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DETECTION OF *B. ABORTUS* S19 ON UTERINE RAT (*RATTUS NORVEGICUS*) WITH IMMUNOHISTOCHEMICAL METHOD

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ABSTRACT: Objective: Brucellosis in humans is the most common zoonotic infection in the world. Brucellosis can be prevented by vaccination but no effective vaccines are known for prevention of human brucellosis and minimal impact of animal brucellosis. Outer membrane proteins (OMPs) of *Brucella sp.* have been the focus of vaccine development and the diagnosis of brucellosis. **Methods:** The pathogenesis study of brucellosis used rat by intraperitoneal injection. Profile OMP detected by SDS-PAGE. The antibodies took from rabbit and the immune response did with western blotting, dot blot and immuno histochemistry. **Results:** *B. abortus* S19 cell to the rat show the presence of inflammatory cells and bleeding in the uterine rat. The 36 kDa OMP could isolated from *B. abortus* S19. Rabbit antibody to 36 kDa OMP can detect *B. abortus* of this histological of the uterus. OMP 36 kDa *B. abortus* S19 is antigenic characterized by its ability to stimulate antibodies in the host's body (*Oryctolagus cuniculus*). **Conclusion:** *B. abortus* S19 can be detected in histopathology of rats uterus shown by interaction of antigen and antibody by using immunohistochemical method.

INTRODUCTION: The genus Brucella consisted of six species, that is *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*. *B. melitensis*, *B. suis* and *B. abortus* are considered the most pathogenic species for humans [1, 2]. The cow is the preferential host for *B. abortus*. The most important animal symptoms of *B. abortus* infection are miscarriage. Bacterial growth in the pregnant uterus is supported by erythritol, which is a fetus product concentrated in chorion and cotyledon [3]. Clinical manifestations in humans are varied and non-specific, usually acute or subacute fever accompanied by malaise, anorexia, weight loss, headache, artalgia and lethargy [4].

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Irregular hepatomegaly is followed by splenomegaly, arthritis, swelling of the scrotum, neck stiffness and lymphadenopathy [5]. The true incidence of human brucellosis is not known, but WHO reported 500,000 cases a year from around the world [5]. *B. abortus* is more common in America and northern Europe. Initially human brucellosis occurs in endemic areas of France, Israel and most of Latin America and is now found in Asia [6].

The prevalence of *B. abortus* in 2014 range between 1% and 2% in Thailand and Indonesia, and 4% - 5% in Malaysia and Myanmar [7]. Brucellosis has been reported from all provinces in Indonesia except Lombok, Bali, Subawa, Kalimantan, West Sumatra, Riau, Jambi and Riau Islands in 2010 [8]. Brucellosis can be prevented by vaccination but no effective vaccines are known for prevention of human brucellosis and minimal impact of animal brucellosis [9, 10]. All available brucellosis vaccine is a live attenuated Brucella. Although effective, these vaccines have

disadvantages: causes of disease in humans, confusing the diagnosis, cause abortion to pregnant animals and spread of disease due to vaccine strains [11]. *B. abortus* S19, live attenuated vaccine, stimulates immune response broadly, including response to $CD8^+$, this vaccine is effective against intracellular bacteria [11].

Outer Membrane Protein (OMP) *Brucella* sp. very important because it is a potent antigen for the make of vaccines and diagnostic reagents [12]. Brucella OMP was first identified in 1980 and classified by molecular mass in SDS-PAGE as group 1 (94 or 88 kDa), group 2 (36 - 38 kDa) and group 3 (31 - 34 and 25 - 27 kDa) [13]. Brucella OMP major gene products are Omp2a and Omp2b for 36-38 kDa porin protein, Omp25 for 25 - 27 kDa OMP and Omp31 for 31 - 34 kDa OMP [14]. Omp2b protein was detected only in *B. abortus* biovar 1, but omp2a protein was undetected [14].

METHOD:

***B. abortus* S19 Culture:** *B. abortus* S19 was grown on Trypto Soy Broth (TSB) at 37 ºC for 3 days, then continued on Trypto Soy Agar (TSA) at 37 ºC until growing yellowish colony like honey, small and convex surface.

Isolation of OMP *B. abortus* S19: Outer membrane proteins (OMPs) *B. abortus* S19 were extracted following method described by Munir15. The harvested cells (0.5 g) were then suspended with 4 ml of 10 Mm Hepes buffer (pH 7.4) and sonicated at 100% amplitudes at 0.5 min intervals for eight minutes. The cell debris was removed by centrifuge at 1700×g. The supernatant was centrifuged at 15,000 rpm for 30 min at 4 °C and pellets which contained total membrane protein were suspended in 2% sodium lauryl sarcosinate (sarkosyl) detergent and stored at 22 °C for 60 min to dissolve inner membrane proteins. Sarkosyl insoluble fraction was then sedimented by centrifugation at 15,000 rpm for 30 min at 4 °C. The pellets are washed twice with PBS and stored at -20 °C.

