Research Article

Meningoencephalitis and Arthritis in Post-Weaning Piglet with Streptococcal Infection

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Abstract | *Streptococcus suis* is the main agent of streptococcosis in pigs, the infection is systemic with high morbidity and mortality, especially in young pigs. This disease causes significant economic losses to the swine industry worldwide. This study aims to determine the histopathological changes of meningoencephalitis and arthritis in post-weaning piglets suspected of streptococcosis. The samples were 35 cadaver of post weaning piglets that showed muscle spasm and swollen joint at several villages in Badung Regency, Indonesia. Samples of the brain and synovial tissue were processed using hematoxylin-eosin dyes (HE). On histopathological examination, congestion, edema, mild infiltration of neutrophils and macrophages in the meningen, and ischemic neuronal pyknosis were found in the brain. In the synovial tissue, there is a heavy infiltration of neutrophils with few macrophages. Microbiological examination showed the growth of small round colonies, grayish-white, mucoid but hemolysis reaction was not clearly visible. On Gram staining, the bacteria looks purple, the coccus arranged like a chain, reacts negatively in the catalase and oxidase tests. The findings of meningoencephalitis in the brain, arthritis in the leg joints, and microbiological tests suggest that post-weaning piglets suffer from streptococcosis.

Keywords | Histopathology, Meningoencephalitis, Post-weaning piglet, Streptococcosis, *Streptococcus suis*

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INTRODUCTION

Streptococcosis is a group of infectious diseases mainly found in young animals of various animal species. This disease is caused by bacteria of the genus Streptococcus spp., that may infection in acute, sub-acute, or chronic forms. *Streptococcus suis* is one of the most important pathogenic bacteria in pigs that can cause significant economic losses to the swine industry worldwide (Staat et al., 1997). *S. suis* is a Gram positive pathogenic bacterium, cocci-shaped, with small colorless colonies, causing disease in pigs with symptoms of meningitis, bronchopneumonia, arthritis, and death, especially in young pigs (Wisselink et al., 2000; Gottschalk and Segura, 2000). *S. suis* bacteria can be found in the upper respiratory tract, especially in the tonsils and nasal cavity, genital organs, and digestive tract. *S. suis* is also a zoonotic pathogen due to direct contact with pigs or consuming pig-derived products (Dutkiewicz et al., 2018). Three protein factors associated with virulence in *S. suis* such as extracellular protein (EF) (Smith et al., 1993), muramidase released protein (MRP) (Vecht et al., 1991) and suilysin (SLY) (Jacobs et al, 1995).

Post-weaning piglets are most susceptible because of decreased maternal antibodies (Cloutier et al., 2003). This disease in pigs was reported for the first time by Field et al, (1954), occurring mainly in piglets up to 10 weeks of age. Economic losses due to this disease are estimated at 300 million dollars per year (Staat et al., 1997). Bacteria can also infect mammals and birds (Hommez et al., 1988; Devriese et al., 1994), and since 1968, it has been recognized as zoonotic pathogens in humans and often cause meningitis with hearing loss, septicemia, or other diseases occurring in pig farmers, butchers and abattoir workers worldwide (Goyette-Desjardins et al., 2014; Van Samkar et al., 2015). There has been an exponential increase in the incidence of *S. suis* infection with high mortality after two epidemics in China in 1998 and 2005 (Lun et al., 2007; Feng et al., 2014). The incidence of Streptococcosis caused by *S. suis* is commonly found in Southeast Asian countries such as Thailand and Vietnam, where people raise pigs traditionally and have a habit of consuming incompletely cooked pork (Takeuchi et al., 2012; Nghia et al., 2011).

The most common lesion found in *S. suis* infection were meningitis (68%) followed by other lesion such as arthritis (12.9%), sepsis, and endocarditis (Huong et al., 2014).

In Indonesia, the first reported incidence of Streptococcosis was in Timika Papua in 2008 with the successful isolation of *S. suis* from pig joints. The incidence of *S. suis* infection in humans has been reported in Bali with clinical symptoms of acute meningitis and septic arthritis. *S. suis* was isolated from cerebrospinal fluid (CSF) but could not be isolated from joint fluid (Aryasa et al., 2019). Infection by *S. suis* in pigs has also been reported in Bali with clinical signs of skin redness, anorexia, diarrhea, exudate of the nose and eyes, swelling of the joints and lethargy. *S. suis* was isolated from sick pigs by the polymerase chain reaction (PCR) method using the glutamate dehydrogenase (GDH) and recombination/repair protein (recN) gene fragments (Besung et al., 2019). The purpose of this study was to determine the cause of death in young pigs with clinical signs of seizures and swelling in the joints.

