Isolation and Identification of Streptococcus suis from sick pigs in Bali, Indonesia

Nengah I. K. Besung
Faculty of Veterinary Medicine Udayana University of Bali, Indonesia
Gusti Ketut Suarjana
Faculty of Veterinary Medicine Udayana University of Bali, Indonesia
Karang Kadek Agustina
Faculty of Veterinary Medicine, Udayana University of Bali
Oka Ida Bagus Winaya
Faculty of Veterinary Medicine, Udayana University of Bali, Indonesia
Hamong Soeharsono
Faculty of Veterinary Medicine, Udayana University of Bali, Indonesia
Ketut Ni Suwiti
Faculty of Veterinary Medicine, Udayana University of Bali, Indonesia
Gusti Ngurah Mahardika (gnmahardika@unud.ac.id)
Universitas Udayana Kampus Surdiman https://orcid.org/0000-0001-5525-0793

Research note

Keywords: Streptococcus suis; sick pigs; Bali – Indonesia;

Posted Date: August 3rd, 2019

DOI: https://doi.org/10.21203/rs.2.11490/v1

Version of Record: A version of this preprint was published at BMC Research Notes on December 1st, 2019. See the published version at https://doi.org/10.1186/s13104-019-4826-7.

Abstract

Objective Streptococcus suis (S. suis) is a causative agent for various syndromes in pigs. It can be transmitted to humans with typical symptoms of meningitis and death. Data in Bali Referral Hospital showed more than 40 confirmed human cases of S. suis meningitis in 2014. Cases of S. suis infection in pigs have never been confirmed in the province. Here we provide evidence of the bacteria in sick pigs in that world tourist destination province. Results The S. suis was confirmed in eight out of 30 cases. Prominent histopathological lesions of confirmed cases were meningitis, endocarditis, pericarditis, bronchopneumonia, enteritis, and glomerulonephritis. The dominant inflammatory cells were neutrophils and macrophages. Further research is needed to understand the risk factor of human infection. Awareness of the community on the risk of contracting S. suis is needed to prevent human infections.

Introduction

Streptococcus suis (S. suis) is the important agent of emerging zoonotic, community-acquired bacterial meningitis [1-3]. It is a global zoonotic agent of pig origin [4]. Asia is a unique hotspot of *S. suis* infection related to the traditional pork consumption customs [5, 6]. The bacteria caused significant economic loss due to mortality and decrease production [7-9], as well as indirect loss due to the decrease demand of pork if an outbreak occurs. *Streptococcus suis* can caused significant economic loss in the world [3, 10].

This emerging zoonotic bacterium should be a of particular concern in a tourist destination area, as it poses a risk of traveler infection as well as of local people. Bali Province of Indonesia is well-known as one of world prominent tourist destination. More than six million travelers visited Bali in 2018 (https://bali.bps.go.id). On the issue of *S. suis*, data in Bali Referral Hospital showed more than 40 confirmed human cases of *S. suis* meningitis in 2014 (Dr. Made Susilawathi, Neurology Department of Bali Referral Hospital, personal communication). Cases of *S. suis* infection in pigs with encephalitis and arthritis signs have long been suspected in the province. Attempt has been made to detect the bacteria but without any convincing result. This seems to occur due to identification problem of *S. suis*. *S. suis* growth characteristic forming small colonies [11] can be unclear in a culture with mixed bacterial species. Misidentification of *S. suis* is not uncommon [12-14]. This false negative results are to be solved through a complete serotyping system [15], which is not available in Indonesia.

Methods

Source of animals

Sick pigs were owned by individual farmers, who agreed their sick and dead animals to be included in the study. The farmers origins were various districts in Bali Province as in Table 1.

During January to July 2018, there have been 30 cases of suspect *S. suis* recorded. The criteria were sick pig in a flock with at least one clinical signs of neurological disorder, reddish discoloration of skin and arthritis. The animals were not treated with antibiotic.

Freshly dead animal was necropsied. Organs with clear pathological lesions were collected in Stuart Transport Medium (CM0111 Oxoid) and buffered formalin. Isolation and biochemical characterization was conducted according standard protocol [16]. The tissues from one animal were pooled and extracted. The suspension was platted into a 5% defibrinated sheep blood agar plate. The plate was incubated 37ºC for 18-24 hours. Some suspected colonies were Gram stained, and growth in Triple Sugar Iron Agar (TSIA), and sulfide indole motility (SIM) medium. Other tests were catalase, oxidase, citrate, Methyl Red (MR), Voges–Proskauer (VP), glucose, and lactose tests. Three suspected colonies were injected to separate tryptic soy broth (TSB) (Sigma Aldrich MFCD00132536), and incubated in 37ºC for 18-24 hours. DNA was isolated using 10% chelex-100 (Biorad) [17, 18]; The glutamate dehydrogenase (GDH) and recombination/repair protein (recN) gene fragments were amplified using published specific primer sets for *S. suis* [19, 20].