OMP Protein Profile with SDS-PAGE: OMP protein profile performed electrophoresis with SDS-PAGE to see the OMP protein bands in *B. abortus* S19. There are several steps of electrophoresis with SDS PAGE, gel preparation, sample injection, gel staining and molecular weight determination. The protein sample containing 3 µL protein added with 12 µl Tris - HCl and 20 µL RSB (volume ratio 1:1), heated at 98 ºC for 5 min, after that loaded 30 µL protein in wells. The gel was running at 200 V in running gel buffer until the blue color is ± 0.5 cm from the bottom plate gel. The gel was stained for 20 min with coomassie brilliant blue. Destainning was done until the gel bands become clear with continuous shaking.

Isolation of Molecular Weight 36 kDa OMP *B. abortus* S19 by Electro - Elution Method: The gel contained with 36 kDa OMP *B. abortus* S19 was loaded into the cellophane contained phosphate buffer 0.2 M. Electro - elution was performed by using a electrophoresis apparatus contained phosphate buffers 0.1 M at 30 V, 90 mA overnight. The cellophane is added cold EtOH and incubated -20 ºC, overnight. The precipitate was loaded TrisCl 0.5 µL buffer and stored at -20 °C.

Preparation of *B. abortus* S19 Cell and Injected of Experimental Animals *Rattus norvegicus*: All bacterial cell antigen preparations were harvested from growth medium and heated for 30 min at 63°C [16]. Bacterial cells with concentrations of 10^9 organisms/mL in PBS with optical density measurements at 600 nm in a spectrophotometer (OD = 0.165 for 10^9 cells per mL for 1cm light path) and stored at 4 °C.

White rats (*Rattus norvegicus*) were divide into 2 groups and injected by antigens, each group contained 5 rats. Group A was injected intraperitoneal with PBS. Group B was injected intraperitoneal with109 organism / mL cell *B. abortus* S19.

Production of Polyclonal Antibodies Whole OMP and 36 kDa OMP *B. abortus* S19: Rabbits (*Oryctolagus cunniculus*, male, 2 months old, body weight 2.5 - 3.0 kg) were divided into two groups, each group contained two rabbits and subcuntaneous immunized by injecting the antigens. Group A was immunized with whole OMP *B. abortus* S19. Group B was immunized with 36 kDa *B. abortus* S19. The antigen in the syringe was emulsified Freud's Complete Adjuvant (CFA). Booster injection was performed with Incomplete Freud's Adjuvant (IFA) with same

dose. Immunization was performed within 70 days according to Custom Rabbit Polyclonal Antibody Production Protocols (Thermo Fisher Scientific).

Rabbit (*Oryctolagus cuniculus*) Antibody Purification by SAS 50% Method: Rabbits (*Oryctolagus cuniculus*) sera (IgG *i.e.* whole OMP and OMP 36 kDa *B. abortus* S19) 200 µL were added with 50% ammonium sulfate (homogenized with vortex 5 minutes and centrifuged 10.000 rpm for 10 min of temperature 4 ºC). The supernatant was added with 50% ammonium sulphate (homogenized with vortex 5 minutes and centrifuged 10,000 rpm for 10 minutes at 4 °C). The first and second precipitate added phosphate buffer 0.2 M, pH 8 and put into a cellophane bag. The bag was stirred in phosphate buffer 0.1 M for 15 hours, 4 ºC. The supernatant was added with ethanol (1:1) and stored at 4 ºC for 24 h (sentrifuged 10.000 rpm for 10 minutes, 4 ºC. The precipitate is dried and dissolved in tris-HCl 20 Mm pH 6.8. Stored -20 °C.

Test of Polyclonal Antibody Whole OMP and 36 kDa OMP Immunized in Rabbit (*Oryctolagus cuniculus*): Test of polyclonal antibody titer to whole OMP and 36 kDa OMP *B. abortus* S19 in rabbit by ELISA method. Antigens were used whole OMP and 36 kDa OMP *B. abortus* S19. The antigen 1 µL were added into coating buffer (1:9) in well plate. The eppendorf was wrapped in aluminium foil and incubated overnight at a 4 °C.

It was washed with PBS-Tween 20 three times for three minutes. Primary antibody *i.e.* serum (IgG) diluted (1:500 in PBS-BSA) against whole OMP and 36 kDa OMP were added and incubated for two hours at room temperature. It was washed in PBS - Tween three times for three minutes. Secondary antibody, anti-rabbit IgG AP labelled, diluted (1:500) in TBS were added and incubated for an hour at room temperature. It was washed in PBS Tween three times for three minutes. The substrate (pNPP) in 10% diethanolamine (100 µL/well) was added and incubated for 30 minutes at room temperature. The reaction was stopped by 100 µL 3N NaOH .