MATERIAL AND METHODS

Samples

Thirty-five (35) samples of post-weaning pig cadaver used in this study came from several villages in Badung Regency, Indonesia. Dead piglets showed clinical signs of spasms and swelling in the joints. The cadaveric samples of piglets aged between 8-10 weeks were necropsied using a predetermined procedure followed by an anatomical and histopathological examination to determine the inflammatory reaction in the brain and synovial tissue.

Methods

For arthritis, the skin was incised at the tarsus or carpal joint that looks swollen with a sterile scalpel to take out the membrane joint with a thickness of less than 1 cm into a pot filled with 10% neutral buffered formalin (NBF). Brain samples were obtained by splitting the head in a sagittal direction using bone scissors; a small part was taken and put into a pot filled with 10% NBF. The formalin-fixed tissue was then processed and histological preparations were made using hematoxylin-eosin (HE) dye (Kiernan, 2001). The stained brain and synovial tissue preparations were examined under a light microscope. Brain and synovial membrane were suspended and plated on sheep blood agar media. Bacterial identification of *Streptococcus suis* was carried out using macroscopic observations of colonies, gram staining, and primary tests of catalase and oxidase using Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

RESULT

This research was initiated by surveying pig farms in several villages in Badung Regency. The survey was conducted to obtain sick or dead post-weaning piglets suspected of being infected with *Streptococcus* spp. with clinical signs of seizures and joint swelling. Field observations were carried out from January to September 2020. One hundred post-weaning piglets were found sick, while 35 died with clinical signs of seizures and swollen joints. Among these piglets 20 were females and 15 were males with landrace races (Table 1).

Table 1: Number of Post-weaning piglets (sick and died) with clinical signs of seizures and joint swelling in several villages of Badung Regency (January-September 2020).

No	Sex	Total suspected	Died with clinical signs of spasms and joint swelling	Description
1	Female	60	20	Dead
2	Male	40	15	Dead
Total		100	35	

Histopathological examination of brain tissue showed congestion, infiltration of neutrophils and macrophages in the meninges accompanied by degeneration of neurons and mild proliferation of microglia in the brain (meningoencephalitis). Significant neutrophil inflammatory cell infiltration was found in the synovial membrane. Apart from neutrophils, a few macrophages and lymphocytes were also found. Histopathological changes of brain and synovial tissue can be seen in Figure 1. Microbiological examination showed the growth of small round colonies, grayish-white and mucoid. On gram staining, the colony looks purple, the coccus was arranged like a chain, reacts

negatively in the catalase and oxidase tests. These findings confirm it as *Streptococcosis* spp. (Goyette-Desjardine et al, 2014). The microbiological test of brain and joint suspension can be seen in Figure 2.

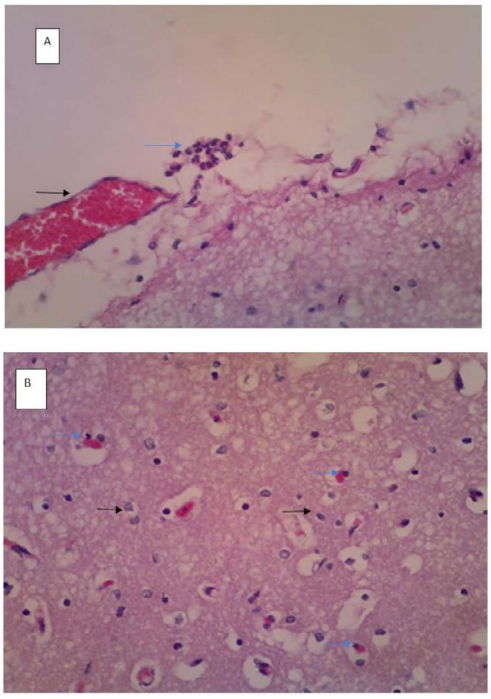

Figure 1: Histopathological photomicrograph of the brain and synovial tissue of post-weaning piglets in several villages in Badung Regency. In brain tissue, there was congestion (black arrow), mild infiltration of neutrophils and macrophages in the meninges (blue arrow) (A), proliferation of microglia (black arrow) and ischemic neuronal pyknosis (blue arrow) (B)

