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Drug resistant parasites and fungi from a one-health perspective: A global concern that needs transdisciplinary stewardship programs



A large cluster of human infections of *Streptococcus suis* in Bali, Indonesia

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ABSTRACT

A cluster of 18 inpatients and 21 outpatients with a major complaint of meningitis and a history of sharing traditional delicacies of raw pork and pig blood in a village festival led to the suspicion of *Streptococcus suis* (*S. suis*) meningitis in Sibang Kaja Village, Badung, Bali, Indonesia. We conducted an investigation and case finding to prevent human fatalities. Demography and laboratory examinations of the inpatients were recorded. Bacterial culture, identification, and sensitivity tests were conducted using a VITEK 2 Compact machine (Biomérieux®) with cerebrospinal fluid (CSF) or blood from the inpatients. The bacterial species were confirmed via PCR. A subsequent investigation was also conducted to identify the source of the meat, the presence of *S. suis* in slaughtered pigs, and the zoonosafety measures at pig farms and slaughterhouses. The five most common clinical signs were fever (92.3%), myalgia (46.1%), neck stiffness (25.6%), headache (23.1%), and nausea/vomiting (20.5%). *S. suis* was confirmed in two CSF and one blood specimens. All inpatients recovered following intravenous treatment with ceftriaxone every 12 h for 14 days and dexamethasone every 6 h for 4 days, while the outpatients were treated with 500 mg of amoxicillin three times daily for 7 days. The veterinary and environmental investigation identified the source of the pork for the festival, confirmed the presence of *S. suis* in the slaughtered pigs, and elucidated the sanitary measures applied at the pig farms and slaughterhouses. We conclude that infections of *S. suis* in humans can spread in cluster phenomena following the sharing of high-risk food. A prompt and early response, as well as early treatment of patients, is paramount in case finding to enable a favourable outcome of full recovery. Additionally, the implementation of the “One Health” approach provides a comprehensive picture of the management of *S. suis* infection in humans.

1. Introduction

Streptococcus suis (*S. suis*) is a zoonotic meningitis infection that is mainly transmitted through either direct contact with pigs or the consumption of pork products [1–3]. *S. suis* is a gram-positive facultative bacterium that is found not only in pigs but also in horses, dogs, and cats [4,5]. *S. suis* infections in humans have been reported in sporadic cases, although significant outbreaks are considered unusual. Since its first description, there have been more than 700 cases reported worldwide, with the majority reported in Southeast Asia and countries with a high

rate of pig breeding and contact with pigs, as well as pork products [6]. When transmitted to humans, *S. suis* may cause a wide range of clinical manifestations, including septicaemia, arthritis, endocarditis, and endophthalmitis [7]. However, *S. suis* infection is most commonly associated with meningitis. At Bali Provincial Sanglah Hospital, 71 patients had suspected bacterial meningitis between August 2014 and December 2017 and 44 were confirmed to have *S. suis* infections [8]. The wide spreading of the bacteria in pigs in the province has been confirmed [9].

Here, we report a large cluster of *S. suis* infections involving 18

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hospital-admitted patients and 21 outpatients who shared traditional food made of raw chopped pork mixed with spicy vegetables and fresh pig blood.

2. Methods

2.1. Ethical approval

This retrospective study was approved by the Research Ethics Committee of the Faculty of Medicine, Udayana University of Bali, Indonesia (Number 231/UN.14.2/KEP/2018) on February 5th, 2018. As per the standard operation procedure of the Mangunsada Hospital of Badung District, cerebrospinal fluid (CSF) or blood specimens were collected following informed consent.

2.2. Epidemiological and clinical investigation

Multiple collective reports of a febrile illness with signs of meningitis were collected among subjects in Sibang Gede, Badung Regency, Bali. The corresponding hospital immediately reported these unusual findings to the health office in the Badung district, which was then forwarded to the provincial health office. An investigation team was formed and consisted of an epidemiologist, a neurologist and a microbiologist. The team investigated and found additional cases of the febrile illness.

All subjects underwent complete blood count, CSF analysis, blood culture, CSF culture and Gram staining and antibiotic sensitivity tests. All subjects subsequently received empirical antibiotic treatment with adjunctive dexamethasone until their blood and/or CSF culture results were available.