Immunogenicity Test of *B. abortus* S19 Cell, Whole OMP and 36 kDa OMP by Western Blot Technique: The stages of the Western Blot work consist of several steps, namely: protein preparation (*B. abortus* S19 cell, whole OMP and 36 kDa OMP *B. abortus* S19), preparation of SDS-PAGE, membrane preparation and protein transfer in membrane and incubation with primary antibody IgG Anti-rat *B. abortus* S19 cell, IgG Anti-rabbit whole OMP and IgG Anti-rabbit 36 kDa OMP. OMPs fractionated by SDS-PAGE were transferred on to NC membrane (nitro - cellulose) using transblott apparatus at 90 V at 4 ºC for 12 h in chilled transfer buffer. After transfer, the membranes were placed in PBS-T Skim Milk 5% for 1 h with continuous shaking in separate plastic boxes. Primary antibody *i.e.* serum (IgG) diluted (1:200 in 5% TBS-T skim) against *B. abortus* S19 cell, whole OMP and 36 kDa OMP were added and the membranes were incubated at 4 ºC overnight. The membranes were washed three times with TBS for 5 minutes. Secondary antibody IgG *i.e.* anti-rat IgG and anti-rabbit IgG concentration of 1:2500 in TBS were added and incubated at room temperature for an hour. The membranes were washed four times with PBS-T for 5 minutes and placed in Western Blue substrate. The reaction was stopped by rinsing in aquadest.

Immunogenicity Test of *B. abortus* S19 Cell, Whole OMP and 36 kDa OMP by Dot Blotting: The protein antigen *B. abortus* S19 cell, whole OMP and OMP 36 kDa OMP were dissolved in PBS contained NaN3 (1 mL Na-azide 1% add 9 mL PBS) on the nitrocellulose membrane. The membranes were inserted to the dot blotter apparatus and the protein antigens with volume 50µL were added. Further blocking buffer was carried out for 1 h and washed with 0.05% PBS-Tween 20 three times for three minutes. Primary antibody *i.e.* serum (IgG) diluted (1:100 - 1:250 in 1% TBS-T skim milk) against *B. abortus* S19 cell, whole OMP and 36 kDa OMP were added and incubated for an hour. The membranes were washed with 0.05% PBS-Tween 20 three times for three minutes. Secondary antibody IgG *i.e.* anti-rat IgG and anti-rabbit IgG (conjugate alkalin-fosphatase or SA-HRP) were added and incubated for an hour. The membranes were washed with 0.05% PBS - Tween 20 three times for three minutes and placed in Western Blue substrate.

Rattusnorvegicus Uterus Preparation for Histopathology and Expression of 36 kDa OMP *B. abortus* S19: Uterus was fixed with 10%

Buffered formalin and processed by dehydration, rehydration, embedding and uterine tissue cutting.

Preparation of Uterine Rat Paraffin Blocks (*Rattus norvegicus*): Fixation and treatment of uterine tissue of rat injected with *B. abortus* S19 cell and uterine tissue of rat injected with PBS using paraffin. Uterine tissue were fixed in formalin 10 for 8 h not more than 24 h at room temperature and washed with tap water for 1 h.

Cutting of Uterine Rat (*Rattus norvegicus*) on Paraffin Block: The uterine tissue were cut in thicknesses 4 - 5 mm with microtom and floated on top of a water bath containing distilled water at 40°C. The uterine tissue were transferred to the slide section and dried overnight.

Deparafination and Dehydration of the Uterine Tissue Slide: Deparafination and dehydration of the uterine tissues was performed using standard methods. It is necessary to remove the embedding material before immunostaining. The uterine tissue slides were perform through deparafination in xylol, storey ethanol (98%, 95%, 90%, 80%, 70%) and washed with distilled water for each 5 minutes, respectively.

Histopathology of Rat Uterus by Hematoxylin-Eosin Stainning Method: Hematoxylin were added for 3 - 5 min and washed with running water for 5 min until the blue color runs out or decreases and differentiated in alcoholic acid 1% (HCl 1%) for 5 min. Then washed with running water and dipped in an alkaline solution (ammonia) and then washed with flowing flow. It was then stained with Eosin Y for 10 min and continued by washing with running water for 1 - 5 min. Tissue slides were mounted with entellan and covered with glass cover.

