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Case Report

Misidentification of *S. suis* as a Zoonotic Agent

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Abstract

BACKGROUND: *Streptococcus suis* is an emerging zoonotic pathogen. This bacterium commonly causes meningitis in human and is often associated with hearing and vestibular dysfunction. *S. suis* tends to be misidentified, leading to under-diagnosis.

CASE PRESENTATION: A previously healthy 50-year-old man was admitted to one of the district hospitals in Bali Province, Indonesia, due to meningitis. He had a history of consuming homemade raw pork product two days before the onset of illness. Streptococcus mitis was identified from the cerebrospinal fluid culture by using VITEX 2 COMPACT (Biomeriuex) with a 99% probability score. This patient had clinical symptoms and risk factor identical to *S. suis* infection. Therefore, we performed confirmation tests for the cerebrospinal fluid by PCR (using primer specific for *gdh* and *recN*) and sequencing of those PCR products. Both of the confirmation tests showed a positive result for *S. suis*.

CONCLUSION: There are few reports of *S. suis* infections in Indonesia, but we believe that the cases in Indonesia, especially Bali, are not uncommon. The under-reported cases are perhaps due to the difficulties in differentiating *S. suis* from other Streptococcus species by culture method, particularly *Streptococcus mitis*. Therefore, confirmation by PCR is necessary.

Introduction

Streptococcus suis (*S. suis*) is the main pathogen in pigs that can cause meningitis, endocarditis, and sepsis in humans [1], [2], [3]. *S. suis* infection is an emerging infectious disease whose incidence is reported to increase and causes public health problems [4], [5], [6]. There are two outbreaks of *S. suis* infection in China that affect more than 200 people with a mortality rate of almost 20%, which have since changed the views of experts on the threat of this pathogen to human health [1]. The first case of *S. suis* infection was reported in 1954 after an outbreak of meningitis, septicemia, and arthritis in piglets. The first case of *S. suis* meningitis in a human was reported in Denmark in 1968 [7], and it has occurred in Bali in 2014, which was followed by an increase in cases of infection in Bali [8].

S. suis is a gram-positive coccus, facultatively anaerobic, and based on Lancefield's classification it has a cell wall structure by group D *Streptococcus* [9]. These bacteria are often reported as *Streptococcus viridans* in their initial identification from CSF culture due to the similarity [10], [11].

Misidentification has an impact on the case finding and to control the transmission, which is highly

important in the effort to comprehensively manage *S. suis* as one of the zoonotic agents. The discussion of the following case is expected to enhance the understanding of the characteristics of *S. suis* and the possibility of identification errors that might occur.

Case Illustration

A 50-year-old male was brought to the emergency department in one of the regional hospitals in Bali with a decrease of consciousness accompanied by headache, fever, nausea, vomiting, and anorexia for 2 days before hospitalisation. He had a history of consuming homemade red *lawar* (a traditional Balinese food made by mixing chopped pork with fresh blood) three days before admission. He had no history of other acute infections. His past medical history was not significant.

On examination, his condition was confused with a Glasgow Coma Scale (GCS) E3V3M5, fever (38°C), and neck stiffness. Routine laboratory test showed leucocytosis (15.9 x 10^3/µL) with neutrophil predominant (93.6%) and thrombocytopenia (125 x 10^3/µL). A brain computed tomography scan (CT scan) demonstrated cerebral oedema. The Cerebrospinal fluid (CSF) had a turbid color, an increased leukocyte count (1080 cell/mm^3) predominating polymorphs (71%), an increased protein level (226 mg/dL) and a decreased glucose level (65 mg/dL) with a decreased CSF / serum glucose ratio (0.38). The CSF culture showed Gram-positive cocci, negative catalase, and colony on blood agar showed hemolytic alpha. Identification with VITEK 2 COMPACT (bioMériux) shows the results of *Streptococcus mitis (S. mitis)* with a probability of 99%.

The diagnosis of acute bacterial meningitis was established, and the patient was treated with 2 gram of ceftriaxone as empirical antibiotic at 12-hour intervals until the culture's result was done. After 3 days of treatment, the patient experienced hearing impairment. Based on those clinical features and history of consuming food containing raw pork products, *S. suis* meningitis was suspected. Furthermore, *S. mitis* as the culture result was known as a commensal bacteria and had never been reported as a cause of meningitis. We suspected there was a misidentification of the causative agent in this case. Hence, a confirmation examination was performed by PCR using the specific primers of the glutamate dehydrogenase (Gdh) and the recombination/repair protein (RecN) encoding gene in *S. suis*. The sequencing of those PCR products was also carried out to confirm whether the causative agent in these cases was *S. mitis* or *S. suis* [12], [13]. From the PCR results, it was found that the isolates showed the positive result in both amplified *S. suis* specific genes.