Tissue processing was conducted and stained haematoxylin and eosin (H&E stain) based on published protocol [21].

Out of 30 cases, eight were found indicative for *S. suis*, forming small and non-hemolytic colonies, Gram positive with coccus-chain forming appearance. Only the positive cases are described further in this manuscript. The epidemiological and clinical data of the presumably positive cases are presented in Table 1. Listed from the most frequent, clinical signs were reddish skin discoloration, anorexia, nasal exudate, diarrhea, limping, eye exudate, lethargic, swollen joint, shivering, weakness, cough, in-appetence, depression, dyspnea, tremor, fever, and snot. Animal age was 3 – 12 months. Counting from the total animal number, number of sick and dead animal in the presumably positive cases, morbidity, mortality, and case fatality rate were 18.7%, 8.4%, and 44.9%, respectively. The cases were from Denpasar, Gianyar, Tabanan, and Karangasem regencies.

The result of Gram staining and a series of biochemical test of suspected colonies from eight cases of suspected S. suis infection in pig in Bali is presented in Table 2. All isolates were Gram (+), coccus form, short chained, and positive in acid slant, catalase, and lactose, but negative in VP test.

Upon electrophoresis of PCR products (not shown), positive samples showed single band of around 700 bp for GDH and 350 bp for recN, which forms equal bands compared with human S. suis isolate as positive control.

A panel of histological pictures of confirmed cases is depicted in Figure 1. The histopathology of confirmed cases was similar to each other with different severity. The prominent pictures were congestion in brain and meningitis, bronchopneumonia, endocarditis, myocarditis, pericarditis, erosion and enteritis along gastrointestinal tract with obvious depletion of Payer patches, hemorrhagic hepatitis and glomerulonephritis, as well as lymphoid depletion, hemorrhage and accumulation of inflammatory cells in the spleen. Dominant inflammatory cell infiltration in those tissues was neutrophil and macrophage.

We confirmed that *S. suis* does present and cause illness in pig in Bali, Indonesia. Human cases confirmed in Bali Referral Hospital must have been originated from pig. *Streptococcus suis* meningitis is

a global zoonotic community-acquired bacterial meningitis [1, 2, 4]. The bacterium is of an extra-ordinary issue in Asia as human outbreaks are related to the traditional pork consumption practices [5, 6]. The practice of consuming raw pork with raw blood delicacy is also common in Bali.

In this study, we carefully selected suspected cases, especially that of acute cases with no history of antibiotic medication. We recorded that the cases impacted in pigs under one year old. *S. suis* can be isolated from sick and healthy pigs at various age [22]. Clinical manifestation seems more frequent in young animals [23]. A study in Canada [24] showed that Typable *S. suis* isolates were more frequently isolated from pigs between five and ten weeks of age. That the ages of our cases were under one year seem to be appropriate for *S. suis* illness in pig.

We recorded clinical signs in our cases involving many organs, central nervous system, respiratory system, gastro-intestinal tract, urogenital, and circulatory system. Recorded clinical signs of *S. suis* infection in literature can be indeed multi-organ. The signs can be pyrexia, inappetence, depression, nasal discharge, dyspnea, tremors, seizures, incoordination, unusual stances (eg, dog-sitting), inability to stand, paddling, opisthotonos, convulsions, nystagmus, skin disease , swollen limbs and death [25]. In some cases, the disease goes per-acute and ended with sudden death without obvious signs [25]. Although septicemia and meningitis are known as the most striking manifestations of the disease, but endocarditis, pneumonia and arthritis have been reported [26]. In per-acute cases, pigs are often found dead with no obvious sign [26]. Other review article is also recorded that the disease syndromes caused by *S. suis* in swine include arthritis, meningitis, pneumonia, septicemia, endocarditis, polyserositis, abortions and abscesses [27]. In recent experimental infection, affected pigs presented clinical signs of anorexia, depression, fever, the eyes of the diseased pigs were glazed, with reddening of mucous membranes, severe nervous symptoms (incoordination, lateral prostration, paddling, opisthotonus, convulsions, lameness in posterior limbs) [28].