Expression of 36 kDa OMP in Rat Uterus (*Rattus norvegicus*) by Immunehistochemistry Methods: Expression of 36 kDa OMP in rat uterus (*Rattus norvegicus*) by immunohistochemistry methods is performed through deparafination. Tissue slides were washed with PBS for 5 min. Hydrogen peroxide (H_2O_2) were added and incubated for 10 - 15 min. Tissue slides were washed 4 times in PBS. Super Block (blue cap) were added and incubated for 5 min at room temperature. The tissue slides were washed once in the buffer. Primary antibody *i.e.* serum (IgG) against *B. abortus* S19 whole bacteria was added and incubated for overnight at room temperature. Tissue slides were washed 4 times in buffer. UltraTek Anti-Polyvalent (yellow cover) were added and incubated for 10 min at room temperature and washed 4 times in buffer. UltraTek Horseradish Peroxidase (red cap) were added and incubated for 10 min at room temperature. Tissue slides were washed 4 times in buffer and added 4 drops (200 μL) DAB chromogen to DAB substrate and incubated for 5 - 15 min. Tissue slides were counter stain with Hemotoxylin Mayer for 10 min and were washed distilled water and dried. Tissue slides were mounted with entellan and covered with glass cover.

RESULT:

Profile of OMP *B. abortus* S19 and Western Blotting Results: Based on **Fig. 2** *B. abortus* S19 exhibited four protein bands. These band included protein with molecular masses 36 kDa, 48 kDa, 63 kDa and 75 kDa, while the protein bands 36 kDa seemed to have the highest concentration than the others proteins. This protein is a protein that will be used as a target protein used for the determination of polyclonal antibodies.

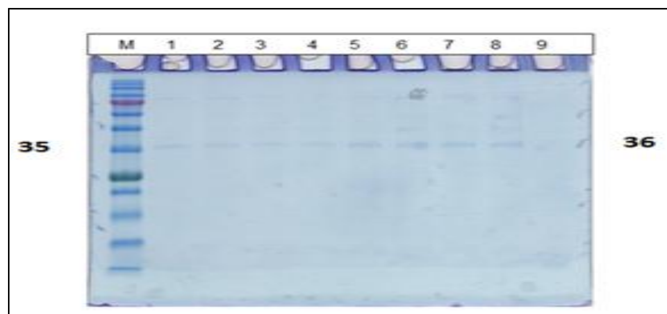

FIG. 1A: PROTEIN PROFILE OF CRUDE OMP *B. ABORTUS* S19. M: Marker protein, 1 dan 3: LBA 0, 5 μL, 2 dan 4: TSA 0. 5 uL. 5 dan 7: LBA 1 uL and. 6 dan 8: TSA 1 uL

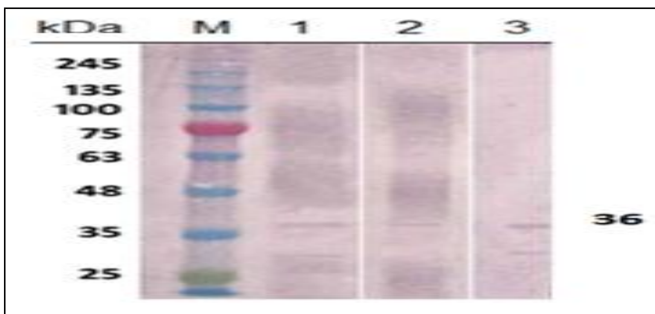

FIG. 1B: PROTEIN PROFILE OF WESTERN BLOTTING *B. ABORTUS* S19. M: Marker proein 1. Crude OMP, 2 Whole cell bacteria and, 3 Purified OMP

Immunogenicity Test of *B. abortus* S19 Cell, Whole OMP and OMP 36 kDa by Dot Blotting: The result of dot blot method obtained change colors on a nitrocellulose membrane showed specific binding reaction between proteins antigen and antibodies from blood sera dipegted in **Fig. 2**.

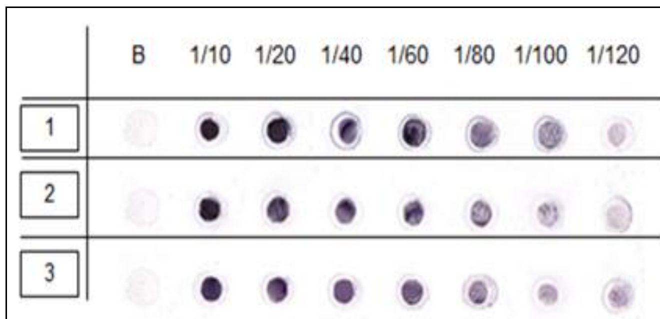

FIG. 2: RESULTS OF IMMUNOGENICITY TEST DOT BLOTTING WITH DIFFERENT ANTIGEN WITH DILUTION OF PRIMARY ANTIBODY'
1: Antigen against serum rabbit antibody (IgG) whole OMP *B. abortus* S19, 2: Antigen against serum rabbit antibody (IgG) OMP 36 kDa *B. abortus* S19 and, 3: Antigen against serum rat antibody *B. abortus* S19 cell

Histopathology Examination of Uterine Rat by Hematoxylin-eosin Stainning: Black arrows show inflammatory cells and bleeding in uterine cells. A. Rat group A (without injection *B. abortus* S19 and B. Rat group B (at injection with *B. abortus* S19). Histologically, there is a difference shown between group A and group B. **Fig. 3A** below shows the presence of inflammatory cells and bleeding in the uterine cells (with black arrows) in Group B, while the rat uterus in group A is not shows the presence of inflammatory cells and bleeding in the uterine cells.