2.3. Microbiological workups, cultures and PCR confirmation tests

All samples were inserted into BacTAlert media and incubated in a BacTAlert machine (Biomerieux®). Positive samples were continued with Gram staining and were grown on 5% goat blood agar plates and incubated aerobically at $35 \pm 2^\circ\text{C}$ for 18–24 h. Identification and sensitivity tests were performed using the VITEK 2 Compact machine (Biomerieux®). Colonies of bacteria that grew on the blood agar media were also confirmed by PCR using published glutamate dehydrogenase (GDH) and recombination/repair protein (recN) primer sets [10,11].

2.4. Veterinary and environmental investigation

The source of pork for the festival was investigated. Pig tonsil tissues were collected from private pig slaughterhouses in Dharmasaba village, which is close to the affected villages. Samples were collected from seven out of 34 slaughterhouses (20%). An environmental survey was conducted through interviews with both village leaders to determine the number of households engaging in small-scale pig husbandry. *S. suis* was detected using PCR as described above.

3. Results

The earliest reports of *S. suis* infection were on March 4 and 5, 2017. Four patients from Sibang Gede complained of illness and explained the history of sharing a “lawar” in a wedding ceremony in the previous one or two days before onset. The symptoms were fever, nausea, vomiting, and headache on the first and second day of onset, respectively. Subject 1 had a high fever (39°C) accompanied by haemodynamic compromise (BP: 60/40 mmHg, pulse rate: 96 bpm) and later developed signs of meningitis with altered mental status and neck stiffness, whereas Subject 2 had similar vital signs, albeit to a milder extent (37.8°C ; 100/60 mmHg; 92 bpm). Subjects 3 and 4, who were admitted on the second day of onset, experienced a decrease in consciousness with irritability, with Subject 4 having neck stiffness that was discovered upon physical

examination. On March 6, enhanced surveillance was conducted. Another 14 patients with similar clinical features were identified from March 6 to 18, 2017. All of them had a minimum of two of the classic symptoms of meningitis. Additionally, 24 cases of febrile illness were found upon investigation. All outpatients had shared the traditional lawar delicacy as described above. Two outpatients were from the neighbouring village of Sibang Kaja. The map of Sibang Gede and Sibang Kaja of Badung Regency, Bali, Indonesia, is presented in Fig. 1. The total cumulative number of inpatients and outpatients with *S. suis* meningitis in both villages is presented in Fig. 2. The food sharing ceremony occurred on March 3, 2017. The investigation team was formed on March 5, 2017, to find other cases. On March 7, 2017, the subdistrict community health centre ordered that 500 mg of amoxicillin be given three times daily for seven days to any patient complaining of febrile illness who had a history of sharing traditional food on March 3, 2017. The histories of the inpatients are listed in Table 1. The first four patients were admitted on March 4 and 5, 2017. The last patient was admitted on March 18, 2017. The standard therapy of Mangunsada Hospital of Badung District, intravenous treatment with 2 g of ceftriaxone every 12 h for 14 days and 10 mg of dexamethasone every 6 h for 4 days, was immediately applied. Specimens of CSF or blood were collected 1–5 days following admission. All inpatients were diagnosed with meningitis, with various additional signs, complaints or blood test results showing septic shock, thrombocytopenia, seizure, conjunctivitis, vertigo, and hearing disturbances.

The demographic data and clinical signs of the inpatients and outpatients with *S. suis* infection from the clusters of Sibang Gede and Sibang Kaja of Badung District, Bali, Indonesia are presented in Table 2. All the inpatients were adult males, while the outpatients were mostly adult males (66.6%), with adult females accounting for 33.3%. The five most common clinical signs were fever (92.3%), myalgia (46.1%), neck stiffness (25.6%), headache (23.1%), and nausea/vomiting (20.5%). Other signs included decreased consciousness, seizure, vertigo, cough, hearing disturbances, diarrhoea, and conjunctivitis. The demographic data and laboratory results for *S. suis* meningitis patients from the Sibang Badung cluster are presented in Table 3. The inpatients were 52.6 ± 13.1 years old, and the onset of illness occurred at 5 ± 3.4 (range 1–15) days. The complete blood count results showed the leucocyte count was $16,700 \pm 7300/\mu\text{L}$, predominantly showed neutrophils (90.3% \pm 4.9%), and the thrombocyte count was $137,200 \pm 58,600/\mu\text{L}$. The CSF examinations showed pleocytosis. The median CSF cell count was 326.3 cells/ μL (range: 8–803 cells/ μL); the median CSF neutrophil count was 43% (range 0–83%); the median CSF lymphocyte count was 56% (range 15–100%); the median CSF glucose level was 72 mg/dL (range 35–112 mg/dL); the median CSF/blood glucose ratio was 0.3 (range 0.2–0.5); and the median CSF protein level was 146 mg/dL (range 29.7–261 mg/dL).