Our suspected cases were from all regencies in Bali Province. However, the confirmed cases were from four regencies/municipality, namely Tabanan, Denpasar, Gianyar, Karangasem. Considering that Bali is a small island with 5.600 square kilometer with high inhabitant density (http://www.baliprov.go.id/v1/geographi), and understanding the free movement of animal in the province, we assume that the disease of *S. suis* infection is distributed through-out the province. Counting from the total animal number, number of sick and dead animal in the presumably positive cases, morbidity, mortality, and case fatality rate (CFR) were 18.7%, 8.4%, and 44.9%, respectively. Morbidity, mortality, and CFR of *S. suis* in pig are variable [29]. Other data are not available on this issue in the literature survey. Therefore, we assumed that our observation on the case epidemiology is plausible.

Our microbiological data confirms the *S. suis*. The result of Gram staining, chain formation, and biochemical characterization shows that identified isolates were Gram (+), coccus with grape-like or short chain. All were acid slant, catalase, and lactose positive, while VP test was negative. *S. suis* is an encapsulated gram-positive bacterial coccus that occurs singly, frequently in pairs, or occasionally in short chains [3]. The majority of strains are alpha-hemolytic on bovine and sheep blood agar plates after

24 h of incubation at 37°C [3]. Four tests are used for a presumptive identification of *S. suis, i. e.* no growth in 6.5% NaCl agar, a negative Voges-Proskauer (VP) test, and production of acid in trehalose and salicin broths [24 , 30]. The VP test is critical in differentiating *S. suis* with other Streptococcus [15].

Final confirmation was made using PCR of GDH and recN. Both gene fragments are proposed as a system for the reclassification of *S. suis* or as a specific PCR system for *S. Suis* [19, 20]. We have established the system for *S. suis* detection from human and animal samples in Udayana University of Bali. We have sequenced both gene fragments from three pig cases and under submission to GenBank (Acc. No. ########). Both gene fragments were homogenous to each other as well as to human isolates, which are available in GeneBank (Acc. No. MK161045-MK161054; unpublished data).

We recorded prominent histologic pictures were congestion in brain and meningitis, bronchopneumonia, myocarditis, erosion and enteritis along gastrointestinal track, hemorrhagic liver and kidney. Dominant inflammatory cell infiltration in those tissues was neutrophil. Gross and microscopic findings of *S. suis* are published to include one or more of fibrinous polyserositis, fibrinous or hemorrhagic bronchopneumonia, purulent meningitis, myocardial necrosis, focal myocarditis and valvular endocarditis [29]. Moreover meningoencephalitis has been a striking lesion found in China [28]. Our meningitis histology in one case (Figure 1, panel A1-2) resembles meningitis picture described by that group.

In conclusion, *Streptococcus suis* has been confirmed in sick pig in Bali, Indonesia. Further research is needed to understand the risk factor of human infection. Vaccine should be immediately developed to reduce economic loss and its transmission to human being.

Further research is needed to elucidate the risk factor of human infection, as well as to map the distribution of *S. suis* in Indonesia. The fragment of GDH or recN might be used to map the infection in Indonesia. The fragment seems specific to *S. suis*. Moreover, the serotype of *S. suis* found in this study needs to be elucidated to. Human infection is mostly attributed to serotype 2 infections. Vaccine can be developed using inactivated strain to reduce economic loss as well as the risk of human infection, including national and international travelers.

Limitations

The distribution of S. suis in Bali and Indonesia is not yet available.

List Of Abbreviations

CFR: case fatality rate

GDH: glutamate dehydrogenase

MR: Methyl Red

PCR: polymerase chain reaction

recN: recombination/repair protein

SIM: sulfide indole motility

TSB: tryptic soy broth

TSIA: Triple Sugar Iron Agar

VP: Voges–Proskauer

Declaration

Ethics approval and consent to participate

The study was not dealing with life animal. The ethical approval of this study was issued by The Ethical Committee on the use of animal in research of the Faculty of Veterinary Medicine Udayana University of Bali. The owners signed informed consent to use of the sick and dead animals in the study.

Consent for publication

Not applicable

Competing interests

None

Data availability statement

All sequence data generated in this study are available in GenBank (Acc. No. #######; to be provided after the accession numbers are issued)

Funding

The study was funded by Directorate General of Research and Development, The Ministry of Research, Technology, and Higher Education of Indonesia, with research contract no. 171.53/UN.14.4.A/LT/2018 dated 19 February 2018.

