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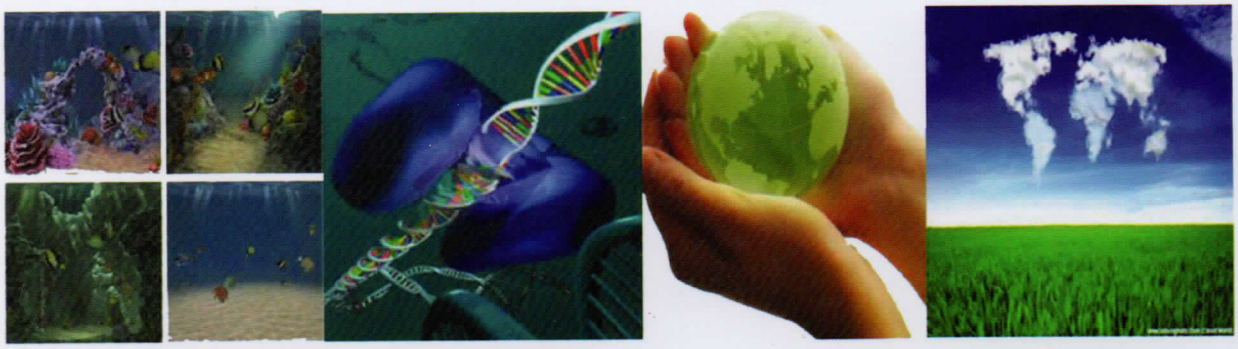

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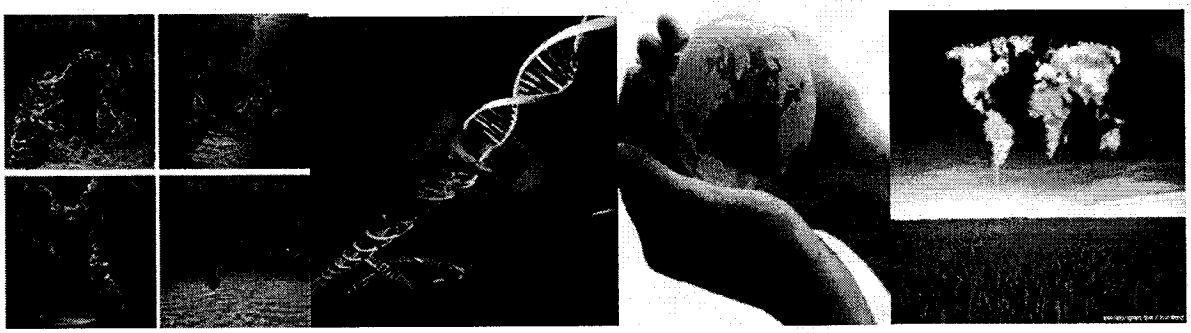

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Detection of OMP31 Gene Encoding Brucella Suis's Local Isolates OMP 31kDa Protein with Polymerase Chain Reaction

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ABSTRACT

Swine brucellosisi is a zoonosis affecting pigs, caused by the bacterium Brucella suis, and an economically important cause of reproductive losses in pigs. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. In pigs, B. suis occurs in the fetus, placenta, fetal fluids and vaginal discharges after an abortion or stillbirth. This study used aborted fetus as sample. For the isolation of Brucella suis, material was inoculated on sterile brucella broth and then on plates of brucella selective agar media. The isolates suspected for Brucella suis were subjected to Gram staining and Stamp's modified Ziehl-Neelsen (MZN) staining for confirming the purity of cultures and morphological characters. Pure suspected Brucella isolates, were analysed for their biochemical profiles for the differentiation of Brucella species on the basis of biochemical tests. The results showed and assuring that the isolates obtained were B. suis. PCR assays resulted in the amplification of 723-bp bands from the targeted omp31 genes of the Brucella suis as a local isolates. Well-established immuno dominant outer membrane protein (31 kDa omp) can be a milestone for the development of effective diagnostic to eradicate the disease.