Examination of 36 kDa OMP Protein Expression in Rat Uterus (*Rattus norvegicus*) by IHC: In this study, IHC on rat uterine tissue (*Rattus norvegicus*), the rat were injected with *B. abortus* S19 cell with primary antibodies (IgG) obtained by immunization of rabbits (*Oryctolagus cuniculus*) subcutaneously using antigen 36 kDa OMP *B. abortus* S19. IHC is a qualitative test, but can be quantitative by using software. The results obtained from this examination can be seen in **Fig. 3B** group A IHC images show a difference when compared to group B.

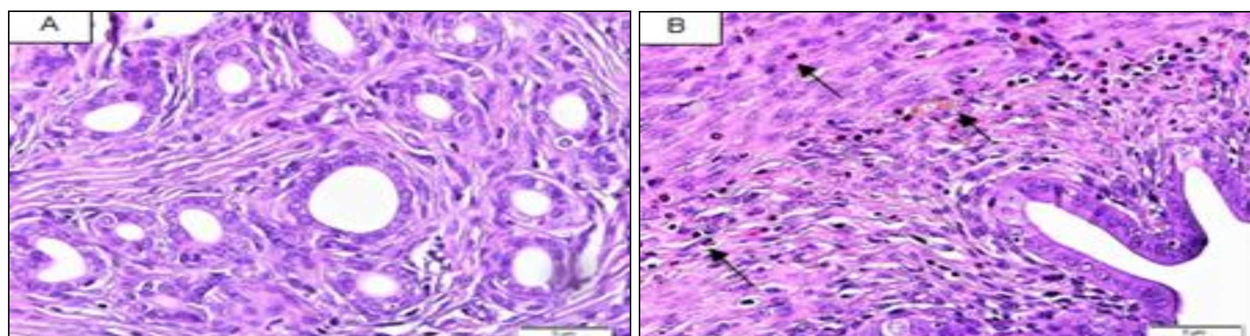

FIG. 3: RESULTS OF HISTOPATHOLOGICAL EXAMINATION OF RAT (HE, 400x, CROSS SECTION)

In group B, the brown color was strong enough qualitatively compared to group A. The percentage of DAB / nuclear in group B was 41.8% while in group A only 1.2%, based on software. The percent value between DAB / nuclear indicates the amount of bonding antigen present with antibodies. So the high percentage value, the more bonds that occur between antigens with antibodies.

Fig. 4 shows an expression of 36 kD OMP in a group B uterine tissue with cross section (100x) by IHC. Expression of 36 kDa OMP antigens can be detected in both uterine and inflammatory cells (indicated by black arrows). This is visualized by the presence of brownish color, indicating a bond between the 36 kDa OMP antigens with 36 kDa OMP antibodies in rat tissue.

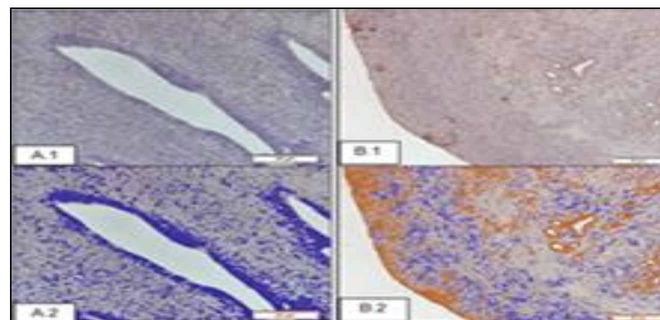

FIG. 4A: OMP EXPRESSION OF 36 kDa ON THE UTERINE RAT (IHC, 100x, CROSS). A1 IHC Rat group A (inner edge). A2 Immunoratio rat group A. B1 IHC rat group B (outer edge) B2 Immunoratio rat group B

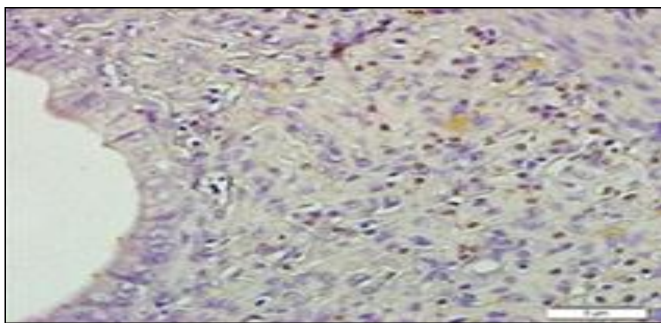

FIG. 4B: POSITIVE IMMUNOREACTIVITY TO THE ANTI-OMP 36 kDa *B. ABORTUS* S19 OF MACRO-PHAGE IN UTERINE TISSUE (IHC, 400x). The arrows show an expression of 36 kDa OMP in uterine cells and inflammatory cells. The presence of a bond between the 36 kDa OMP antigen with 36 kDa OMP antibody is visualized by brownish color.