Authors' contributions

INKB and GNM designed the study and were major contributor in writing the manuscript. IGKS supervised the microbiology work. KKA, HS, and NKS collected field samples and data. IBOW conducted and judged the pathological examination. All authors read and approved the final manuscript.

Acknowledgements

The authors thank to Mrs Krisna Dewi and Mrs Sutrisna Dewi for the laboratory works. The grammar and spelling of this manuscript has been edited professionally in Edanz Editing (https://www.edanzediting.com).

References

1. Brouwer MC, van de Beek D: Epidemiology of community-acquired bacterial meningitis. *Curr Opin Infect Dis* 2018, 31(1):78-84.

2. van de Beek D, Brouwer M, Hasbun R, Koedel U, Whitney CG, Wijdicks E: Community-acquired bacterial meningitis. *Nat Rev Dis Primers* 2016, 2:16074.

3. Goyette-Desjardins G, Auger JP, Xu J, Segura M, Gottschalk M: Streptococcus suis, an important pig pathogen and emerging zoonotic agent-an update on the worldwide distribution based on serotyping and sequence typing. *Emerg Microbes Infect* 2014, 3(6):e45.

4. Zou G, Zhou J, Xiao R, Zhang L, Cheng Y, Jin H, Li L, Zhang L, Wu B, Qian P *et al*: Effects of Environmental and Management-Associated Factors on Prevalence and Diversity of Streptococcus suis in Clinically Healthy Pig Herds in China and the United Kingdom. *Appl Environ Microbiol* 2018, 84(8).

5. Nghia HD, Tu le TP, Wolbers M, Thai CQ, Hoang NV, Nga TV, Thao le TP, Phu NH, Chau TT, Sinh DX *et al*: Risk factors of Streptococcus suis infection in Vietnam. A case-control study. *PLoS One* 2011, 6(3):e17604.

6. Takeuchi D, Kerdsin A, Akeda Y, Chiranairadul P, Loetthong P, Tanburawong N, Areeratana P, Puangmali P, Khamisara K, Pinyo W *et al*: Impact of a Food Safety Campaign on Streptococcus suis Infection in Humans in Thailand. *Am J Trop Med Hyg* 2017, 96(6):1370-1377.

7. Ma F, Chang X, Wang G, Zhou H, Ma Z, Lin H, Fan H: Streptococcus Suis Serotype 2 Stimulates Neutrophil Extracellular Traps Formation via Activation of p38 MAPK and ERK1/2. *Front Immunol* 2018, 9:2854.

8. Ma Z, Zhu H, Su Y, Meng Y, Lin H, He K, Fan H: Screening of Streptococcus Suis serotype 2 resistance genes with GWAS and transcriptomic microarray analysis. *BMC Genomics* 2018, 19(1):907.

9. Zhou Y, Dong X, Li Z, Zou G, Lin L, Wang X, Chen H, Gasser RB, Li J: Predominance of Streptococcus suis ST1 and ST7 in human cases in China, and detection of a novel sequence type, ST658. *Virulence* 2017, 8(6):1031-1035.

10. Yu Y, Qian Y, Du D, Li Q, Xu C, Liu H, Chen M, Yao H, Lu C, Zhang W: Infection and adaption-based proteomic changes of Streptococcus suis serotype 2 in a pig model. *J Proteomics* 2018, 180:41-52.

11. Amass SF, Wu CC, Clark LK: Evaluation of antibiotics for the elimination of the tonsillar carrier state of Streptococcus suis in pigs. *J Vet Diagn Invest* 1996, 8(1):64-67.

12. Huong VT, Ha N, Huy NT, Horby P, Nghia HD, Thiem VD, Zhu X, Hoa NT, Hien TT, Zamora J *et al*: Epidemiology, clinical manifestations, and outcomes of Streptococcus suis infection in humans. *Emerg Infect Dis* 2014, 20(7):1105-1114.

13. Fongcom A, Pruksakorn S, Netsirisawan P, Pongprasert R, Onsibud P: Streptococcus suis infection: a prospective study in northern Thailand. *Southeast Asian J Trop Med Public Health* 2009, 40(3):511-517.

14. Tsai HY, Liao CH, Liu CY, Huang YT, Teng LJ, Hsueh PR: Streptococcus suis infection in Taiwan, 2000-2011. *Diagn Microbiol Infect Dis* 2012, 74(1):75-77.