KEYWORDS: B. suis, omp31, PCR, 723 bp

INTRODUCTION

Brucellosis is a zoonotic disease that could affecting pigs, caused by *Brucella suis*. Other species could be infected with *B. suis* after direct contact with genitals or body fluid from infected pigs and it can be transmitted from one human to another. Porcine brucellosis can be difficult to diagnose with isolation and identificaton technique and it takes time to obtain the results. Therefore, serology was generally considered to be more reliable technique for identifying specific antibody against Brucella sp. Currently, a direct serological test for porcine brucellosis detection is not yet available [1,2,3,4,5].

The Brucella cell membrane comprised of 3 layers; the cytoplasmic membrane, the peripheral cytoplasmic membrane, and the outer membrane [6]. Protein in the outer membrane called as outer membrane proteins (OMPs) [7,8]. OMPs of Brucella showed very strong immunogenicity, which might be associated with the survival of Brucella in macrophages [9]. In Brucella suis, one of specific protein is encoded by OMP 31 gene.

A recent study indicated that proteins in the OMP25/OMP31 family were highly conserved as an immunodominant antigen and are related to the Brucella virulence [10]. These characteristics support Omp31 as a promising subunit for detection kit and vaccine candidate against brucellosis.

To obtain a new diagnostic tools, initial steps including isolation, extraction and purification are necessary. The objective of this research was detection of OMP31 gene encoding Brucella suis's local isolates by polymerase chain reaction (PCR). Results obtained from this research is expected to be used as swine brucellosis diagnosis.

MATERIALS AND METHODS

Sample collection

Samples collected assepticaly from fetus and sow just after abortion (fetal membrane, fetal stomach content, and vaginal swabs) were subjected to isolation of bacteria and its molecular characterization through PCR.

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Bacteriological isolation and identification of B. suis

For the isolation of Brucella suis, material was inoculated on sterile brucella broth and then on plates of brucella selective agar media and incubated at 37°C for 48 h. The plates were observed at every 24 h for the development of growth. After the growth, the colonies suspected for Brucella on the basis cultural characteristics [11] were picked up and streaked to another Brucella selective agar plates and incubated at 37°C for 2 days to obtain pure culture.

Morphological Characterization of Isolates

The isolates suspected for Brucella suis were subjected to Gram staining and Stamp's modified Ziehl-Neelsen (MZN) staining [12] for checking the purity of cultures and morphological characters. Stamp modified Ziehl-Neelsen staining method was performed with 0.4% basic fuchsin solution, followed by rapid decolouration with 0.5% acetic acid solution, and counterstaining with 1% methylene blue solution. The smears were examined microscopically with an oil-immersion objective lens (×100).

Biochemical confirmation of isolate

Pure suspected Brucella isolates, were analysed for their biochemical profiles for the differentiation of Brucella species on the basis of biochemical tests, namely, catalase, oxidase, urea hydrolysis, nitrate reduction tests, indole production, and citrate utilization as per the standard methods [11,12].

Molecular Characterization of Brucella suis isolates

PCR primers used to detect DNA sequence of the gene coding for the outer membrane protein-31 (OMP-31) reported for Brucella in GenBank database located at NCBI (Leal-Klevezas et al., 1995). The forward primer sequence were forward 5'ATG AAG TCC GTA ATT TTG GCG TCC3' and reverse 5'TTA GAA CTT GTA GGT CAG ACC GAC 3'. For molecular confirmation of these isolates, amplification of OMP-31 genes was performed using PCR (Qiagen). DNA was extracted from the brucella isolates by using the QIAMP DNA minikit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions.

Reaction compositing using Top Taq Master Mix is Master mix (intron)12.5 µl, DW 0.5 µl, Primer EAE1 1 µl, Primer EAE2 1 µl, template 5 µl (total 20 µl). Gentle vortex and spin down. The thermal cycling conditions were as follows: an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min, with final extension at 72°C for 5 min.

PCR product were assessed by electrophoresis in a 2% agarose gel and then stained with ethidium bromide (0.5 µg/mL). Gels were photographed under ultraviolet light [13].