DISCUSSION: OMPs isolation in this study was based on Munir method [15] using sarkosyl and sonication, this procedure is useful to avoid the enzymatic treatment that can change the OMP structure. However, in other studies, OMP isolation performed by the n-octylglucoside (NOG) method gave a larger protein pattern, especially the major protein when compared to OMP isolated by using sarcosyl or SDS [17]. Further research is needed on the comparison of OMP isolation procedure using sarcosyl and NOG. Several Brucella immunogenic antigens have been identified in the outer membrane of this pathogen [18]. OMP is immunogenic because it produces a high immune response [15].

In this study, protein bands of 48 kDa, 63 kDa and 75 kDa were weakly stained, while the protein bands 36 kDa seemed to have the highest concentration than the others proteins. This protein is a protein that will be used as a target protein used for the determination of polyclonal antibodies. The first OMP Brucella identification in 1980 with detergent extraction, the 36 kDa molecular weight was included in group two [19]. Brucella OMP with a molecular weight of 36 kDa is immunogenic / antigenic, in accordance with the requirements that major *B. abortus* OMPs of 36 - 38 kDa and 25-27 kDa are antigenic proteins [14]. They are also called group 2 porin proteins and group 3 proteins respectively. The gene products of Brucella major OMPs have been designated as Omp2a and Omp2b for the 36-38 kDa porin proteins, Omp25 for the 25 - 27 kDa Omp, and Omp31 for the 31 - 34 kDa OMP [14]. The identification of Brucella protective antigens shows that Omp2b is the priority antigen for designing the recombinant protein vaccine [14]. In addition, the results of research conducted by Sung *et al.*, [24] show that Omp2b is a lipopolysaccharide-free protein for developing diagnostic tests.

B. abortus S19 has molecular weight that varies 89.0, 73.0, 53.7, 49.0, 38.0, 27.0, 22.3, and 17.7 kDa [15]. This difference is probably due to environmental differences that result in differences in gene expression affecting protein synthesis and then affect virulence of bacteria. The Omp2b protein (36 kDa OMP) has the potential to induce immune response of B cells and cells and possibly a candidate sub vaccine unit against brucellosis [14]. Aulanni'am (2017) [21] study has been successful in predicting epitopes of OMP 36 kDa *B. abortus* of local isolates that have immunogenic characteristics in its ability to bind to MHC I, MHC II and B cells. The ELISA results showed that antibody against whole OMP and 36 kDa OMP *B. abortus* S19 were reached the peak at fourth bleeding (7 weeks) or a week after second IFA.

The absorbance value whole OMP was 0.104 and 36 kDa OMP was 0.102. This is consistent with Tabynov (2014) [22] is suggesting that a second exposure to the same immunogen would lead to the addition of a striking immune response in the form of emerging immonocompetent cells and antibodies. Therefore, the rabbit serum taken on the fourth bleeding (1st week after the 2nd IFA) is used as the primary antibody by IHC rat uterus.

The result immunogenicity test **Fig. 1B** shows of expression of antigen-antibody (whole cell proteins against whole cell antibodies, total OMP proteins against total OMP antibodies and 36 kDa OMP protein against 36 kDa OMP antibodies) reaction of *B. abortus* S19. The dominant band appears on the NC membrane show a molecular weight 36 kDa in all protein. Immunoblotting assays in cows immunized with *B. abortus* S19 also showed an antigen-fighting reaction against antibodies in proteins with a molecular weight of 36 kDa [24]. Antigenic proteins are characterized by their ability to stimulate antibodies in the host's body [23]. In accordance with this statement indicates that the 36 kDa protein has antigenic properties because it can cause antibodies on the host's body.

In this study, **Fig. 2** shows the titer at dilution 1/120 still can be seen any reaction between antigen with antibody. This result also illustrates that immunization proceeded well and confirmed that *B. abortus* S19 had an immunogenic antigen / protein that released the homologous antibody and was able to react with OMP 36 kDa. This result also illustrates that immunization proceeded well and confirmed that *B. abortus* S19 had an immunogenic antigen / protein that released the homologous antibody and was able to react with OMP 36 kDa.

Results of Histological Examination of Rat Uterus with Hematoxylin Eosin Staining: Histologically, there is a difference shown between group A and group B. **Fig. 3B** below shows the presence of inflammatory cells and bleeding in the uterine cells (with black arrows) in Group A, while the rat uterus in group B is not shows the presence of inflammatory cells and bleeding in the uterine cells. Similar observations were found in dogs and cows that were naturally infected with *Brucella* spp. and cattle infected with *B. melitensis* [24].

