparasuis and Glasser's Disease in Pigs : a Review. Vet. Med. 51(5): 168-179.

[9] Howell, K.J., Peters, S,E,, Wang, J., Garcia, J.H., Weinert, L.A., Luan, S.L., Chaudhuri, R.R., Angen,O., Aragon,V., Williamson, S.M., Parkhill, J., Langford, P.R., Rycroft,A.N., Wren, B.W., Maskell,D.J., W. Tucker,A.W., (2015). Development of a Multiplex PCR Assay for Rapid Molecular Serotyping of Haemophilus parasuis. J. Clin. Microbiol.53(12): 3812-3821.

[10] Bagul, U.S., Sivakumar, S.M., (2016). Antibiotic Susceptibility Testing: A Review On Current Practices. Int. J. Phar. 6(3): 11-17.

[11] Smart, N.L., Miniats, O.P, Rosendal S., Friendship, R.M. (1989). Glassers Disease and Prevalence of Subclinical Infection with Haemophilus parasuis in Swine in Southern Ontario. Can Vet. J. 30: 339-343.

[12] Oliveira, S., Pijoan, C., Morrison, R. (2004). Evaluation of Haemophilus parasuis Control in Nursery Using Vacination and Controlled Exposure. J. Swine Health Prod.12(3): 123-128.

[13] Rajurkar, G., Roy, A., Yadav. M.M., (2010). Antimicrobial Sensitivity Pattern of Haemophilus paragallinarum Isolated from Suspected Cases of Infectious Coryza in Poultry. Vet. World, 3(4): 177-181.

[14] Jain, A., Kumar, P., and Awasthi, S., (2005). High Nasopharyngeal Carriage of Drug Resistant Streptococcus pneumoniae and Haemophilus influenzae in North Indian School Children. Tro.l Med. Int. Health. 10(3): 234–239.

[15] Veloo, A. C. M., Seme, K., Raangs, E., Rurenga, P., Singadji, Z., Wekema-Mulder, G., & van Winkelhoff, A. J. (2012). Antibiotic Susceptibility Profiles of Oral Pathogens. Int. J.Antimicrob.l Agents, 40(5): 450-454.