15. Higgins R, Gottschalk M: An update on Streptococcus suis identification. *J Vet Diagn Invest* 1990, 2(3):249-252.

16. Lehman DC, Mahon CR, Suvarna K: Streptococcus, Enterococcus, and Other Catalase-Negative, Gram-Positive Cocci. In: *Textbook of Diagnostic Microbiology, 5th Edition.* Edited by Mahon CR, Lehman DC, Manuselis G. Missouri: Saunders Elsivier; 2016: 328-348.

17. Giraffa G, Rossetti L, Neviani E: An evaluation of chelex-based DNA purification protocols for the typing of lactic acid bacteria. *J Microbiol Methods* 2000, 42(2):175-184.

18. Reyes-Escogido L, Balam-Chi M, Rodriguez-Buenfil I, Valdes J, Kameyama L, Martinez-Perez F: Purification of bacterial genomic DNA in less than 20 min using chelex-100 microwave: examples from strains of lactic acid bacteria isolated from soil samples. *Antonie Van Leeuwenhoek* 2010, 98(4):465-474.

19. Ishida S, Tien le HT, Osawa R, Tohya M, Nomoto R, Kawamura Y, Takahashi T, Kikuchi N, Kikuchi K, Sekizaki T: Development of an appropriate PCR system for the reclassification of Streptococcus suis. *J Microbiol Methods* 2014, 107:66-70.

20. Okwumabua O, O'Connor M, Shull E: A polymerase chain reaction (PCR) assay specific for Streptococcus suis based on the gene encoding the glutamate dehydrogenase. *FEMS Microbiol Lett* 2003, 218(1):79-84.

21. Zhang Y, Si Y, Zhai L, Yang N, Yao S, Sang H, Zu D, Xu X, Qin S, Wang J: Celastrus orbiculatus Thunb. ameliorates high-fat diet-induced non-alcoholic fatty liver disease in guinea pigs. *Pharmazie* 2013, 68(10):850-854.

22. Luque I, Blume V, Borge C, Vela AI, Perea JA, Marquez JM, Fernandez-Garayzabal JF, Tarradas C: Genetic analysis of Streptococcus suis isolates recovered from diseased and healthy carrier pigs at different stages of production on a pig farm. *Vet J* 2010, 186(3):396-398.

23. Cloutier G, D'Allaire S, Martinez G, Surprenant C, Lacouture S, Gottschalk M: Epidemiology of Streptococcus suis serotype 5 infection in a pig herd with and without clinical disease. *Vet Microbiol* 2003, 97(1-2):135-151.

24. Higgins R, Gottschalk M, Mittal KR, Beaudoin M: Streptococcus suis infection in swine. A sixteen month study. *Can J Vet Res* 1990, 54(1):170-173.

25. Gottschalk M: Streptococcus suis Infection. In: *Merck Veterinary Manual.* Edited by Allen DG, Constable PD, Dart A, Davies PR, Quesenberry KE, Reeves PT, Sharma JM. New Jersey: Merck Sharp & Dohme Corp; 2016.

26. Sanford SE, Tilker ME: Streptococcus suis type II-associated diseases in swine: observations of a one-year study. *J Am Vet Med Assoc* 1982, 181(7):673-676.

27. Staats JJ, Feder I, Okwumabua O, Chengappa MM: Streptococcus suis: past and present. *Vet Res Commun* 1997, 21(6):381-407.

28. Zheng P, Zhao YX, Zhang AD, Kang C, Chen HC, Jin ML: Pathologic analysis of the brain from Streptococcus suis type 2 experimentally infected pigs. *Vet Pathol* 2009, 46(3):531-535.

29. John VS, Wilcock B, Kierstead M: Streptococcus suis Type 2 Infection in Swine in Ontario: A Review of Clinical and Pathological Presentations. *Can Vet J* 1982, 23(3):95-97.

30. Tarradas C, Arenas A, Maldonado A, Luque I, Miranda A, Perea A: Identification of Streptococcus suis isolated from swine: proposal for biochemical parameters. *J Clin Microbiol* 1994, 32(2):578-580.

Tables

Due to technical limitations, tables 1 and 2 are only available as a download in the supplemental files section.