Two out of 7 CSF specimens and one out of 10 blood specimens were positive for *S. suis*. The PCR results for GDH and recN of all the samples produced specific single bands of expected sizes (data not shown).

The source of pork for the festival was a meat vendor that collected pork from private slaughterhouses. Out of 150 pig tonsil tissues, 16 (10.67%) were positive for *S. suis*. The environment survey showed that 70% of households in Sibang Kaja and Sibang Gede kept pigs in their backyard, with 2–5 heads per household. The stalls were mostly built with concrete floors and walls of one metre height. Biosecurity was never implemented. Various animals, such as birds, rats, and pets, roamed freely in and out of the stalls. Disinfectants were never applied. While the slaughterhouses were mostly clean and in good condition with white porcelain floors and walls, sampling of pigs for *S. suis* investigation had never been conducted by the Regency Veterinary Office.

4. Discussion

The unusual high incidence of patients with febrile illness and signs of meningitis originating from a village prompted the suspicion of a

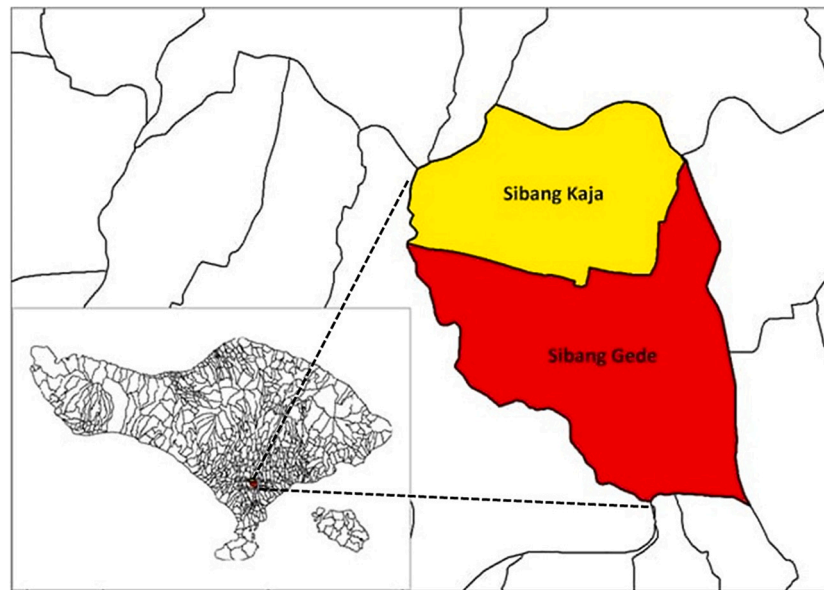


Fig. 1. The map of Bali-Indonesia and the location of affected villages.

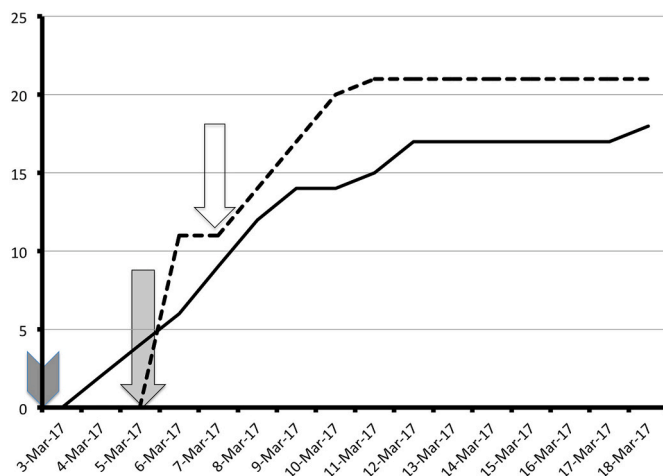


Fig. 2. Total cumulative number of inpatients and outpatients of *Streptococcus suis* infection cluster in Badung District in 2017. Arrow head is the date of communal festivity of preparing traditional delicacies of red “lawar” and “komoh”, at March 3, 2017. The grey arrow indicates the date of surveillance team was formed (March 5, 2017). The close arrow indicates the decision of the head of Subdistrict Community Health Centre to order to give ampicillin 500 mg three time daily for seven days for any patient who had history of sharing the food and complaining flu like syndrome. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cluster of a particular infectious disease. The history of a shared traditional delicacy with raw pork and pig blood in a village festivity led to the suspicion of *S. suis* infection. Global human infections of *S. suis* are almost always related to pig handling, pig slaughtering, or pork consumption [2,12,13]. Consumption of raw pork with uncooked pig blood has been found to be a major risk factor for *S. suis* infection in humans in Asia [13–15].

