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Control And Preventive Study Of Brucellosis By Using Lipopolysacharide Sub Unit Vaccine Brucella abortus Strain S-19

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Abstract

The aims of this research is to determine the ability of sub unit lipopolysacharide(LPS) vaccine of *Brucella abortus* strain S-19 in mice and goat, including IgM and sub classes IgG antibody humoral response, cellular mediated immune response (IL-2, IFN- γ) in mice, also IgG as humoral immunity, IL-4 and IL-12 as cellular immunity, comparison affectivity with *Brucella abortus* strain RB-51 vaccine in goat. This research has two steps methods. Step first, 30 Balb C mice were divided into 3 groups and vaccinated subcutaneously, First group injected *B. abortus S-19*, second group injected LPS and third group injected sodium chloride solution. Booster vaccination was conducted every two weeks till the eight week after first vaccination. The second step performed vaccinated to 30 goats divided into three groups. First group was injected by subcutaneous LPS 50 µg/ml and second group injected LPS 100 µg/ml and the third group injected with sodium chloride as control. Booster vaccination conducted 2 weeks after first vaccination and second vaccination. Result of the research conferred. Result research, antibody response in mice showed vaccination by LPS of *B. abortus S-19* showed higher titer than vaccination by whole cells but inverse cellular response. The both vaccines showed induce subclass antibody response, vaccination by LPS tendency to IgM response but vaccination by Whole cells active vaccine tendency to IgG1, IgG 2a and IgG2b. Response antibody in goat on two weeks after first vaccination, vaccination with LPS of *B. abortus* S-19, dose 50 µg/ml failed or zero titer IgG response but dose 100 µg/ml was 500response antibody on two weeks after second vaccination by dose 50 µg/ml was 340 but by dose 100 µg/ml was 960, while cellular IL-12 response two weeks after first vaccination by dose 50 µg/ml was 22.88 pg/ml but by 100 µg/ml was 62.15 pg/ml. Response cellular IL -12 two weeks after second vaccination 50 µg/ml was 12.04 pg/ml while by dose100 µg/ml was 130.88pg/ml Cellular immune response IL-4 on two weeks after first vaccination, dose 50 µg/ml showed 55.57 pg/ml but by dose100 µg/ml was 49.35 pg/ ml. Response cellular IL-4 on two weeks after second vaccination by dose 50 µg/ml was 22.17 pg/ml but by dose 100 µg/ml was 143.89 pg/ml.

Keywords: Vaccine sub-unit LPS of *Brucella abortus* S-19, Humoral antibody, Cellular antibody.

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1. Introduction

We Brucellosis or abortus infectious disease in cattle as zoonotic disease also as strategic categorical disease as important to eradicated. The disease required regulation of cattle moving from area to others area (DITKESWAN, 2011). Percentage of Brucellosis at Indonesia in cattle caused *B. abortus* biotype 1 (77.6%), 2 (13.2%) and 3 (9.2%) (Sudibyo,1995).

According Samkhan *et al.*, 2011 Brucellosis occur in Madura Island only 0.04% or 3 of 7099 cattle. Occurring disease has a little but need a good control because the disease influence populated cattle in Madura Island. Madura cattle it's once specific cattle in Indonesia there are need attention. Brucellosis in east Java island in cattle showed 1.82% at 2009 and 1.13%at 2010 also 0.51% at 2011.

Preventive brucellosis in cattle at Indonesia has performed by vaccination. Commercial vaccines in Indonesia have two kinds are active vaccine *B. abortus S-19* and active vaccine *B. abortus* RB 51. Both vaccines confer immunity in cattle but antibody resulted was vaccinated by *B. abortus* S-19 cannot differentiated with antibodies from infected by *B. abortus* wild type, the others drawback using *B. abortus* S-19 can cause abortus if this vaccine give to pregnant cattle. So the vaccine *B. abortus* RB-51 more batter than *B. abortus* S-19 vaccine, but *B. abortus* RB-51 vaccine didn't have LPS is virulent factor so this vaccine has question about protectively response. LPS of gram negative *B. abortus* as virulent factors also important for immunologic response and role infection. LPS of gram negative bacteria including *B. abortus* confer antibody response including IgM response to much (Olser, 2012) and followed IgG1 also IgG2. LPS also confer cellular immunity response including Interferon gamma (IFN- γ), IL-2, IL-4 and IL-12 (Foriester et al., 2000). Based on introduction LPS of *B. abortus* S-19 was choice used as vaccine to mice and goats