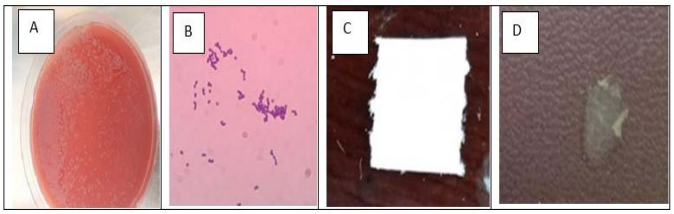

Figure 2: Photomicrograph of microbiological tests: culture of brain and synovial suspension on sheep blood agar showing no hemolysis (A), Gram staining showing purple colored cocci arranged in chains (B), negative oxidase test is indicated by the color of the paper that not changed into blue (C) and negative catalase test was indicated by the absence of air bubbles after being dripped with 3% H2O2 (D)

DISCUSSION

The consistency of meningoencephalitis and arthritis lesions found in cadaveric piglets with clinical spasm and joint swelling is related to environmental stress and management. In the early stages of breeding, piglets will get pressure/stressors in the form of a combination of a new environment combined with an immature immune system. These conditions make pigs highly susceptible to infection by various pathogens, which may lead to disease accompanied by clinical signs. *Streptococcus suis* is one of the most important pathogens affecting the health of piglets in the breeding phase (Goyette-Desjardine et al, 2014). These bacteria can act as commensals or as opportunistic pathogens in the nasal cavity and tonsils of most pigs (Gottschalk, 2012). In most cases, the bacteria remain in the colony of healthy pigs, but over time disease can develop with systemic infection. Infections caused by *S. suis* in breeding pigs are usually characterized by a very low incidence rate (5%) of clinical cases presenting with various signs such as septicemia, arthritis, pericarditis, and meningitis. The onset of clinical signs usually takes place very quickly with sudden death is also common (Gottschalk, 2010).

During the colonization of the host's mucous membrane, the interaction between the pathogenic bacteria and the host structure is very complex. Once able to pass through the mucosal epithelial barrier, *S. suis* can reach and survive in the blood and invade several organs, including the spleen, liver, kidney, lung, and heart, and cause hyper inflammation (Fittipaldi et al., 2012). This pathogen is also able to penetrate the brain microvasculature composed of endothelial cells or epithelial cells of the choroid plexus to gain access to the central nervous system to cause meningitis (Segura et al., 2016). Pigs of all ages can be exposed, but susceptibility generally declines post-weaning (Staats et al., 1997). Despite high carrier rates, morbidity rarely exceeds 5% but can reach more than 50% in reared pigs with poor hygiene and comorbidities. With proper treatment, mortality is usually low (0-5%) but can be up to 20% in untreated herds (Fulde and Valentine-Weigand, 2012).

The morbidity with clinical signs of seizures and joint swelling in post-weaning piglets found in this study was around 35%, this finding is relatively very high this may be related to very poor pen sanitation accompanied by co-infectious diseases (Fulde and Valentin, 2012). High morbidity is also influenced by the density of the population in a pen where transmission occurs through direct contact (oronasal) that cannot be controlled between healthy and infected pigs (Berthelot-Herault et al., 2001). Samples of pigs with clinical signs of seizures and joint swelling were between 8-10 weeks old, this finding is following the opinion of Higgin et al. (1990) who said that *S. suis* was more often isolated from 6-10 weeks old pigs.

Histopathological examination of various organs found inflammation and necrosis with varying degrees of severity. Inflammation of meningoencephalitis in the brain, bronchopneumonia in the lungs, endocarditis in the heart,

gastroenteritis in the gastrointestinal tract, glomerulonephritis in the kidneys, and synovitis in the synovial tissues. Inflammation that occurs is systemic because it is found in multiple organs. Neutrophil inflammatory cells were found to be significant in several organs followed by macrophages and lymphocytes. Based on the distribution of inflammation, streptococcosis is divided into meningitis, respiratory and articular forms. Streptococcal meningitis in piglets is common at the age of 30-35 days with clinical signs of convulsions, loss of coordination, and if inflammation persists, followed by fever, anorexia, and paralysis. Respiratory streptococcosis can be seen at the age of 2-4 weeks, this form of respiration is characterized by the presence of interstitial pneumonia, hemorrhagic fibrinous with necrosis of the alveolar septa. While the articular form is characterized by weakness in one or all of the legs resulting in a lack of food and stunted growth (Feng et al., 2014; Segura et al., 2016).