All the inpatients were adult males, while the outpatients were mostly of the same sex and age group (66.6%), with adult females accounting for only 33.3% of the epidemiological pattern of *S. suis* infections. The consumption of raw pork with raw blood seems to be adult- and male-related customs in Balinese culture. This pattern is consistent

in other Asian cultures [15]. Balinese people also have a unique tradition known as “Mebat”, which is the tradition for food processing and cooking, especially pork, and several people consume this meat as a part of Hindu ceremonies. This tradition includes the slaughtering of pigs, cutting the pork, cooking the meat, and serving it as dishes termed red “lawar” and “komoh” soup. Red “lawar” is prepared from minced pork mixed with raw pork blood and vegetables. Meanwhile, “komoh” soup is prepared using fresh pork blood and herbs [16].

The cluster event of *S. suis* meningitis was convincing. All inpatients and outpatients admitted to participating in the consumption of a shared traditional delicacy. The incubation period after the food festivity ranged from 1 to 15 days. The last patient was admitted on March 18, 2017. The incubation period of *S. suis* meningitis was described to be between 3 h and 14 days [17]. Outpatients complaining of febrile illness were found in the investigation. The last outpatient case was recorded on March 11, 2017, eight days after the ceremony. Although the role of other febrile illness agents was not negligible, the involvement of *S. suis* was not impossible. Having a history of eating the same food as the index case (Patient P4 in Table 1) and a short incubation period of 3–8 days was convincing to include outpatients in the cluster. *S. suis* causes a systemic infection in human-related bacteraemia [6], and febrile illness is also common in *S. suis* infections [17]. Although the most prominent sign of an *S. suis* infection is meningitis, the clinical spectrum of this bacterium expands to include various complaints, such as arthritis, endocarditis, peritonitis and endophthalmitis [18–20]. Due to the decision of the head of the subdistrict community health centre that 500 mg of amoxicillin be given three times daily for seven days for any patient complaining of febrile illness who had a history of sharing traditional food on March 3, 2017, all of the outpatients recovered without any sequelae.

Haematology and CSF data supported the diagnosis of *S. suis* meningitis in the inpatients. Haematologic examinations of the inpatients revealed leucocytosis with predominant neutrophilia and thrombocytopenia. Leucocytosis with predominant neutrophils is a hallmark of bacterial infection [2,21–23]. Leucocytosis and thrombocytopenia are consistently found in *S. suis* infection in other countries [17] as well as in Indonesia [8]. CSF examinations revealed pleocytosis, hypoglycorrhachia and increased protein levels. Increased CSF cell counts with predominant neutrophils, low glucose, and increased protein levels indicated acute bacterial meningitis [2,21]. Some patients with CSF samples predominated by mononuclear cells or lymphocytes might have

Table 1

Time-line of admitted patients of suspected meningitis following traditional festivity and sharing traditional food of “red *lawar*” in March 3, 2017, in Badung District, Bali, Indonesia, 2017.

Patient Number	Admission date	Therapy started	Specimen collection date	Culture (result)	Clinical presentation and complication
P1	4-Mar-17	4-Mar-17	9-Mar-17	Blood (negative)	Septic shock; meningitis; thrombocytopenia
P2	4-Mar-17	4-Mar-17	Not done	Not available	Meningitis; seizure; thrombocytopenia
P3	5-Mar-17	5-Mar-17	8-Mar-17	CSF (negative)	Meningitis; seizure; conjunctivitis; thrombocytopenia
P4	5-Mar-17	5-Mar-17	8-Mar-17	CSF (positive)	Meningitis; thrombocytopenia
P5	6-Mar-17	6-Mar-17	9-Mar-17	CSF (negative)	Meningitis; vertigo; thrombocytopenia
P6	6-Mar-17	6-Mar-17	Not done	Not available	Suspect DHF prior to meningitis; hearing disturbance
P7	7-Mar-17	7-Mar-17	8-Mar-17	CSF (positive)	Meningitis; seizure
P8	7-Mar-17	7-Mar-17	8-Mar-17	CSF (negative)	Meningitis
P9	7-Mar-17	8-Mar-17	9-Mar-17	CSF (negative)	Meningitis
P10	8-Mar-17	8-Mar-17	9-Mar-17	CSF (negative)	Meningitis
P11	8-Mar-17	9-Mar-17	9-Mar-17	CSF (negative)	Meningitis
P12	8-Mar-17	8-Mar-17	11-Mar-17	Blood (negative)	Meningitis
P13	9-Mar-17	9-Mar-17	11-Mar-17	CSF (negative)	Meningitis
P14	9-Mar-17	9-Mar-17	11-Mar-17	CSF (negative)	Meningitis; ataxia
P15	11-Mar-17	11-Mar-17	13-Mar-17	Blood (positive)	Meningitis
P16	12-Mar-17	12-Mar-17	14-Mar-17	Blood (negative)	Meningitis
P17	12-Mar-17	12-Mar-17	Not done	Not available	Meningitis
P18	18-Mar-17	18-Mar-17	Not done	Not available	Meningitis