Figures

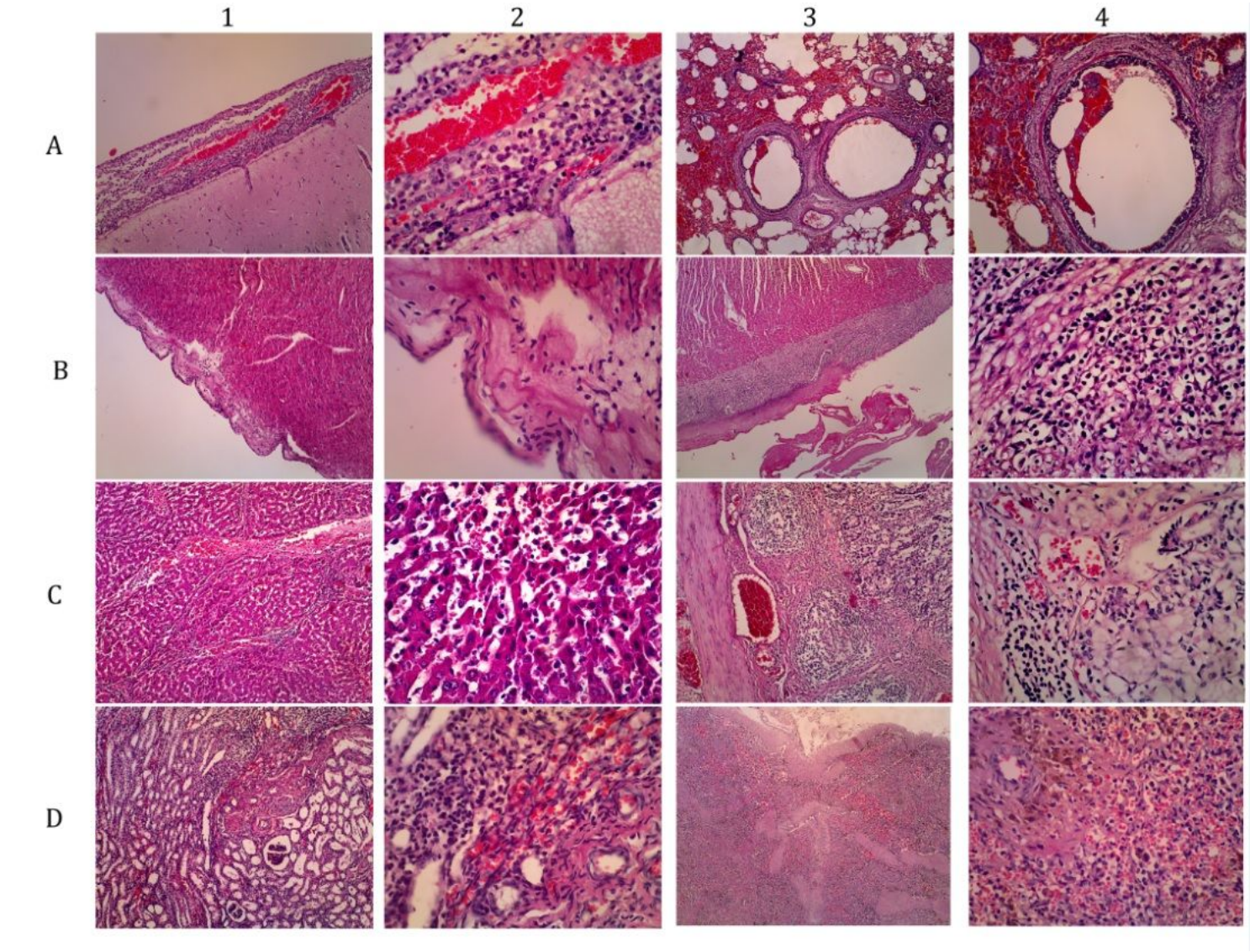

Figure 1

Histopathological pictures of various tissues of confirmed S. suis infection in pig cases in Bali, Indonesia, 2018. Panel A1-2 are brain and meninges showing meningitis; A3-4 are lung tissues showing bronchopneumonia; B1-2 are myocardium showing endocarditis; B3-4 are myocardium showing pericarditis; C1-2 are liver showing congestion and hepatitis; C3-4 are intestine showing hemorrhagic enteritis with depletion of Payer patches; D1-2 are kidney showing hemorrhagic glomerulonephritis; D3-4 are spleen showing perifollicular infiltration of inflammatory cells and hemorrhage; H&E stained. Magnifications in column 1 and 3 are 100X; Column 2 and 4 are 400X;

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.jpg
- Table2.jpg