RESULTS AND DISCUSSION

All the aborted materials collected from the cases of abortions were inoculated on Brucella selective agar plates and the isolates producing characteristic, very small, glistening and smooth, round, and pin-point colonies. Microscopic examination of Gram-stained cultures revealed small Gram-negative coccobacilli and, on modified Ziehl-Neelsen (MZN) staining, organisms stained red against a blue background. These isolates were further assessed for the biochemical characters and like previous research, the isolates were found positive for catalase, oxidase, urea hydrolysis and nitrate reduction tests and negative for indole production, and citrate utilization. All the isolates revealed morphological characters similar to previous findings [11] with biochemical tests in concurrence with the findings of other studies [11,12].

To ensure that the isolates were found is B. suis, urea hydrolysis test just take 20 minute, while another species need 1 - 2 hours. They could grow in the presence of thionin at standard concentrations and without needing supplementary CO2 [14]. The only unequivocal and the most reliable method for the diagnostic of animal brucellosis is based on the isolation of Brucella spp. Therefore, the isolation of B. melitensis and other on appropriate culture media is recommended for an accurate diagnosis [15].

PCR assays resulted in the amplification of 723-bp bands from the targeted omp31 genes of the Brucella suis as a local isolated. PCR analyses were repeated twice. The results are given in Figure 1. The accuracy and reliability of PCR data obtained from the B. suis were confirmed by DNA sequence analysis.

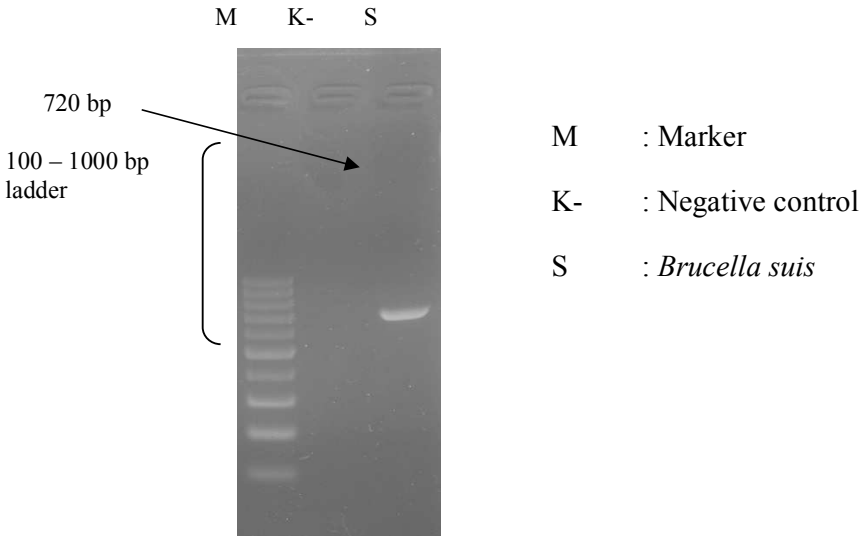

Figure 1. Amplification of omp31 gene of *Brucella suis*

The PCR is a reliable and fast tool for direct detection of *B.suis* in clinical samples. For the diagnosis of brucellosis it has been already established and shown its usefulness. But till now and to the knowledge of the authors there is no description of a single probe PCR that is able to detect all practically relevant *B. Suis*. The assay was tested 100% specific for *B. suis* and negative for other *Brucella* spp. and closely related non-*Brucella* species.

CONCLUSION

Brucella suis was mainly responsible for the brucellosis in swine and also for the transmission of infection to human being. For the control of the *Brucella suis,* effective diagnosis is required and all these can only be decided after epidemiological studies including isolation of etiological agents from the clinical cases. A country like Indonesia, with large swine population being reared in the close vicinity of human is always on the edge of *Brucella* zoonoses. Well-established immuno dominant outer membrane protein (31 kDa omp) can be a milestone for the development of effective diagnostic to eradicate the disease.

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COMPETING INTEREST

The authors declare that they have no competing interest.

AUTHORS CONTRIBUTION

WMY, EKS, DH, WT carried out the main research works, DH performed the statistical analysis and analysed the main data in the experiments. EKS helped to draft the manuscript. All authors read and approved the final manuscript.

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