DHF: dengue haemorrhagic fever; March 25, 2017 all patients were released from hospital.

Table 2

Demographic Data and clinical signs of in- and outpatients *Streptococcus suis* infection from Cluster Sibang Badung.

Demographic Data/Clinical signs	Number	%
Age (years)		
20–24	3	7.7
25–39	0	0
30–34	1	2.5
35–39	3	7.7
40–44	4	10.3
45–49	4	10.3
50–54	6	15.4
55–59	4	10.3
≥60	14	35.8
Sex		
Male	32	82.6
Female	7	17.94
Address		
Sibang Gede	37	94.87
Sibang Kaja	2	5.13
Patient status		
Inpatient	18	46.15
Outpatient	21	53.85
Clinical signs		
Fever	36	92.3
Myalgia	18	46.1
Neck stiffness	10	25.6
Headache	9	23.1
Nausea and Vomiting	8	20.5
Decreased of consciousness	5	12.8
Seizure	3	7.7
Vertigo	2	5.1
Cough	2	5.1
Hearing disturbance	1	2.6
Diarrhoea	1	2.6
Conjunctivitis	1	2.6

reflected delayed specimen collection and prior antibiotic therapy [21].

Demography showed that all inpatients being adult males and most outpatients being male are common features of *S. suis* infection This pattern has been indicated in Thailand [15] as well as the global epidemiology of the infection [2]. The preference for red “*lawar*”, which is uncooked pork with fresh blood, and “*komoh*”, or raw pork soup mixed with fresh blood, seems to be attributed to this age and sex group.

We recorded a wide spectrum of clinical signs of *S. suis* infection in the inpatients. The five most common clinical signs were fever, myalgia, neck stiffness, headache, and nausea/vomiting. Other signs included decreased consciousness, seizures, vertigo, cough, hearing disturbances,

Table 3

Demographic Data and Laboratory Result Inpatients Meningitis *Streptococcus suis* from Cluster Sibang Badung.

Variables	
Age (mean ± Standard Deviation/SD, range); years	52.6 ± 13.1 (37–77)
Sex (number and %)	
Male	18 (100%)
Female	0 (0%)
Onset of illness (mean ± SD, range); day	5 ± 3.4 (1–15)
WBC (x 1000/μL), mean (SD)	16.7(7.3)
Neutrophil (±SD)	90.3% (4.9)
Platelet count (x1000/uL)	137.2 (58.6)
CSF Results, median (range)	
CSF cell count (cells/μL)	326.3(8–803)
CSF PMN (%)	43(0–83)
CSF MN (%)	56(15–100)
CSF Glucose (mg/dL)	72 (35–112)
CSF/Blood Glucose ratio	0.3 (0.2–0.5)
CSF protein (mg/dL)	146 (29.7–261)
Culture positive <i>S. suis</i>	
CSF specimen	2/7 (14.3%)
Blood specimen	1/14 (7.1%)

Note

WBC: white blood cells; CSF: cerebrospinal fluid; PMN: polymorphonuclear cells; MN: mononuclear cells.

diarrhoea, and conjunctivitis. Another report described that the most common presenting symptoms were fever, headache, joint pain, vomiting and hearing loss [22]. Another group found that the majority of meningitis patients developed classic meningitis symptoms, including severe headache, a high fever, neck stiffness and a change in mental status or altered consciousness [2,12].