Examination of 36 kDa OMP Protein Expression in Rat Uterus (*Rattus norvegicus*) by IHC: Some conditions that must be met from the IHC method is that the active ingredient must be able to form antibodies specific to the active ingredient when injected into a second host different from the host from which the active ingredient originated. The active ingredient must also accumulate in sufficient quantities within the cell or tissue so that it can be bonded by specific antibodies and may be visualized. This diaminobenzidine (DAB) chromogen contains H_2O_2 peroxide as a marking substance that will form a complex with peroxidase enzyme in the SA-HRP (Strepvidinhorseradid peroxidase) complex. Complex formed from DAB chromogen will form a dark brown color.

In this study, IHC on rat uterine tissue (*Rattus norvegicus*), the rat were injected with whole *B. abortus* S19 bacteria with primary antibodies (IgG) obtained by immunization of rabbits (*Oryctolagus cuniculus*) subcutaneously using antigen 36 kDa OMP *B. abortus* S19. IHC is a qualitative test, but can be quantitative by using software. The results obtained from this examination can be seen in **Fig. 4A** group A IHC images show a difference when compared to group B. In group A, the brown color was strong enough qualitatively compared to group B. The percentage of DAB / nuclear in group A was 41.8% while in group B only 1.2%, based on software. The percent value between DAB/nuclear indicates the amount of bonding antigen present with antibodies. So the high percentage value, the more bonds that occur between antigens with antibodies. IHC techniques have documented that Brucella antigens are intensely stained within the cytoplasm of phagocytic cells. The present study observed **Fig. 4B** that an expression of 36 kD OMP in a group A uterine tissue with cross section (100x) by IHC. Expression of 36 kDa OMP antigens can be detected in inflammatory cells (indicated by black arrows). This is visualized by the presence of brownish color, indicating a bond between the 36 kDa OMP antigens with 36 kDa OMP antibodies in rat tissue.

Similarly, in other study, Brucella antigens in particular stained in the cytoplasm of macrophages and neutrophils located in the intralobular interstitium, and in the ductal and alveolar lumina in the mammary glands of 3 cows with rare extracellular positivity [25]. *Brucella* sp. is capable of surviving and replicating in epithelial cells, as in phagocytic leukocytes [26]. Accordingly, in this study **Fig. 4B** Expression of 36 kDa OMP antigens can be detected in uterine cells that show intense positivity.

CONCLUSION: This research has shown that 36 kDa OMP *B. abortus* S19 antigen in the uterine tissue of infected rat with *B. abortus* S19 cells can be recognized with antibodies obtained from immunization in rabbits using 36 kDa OMP *B. abortus* S19 antigen.OMP 36 kDa *B. abortus* S19 is antigenic / immunogenic characterized by its ability to stimulate antibodies in the host body (*Oryctolagus cuniculus*). Inflammation of the uterine tissue originating from rats injected with *B. abortus* S19 cell. There is expression of OMP 36 kDa *B. abortus* S19 in rat tissue (*Rattus norvegicus*).OMP 36 kDa *B. abortus* S19 is known to be antigenic. Therefore, it is necessary to do further research whether OMP 36 kDa *B. abortus* S19 is HA protein which is adhesin protein. Further research is needed to determine the protective properties of OMP 36 kDa *B. abortus* S19. Need

further research on organs other than uterus to know the spread of *B. abortus* S19.

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CONFLICT OF INTEREST: There is no conflict of interest.

REFERENCES:

1. Karthik K, Prabakar G, Bharathi R, Khurana SK and Dhama K: Equine brucellosis: Review on epidemiology, pathogenesis, clinical signs, prevention and control. Journal of Experimental Biology and Agricultural Sciences 2016; 4: 2320-8694.
2. Poester FP, Samartino LE and Santos RL: Pathogenesis and pathobiology of brucellosis in livestock. Rev. Sci. tech. Off. Int. Epiz 2013; 32(1): 105-115.
3. Zhang H, Doui X, Li Z, Zhang Y, Zhang J, Guo F, Wang Y, Wang Z, Li T, Gu X and Chen C: Expression and regulation of the ery operon of *Brucella melitensis* in human trophoblast cells. Experimental and Therapeutic Medicine 2016; 12: 2723-2728.
4. Jamal F, Emadi SS and Mosadegh A: Prevalence of *Brucella* species in raw milk produced in the industrial and traditional production units in Yazd. International Journal of Medical Laboratory 2016; 3(3): 191-197.
5. Kurdoglu M, Cetin O, Kurdoglu Z and Akdeniz H: The effect of brucellosis on women's health and reproduction. International Journal of Women's Health and reproduction science 2015; 3: 176-183.
6. Pal M, Gizaw F, Fekadu G, Alemayehu G and Kandi V: Public health and economic importance of bovine brucellosis: An overview. American Journal of Epidemiology and Infectious Disease, 2017; 5(2): 27-34.
7. Saad MZ and Kamarudin MI: Control of animal brucellosis: The Malaysian experience. Asian Pasific Journal of Tropical Medicine 2016; 9(12): 1136-1140.
8. Anonimus: Blue print program swasembada daging sapi. Direktorat Jenderal Peternakan dan Kesehatan Hewan, Kementerian Pertanian, Jakarta 2014.
9. Yang X, Skyberg JA, Cao L, Clapp B, Thornburg T and David W: Pascual DW. Progress in Brucella vaccine development. Front Biol (Beijing) 2013; 8(1): 60-77.
10. Thakur A, Pedersen LE and Jungersen G: Immune makers and correlates of protection for vaccine induced immune responses. Vaccine 2012; 30: 4907-4920.
11. Dorneles EMS, Sriranganathan N and Lage AP: Recent advances in *Brucella abortus* vaccines. Veterinary Research 2015, 46: 76.
12. Denisove AA, Korobovtseva YA, Karpova OM, Tretyakova AV, Mikhina LV, Ivanov AV, Salmakov, KM and Borovick RV: Immunopotentiation of live brucellosis vaccine by adjuvant. Elsevier 2010; 17-22.
13. Ratnasari R, Handijatno D, Suwarno and Rantam FA: Determinan antigen gen omp2a *Brucella abortus* isolatlokal. Acta Veterinaria Indonesia 2014; 1: 17-25.
14. Golshani M, Vaeznia N, Sahmani M and Bouzari S: *In silico* analysis of *Brucella abortus* Omp2b and *in vitro* Expression of SOmp2b. Clin Exp Vac Res 2016; 5: 75-82.
15. Munir R, Afzal M, Hussain M, Naqvi SMS and Khanum A: Outer membrane proteins of *B. abortus* vaccinal and field strains and their immune response in buffaloes. Pak Vet J 2010; 30(2): 110-114.
16. National Centre of Reference for Human Brucellosis: Université Paris- Est / ANSES, Animal Health Laboratory, Bacterial Zoonoses Unit (Maisons Alfort); EU Reference Laboratory for Brucellosis, National and OIE/FAO Reference Laboratory for Animal Brucellosis 2014.
17. Sumarno: Karakterisasi molekuler protein adhesi *Vibrio cholerae* 01 M094V dan protein reseptornya pada sel epitel usus halus tikus putih (Wistar).Tesis. Program Pascasarjana Universitas Airlangga. Surabaya 2000.
18. Calderon AED, Merino LA, Sriranganathan N, Boyle SM and Rodriguez CA: A History of the development of *Brucella* vaccines. Biomed Res Int 2013; 743509.
19. Bellanti JA: Immunologi lll. Terjemahan A. S. Wahab. Gadjah Mada University Press, Yogyakarta 1993.
20. Bashar SN: Determination of ideal time of *Brucella* diagnostic methods in abortion cows. Bas J Vet Res 2015; 14(2).
21. Aulanni'am, Tyasningsih W, Wuragil DK and Rantam FA: Development of brucellosis vaccine based on determinant antigenic of Outer Membrane Protein (OMP) 36 kDa from *Brucella abortus* Local Isolate. International Journal of Pharmaceutical and Clinical Research 2017; 9(3): 201-204.
22. Tabynov K, Sansyzbay A, Kydyrbayev Z, Yespembetov B, Ryskeldinova S, Zinina N, Assanzhanova N, Sultakulova K, Sandybayev N, Khairullin B, Kuznetsova N, Ferko B and Egorov A: Influenza viral vectors expressing the Brucella OMP16 or L7/L12 protein as vaccines against *B. abortus* infection. Virology Journal 2014; 11: 16.
23. Kim WK, Moon JY, Kim S and Hur J: Comparison between immunization routes of live attenuated *Salmonella typhimurium* strains expressing BCSP31, Omp3b and SOD of *Brucella abortus* in murine model. Frontiers in Mycrobiology 2016; 550.
24. Ahmed YF *et al*.: Pathological Studies on Buffalo-Cows Naturally Infected with *Brucella melitensis*, Global Veterinaria 2012; 9(6): 663-668.
25. Vitry MA, Mambres DH, Deghelt M, Hack K, Machelart A, Lhomme F, Vanderwinden JM, Vermeersch M, Trez CD, Morga DP, Letesson JJ and Muraillea E: *Brucella melitensis* invades murine erythrocytes during infection. American Society for Mycrobiology 2014.
26. Pei J, Donagh KM and Ficht TA: Brucella dissociation is essential for macrophage egress and bacterial dissemination. Cellular and Infection Mycrobiology 2014; 4: 23.

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