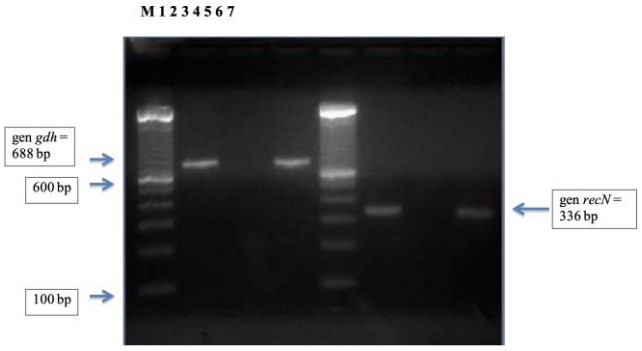

Figure 1: PCR gdh and recN gene detection. The amplicon was electrophoresed on 1 % agarose gel. The sample was positive for gdh gene (688bp) and recN gene (336bp). (M = marker 100 bp (Invitrogen); lane 1 = control positive for gdh gene; lane 2 = negative control; lane 3 = sample (gdh gene was positive); Lane 4 = Marker 100 bp (Invitrogen); Lane 5 = control positive for recN gene and lane 6 = sample (recN gene was positive)

The BLAST result of PCR product sequences showed 100% identity to *gdh* and 99% identity to *recN* of *S. suis* strains (CP020863.1). According to the performed confirmation tests, the patient was diagnosed with *S. suis* meningitis. After the patient had been hospitalized for 14 days, the patient was discharged from the hospital with the complications of hearing impairment.

Discussion

Regarding the case illustration above, our patient exhibits typical symptoms as acute bacterial meningitis including fever, headache, anorexia, hearing loss, and a history of consuming raw pork food products. *S. suis* can cause systemic infections in humans in the form of meningitis, sepsis, endocarditis, arthritis, endophthalmitis, uveitis, spondylodiscitis, ophthalmoplegia, and epidural abscess [7]. The most common manifestation of *S. suis* infection is meningitis that occurs in about two-thirds of patients [1], [2], [14]. One of the typical symptoms of *S. suis* meningitis is hearing impairment, or loss, which occurs in more than 50% of the cases [1], [2], [14]. Various risk factors associated with acquiring *S. suis* infection included the consumption of raw pork products, pig-related occupation, pigs, or pork exposure, alcohol drinking, skin injury especially due to exposure during pork processing, and underlying diseases contributing to immunocompromised conditions [2], [5].

Apparently, the incubation period of *S. suis* infection in humans varies depending on the route of transmission. A shorter incubation period occurs when infection happens through wounds on the skin which rapidly spread hematogenously, while the incubation

period is longer if infected through oral consumption [7], [15], [16]. In the case of outbreaks in China, the incubation period ranged from 3 hours to 14 days (median 2.2 days) [15]. *S. suis* infection often occurs in predominantly healthy adults, men with an average age of 51 years, but very rarely found in children [2], [16].

Microbiological examination plays an important role in establishing the diagnosis of meningitis, especially for identification of causative agents and antimicrobial sensitivity testing. The microbiological examinations of CSF fluid that were performed in this case — including the Gram staining, colony appearance on blood agar with alpha-hemolytic, and negative catalase test — matched the characteristics of *Streptococcus*. The result of bacterial identification using biochemical examination (VITEK 2 Compact) is *S. mitis*. However, the patient's history and clinical symptoms did not match with the bacterial identification result, *S. mitis*. This bacterium has never been reported as the causative agent of meningitis. Suspicion of other causative agents such as *S. suis* bacteria arose due to a history of contact with animals as hosts and hearing impairment that occur in the patient.

S. suis is a coccus Gram-positive bacterium, facultatively anaerobic, negative catalase, and forms alpha hemolysis in blood agar [17], [18]. If we review the results of the microbiological examination, it can be concluded that the bacteria have similarities with other Streptococcus bacteria such as *Streptococcus viridans*, *Streptococcus equid*, *Streptococcus mitis,* and other *Streptococcus* which often cause misidentification [4], [6], [11], [19]. In a study performed by Donsakul et al., from 1993 to 1999 (reported in 2003), 5 of the 8 cases were initially identified as *Streptococcus viridans* infections. The conventional biochemical examination has lower specificity than an examination of molecular biology [12].

In this case, PCR was performed using the specific primer to amplify two specific genes in *S. suis, gdh*, and *recN* [12], [13] and a positive result was shown, indicating that the bacteria isolated from the CSF is *S. suis*. The PCR method provides more specific result based on gene identification that encodes glutamate dehydrogenase (*gdh*) and the gene that encodes recombination, or repairs protein (*recN*) [11], [12], [20]. In 2015, Okwumabua et al. conducted a study that identified 306 *S. suis* isolates using PCR method through identification of the encoding gene *gdh*. The gene identification has been used in diagnosis of other bacterial infections and reported to have a very low mutation rate [12]. Identification of the *recN* gene is also an easy and accurate examination for *S. suis*. PCR based on the *recN* sequence is a better method for identification and detection of *S. suis* compared to *gdh* gene identification [11], because it has been shown to have a lower level of similarity at the species level and a higher divergence value at the subspecies level than other genes [21].

The diagnosis of *S. suis* meningitis is highly important, because it is one of the zoonotic diseases that can cause public health problems and potentially leads to an outbreak. It requires comprehensive prevention of the causative agents by breaking the chain of transmission through intermediary animals.

In conclusion, the low prevalence of *S. suis* meningitis cases reported in Indonesia, especially in Bali, does not indicate the actual condition of the cases. This could happen due to the misidentification of *S. suis* from other *Streptococcus sp.* from conventional cultural method. The choice of a more accurate type of examination such as PCR is highly important to consider as a confirmation test of CSF examination, especially in cases with clinical symptoms that were suspected as *S. suis* infection.

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