S. suis after being able to pass through the mucosal epithelial barrier can reach and survive in the blood circulation before attacking several organs, including the spleen, liver, kidney, lung, and heart, and causing hyper inflammation (Fittipaldi et al., 2012). This pathogen is also able to penetrate the brain microvasculature which is composed of endothelial cells or epithelial cells of the choroid plexus to gain access to the central nervous system to cause meningitis (Segura et al., 2016). Meningitis is the typhical of *S. suis* infection of the central nervous system, whereas encephalitis involving brain tissue is relatively rare (Huong et al., 2014). In this study, meningoencephalitis was seen in 35 post-weaning piglets with infection in the central nervous system. This finding is in line with Zheng et al (2009) who stated that experimentally intranasal inoculation of *S. suis* serotype 2 strain SC19 caused meningoencephalitis lesions in all piglets indicating infection in the central nervous system. Sanford (1987) also reported that meningoencephalitis and arthritis were found in 35 piglets infected with *S. suis* naturally in the central nervous system. Histopathological changes in the form of meningoencephalitis and arthritis in piglets are strong indications of the occurrence of streptococcosis in several villages in Badung regency. Meningoencephalitis and arthritis are characteristic changes due to *S. suis* infection although inflammation of other organs may also be present. Besung et al. (2019) reported that they had successfully isolated and identified sick pigs from several regency in Bali Province such as Denpasar, Tabanan, Gianyar, and Karangasem regency. This finding strengthens the fact that there is a possibility that all districts in the province of Bali have been infected by the bacterium *S. suis*. This is possible because there is no control over traffic movement of pigs between regencies in Bali Province. The occurrence of streptococcosis with clinical signs of acute bacterial meningitis and arthritis in two patients at Denpasar and Badung hospitals (Aryasa et al., 2019) further strengthens that Badung regency has also been infected by *S. suis*. In both patients, *S. suis* was isolated from the cerebrospinal fluid culture but the joint fluid culture was negative due to previous antibiotic therapy. The incidence of streptococcosis in humans is generally preceded by contact between humans and pigs and consuming processed pork or pork products (Dutkiewicz et al., 2018). Disease risk can be minimized by avoiding overcrowding, maintaining well-ventilated air, minimizing mixing and movement (especially during weaning), controlling other swine diseases, incorporating pest control measures, adequate cleaning and drying of pens, and using disinfectants and/or fumigants between groups/cage. It is necessary to inject piglets a few days before weaning with long-acting penicillin. Such injections can prevent disease but this method must be used with care to avoid the risk of developing antibiotic-resistant strains. Vaccine administration is the main requirement to consider both commercial vaccines and attenuated autogenous vaccines where the vaccine material is derived from a virulent strain isolated from sick pigs. Although the use of commercial vaccines is more convenient, they usually provide protection only against certain types of virulence factors from *S. suis*, the use of autogenous vaccines is quite inconvenient because it requires complete laboratory tests to determine the causative serotype, but this type of vaccine provides better protection and prevents spread of disease in flocks during outbreaks of *S. suis* infection. However, commercial polyvalent vaccines are needed to protect piglets from infection with all types of *S. suis* serotypes effectively and safely.

CONCLUSION

Based on histopathological findings and microbiological tests, it can be conclude that post-weaning piglets in several farms in Badung regency are being infected by *Streptococcus suis* which is also clinically evident by the meningoencephalitis, and arthritis in animals. To avoid the further spread of streptococcosis in post-weaning piglets in Badung regency, immediate concrete steps should be taken, such as improving the way of rearing piglets, administering antibiotics and mass vaccination of animals.

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CONFLICT OF INTEREST

The authors pronounce that they have no conflict of interest.

NOVELTY STATEMENT

This research is original and has never been published.

AUTHORS CONTRIBUTION

All authors contributed equally.

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