The species identification of *S. suis* from the confirmed cases was performed using PCR of GDH and recN as previously published [8]. However, the detection rate was relatively low, as only two out of seven CSF specimens and one out of ten blood specimens were positive for *S. suis*. CSF is the sample of choice of *S. suis* meningitis [2], although the bacterium can also be detected from patient blood [2,6,13]. We managed to collect CSF from only seven inpatients, as the others were reluctant to sign informed consent forms. Moreover, the CSF and blood collections were delayed 2–5 days upon admission and the beginning of the treatment. This is indeed the limitation of our study. However, the history of sharing high-risk food and meningitis signs were seen as strong evidence for presumptive diagnosis without any bacterial culture.

Prompt field investigation in this case was paramount in enhancing patient awareness. More patients with meningitis signs were directly

admitted without any delay. This presumably contributed to the lack of fatalities in the cluster. The global case fatality rate (CFR) of *S. suis* is approximately 3% [5], although it can be higher [24]. The standard therapy for *S. suis* meningitis has been in place upon admission (Table 1). Amoxicillin (500 mg) three times daily for seven days was directly given to all outpatients with febrile illness detected during the investigation. This mass therapeutic decision helped avoid the need for patient hospitalization. Lessons learned in our cluster case should be part of the alert system in possible *S. suis* outbreaks elsewhere.

Not all who came in contact with *S. suis*-infected food became sick. As explained above, there was a customary food preparation, so-called “*mebat*”, on March 3, 2017. All attendees ate on location, while community members who did not attend received the same food at home. We can presume that many people contracted *S. suis* infection without any clinical signs. Only approximately 40 individuals were ill. Some community members might have been exposed to the bacterium and were protected. Antibodies against *S. suis* have been detected in United States swine workers [25]. This needs to be elucidated further.

All patients were intravenously treated for bacterial meningitis with 2 g of ceftriaxone every 12 h for 14 days and 10 mg of dexamethasone every 6 h for 4 days, as per the Mangunsada Hospital protocol. Ceftriaxone is a third-generation cephalosporin that is recommended as the drug of choice for bacterial meningitis [6,21]. All patients survived and were discharged after the completion of antibiotic treatment.

This response to this outbreak was a good example of implementation of the “One Health” approach. While the Human Medicine Sector was able to implement a prompt and early response, veterinary and environmental investigation identified the source of pork for the festival, the presence of *S. suis* in tonsil tissues of slaughtered pigs, the condition of backyard pig stalls in both villages, and the sanitary measures applied in slaughterhouses.

Sixteen out of 150 (10.67%) tonsil tissue samples from slaughterhouses were determined to be positive for *S. suis* using PCR. Tonsil tissue is a sample of choice to detect the occurrence of *S. suis* in pigs [26]. The detection level varies from 5 to 40%, depending on the presence of clinical signs [26]. Our results, together with those of a previous study [9], indicate that *S. suis* seems to be enzootic in Bali; this possibility needs to be investigated. Biosecurity in small-scale pig farming needs to be promoted, and regular sampling in slaughterhouses needs to be conducted to improve sanitary measures. A massive campaign to avoid uncooked blood and pork and to promote healthier pig farming should be conducted to prevent human suffering.

5. Conclusions

We concluded that *S. suis* infections in humans can spread in cluster phenomena following the sharing high-risk food. The prompt and early response was paramount in case finding as well as the early treatment of patients to allow a favourable outcome of full recovery. In addition, the implementation of the “One Health” approach provides a comprehensive picture of improved management of *S. suis* infection in humans. The removal of high-risk food should be included in health promotion activities.

Policy and ethics

This retrospective study was approved by the Research Ethics Committee of the Faculty of Medicine, Udayana University of Bali, Indonesia (Number 231/UN.14.2/KEP/2018) on February 5th, 2018. As per the standard operation procedure of the Mangunsada Hospital of Badung District, cerebrospinal fluid (CSF) or blood specimens were collected following informed consent.

Conflicts of interest

None.

CRediT authorship contribution statement

N.M.A. Tarini: Conceptualization, Methodology, Writing – original draft. **N.M. Susilawathi:** Conceptualization, Data curation, Formal analysis, Writing – original draft. **A.A.R. Sudewi:** Conceptualization, Supervision, Writing – review & editing. **A. Soejitno:** Investigation, Data curation. **N.N.D. Fatmawati:** Methodology, Formal analysis. **I.P. B. Mayura:** Methodology, Formal analysis. **A.A.W. Lestari:** Methodology, Data curation. **G. Suputra:** Investigation, Data curation. **I.K. Subrata:** Investigation, Data curation. **C.I.S.D. Astiti:** Investigation, Data curation. **I.N.K. Besung:** Investigation, Data curation. **G.N. Mahardika:** Conceptualization, Supervision, Writing – review & editing.

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