Aims of the research that LPS of B abortus S-19 vaccine can be used as sub unit vaccine for control Brucellosis in cattle at Indonesia, it's also confer humoral sub class antibody response (IgM, IgG1 and IgG2) and cellular mediated immune response (IFN-γ, IL-2) in mice. LPS of B.abortus S-19 also confer antibody response (IgG) and cellular immunity (IL-4 and IL-12) in goats, this LPS of *B. abortus* vaccine more protective and immune response than *B. abortus* RB-51 vaccine.

2. Materials and Methods

2.1. Bacterial strains and growth conditions

B. abortus strain S-19 (Pusvetma Surabaya, Indonesia) were grown in Triptic soya broth medium added 5% horse serum were incubation at 37°C in shaker incubator for three

days. After centrifugation at 10000 rpm for 5 minutes of culture media, sedimented bacteria were harvested and used for LPS extraction and purification.

2.2. LPS extraction and purification

LPS was extracted by cold phenol-water method as described previously with some modifications. In brief, bacterial suspensions (10^8colony-forming units/mL) were centrifuged at 10,000×*g* for 5 *min*. The pellets were washed twice in PBS (pH = 7.2) (0.15 *M*) containing 0.15 *mM* CaCl2 and 0.5 *mM* $MgCl_2$. Pellets were then resuspended in phenol 0.5%. and it was dry. The *B. abortus* was then re-suspended in PBS after that was filtrated by membrane 0.45 µm. In order to eliminate contaminating protein and nucleic acids, treatment with proteinase K, DNase and RNase was performed prior to extraction step. For this purpose, proteinase K (100 *µg/mL*) (Roche, Mannheim, Germany) was added to the cell mixture and the tubes were kept at 65°*C* for an additional hour. Mixture was subsequently treated with RNase (40 *µg/mL*) (Roche, Mannheim, Germany) and DNase (20 *µg/mL*) (Roche, Mannheim, Germany) in the presence of 1 *µL/mL* 20% $MgSO_4$ and 4 *µL/mL* chloroform and incubation was continued at 37°*C* overnight.At the next step, an equal volume of hot (65–70°*C*) 90% phenol was added to the mixtures followed by vigorous shaking at 65–70°*C* for 15 *min*. Suspensions were then cooled on ice, transferred to 1.5 *mL* polypropylene tubes and centrifuged at 8500×*g* for 15 *min*. Supernatants were transferred to 15 *mL* conical centrifuge tubes and phenol phases were re-extracted by 300 *µL* distilled water. Sodium acetate at 0.5 *M* final concentration and 10 volumes of 95% ethanol were added to the extracts and samples were stored at -20°*C* overnight in order to precipitate LPS. Tubes were then centrifuged at 2000×*g* 4°*C* for 10 *min* and the pellets were resuspended in 1 *ml* distilled water. Extensive dialysis against double distilled water at 4°*C* was carried out at the next step until the residual phenol in the aqueous phases was totally eliminated. Final purified LPS product was lyophilized and stored at 4°*C* until was used.(Alton, G.G., L.M. Jones,R.D. Angus and J.M Verger. 1998).

2.3. Formula of the LPS as subunit vaccine

LPS vaccine of *Brucella abortus* 1 volume add 1volume of adjuvant were mixed by vortex until homogenous and then were used for vaccination to animals.

2.4. Treatment LPS vaccine to mice and goats

Vaccination performed to animals by LPS Vaccine has 2 kinds. First was conducted inoculation by subcutaneous in mice. 30 mice were divided three groups. First group was inoculated

B. abortus S-19 active vaccine 10^9 CFU/ml, second groups was inoculated LPS with adjuvant, dose of vaccine 20 µg/ml, The third group was inoculated with aquadest as control. Booster vaccination was conducted twice every 2 weeks. After 2week last booster vaccination were collected serum and determine titer of IgM, IgG1, IgG2a and IgG2b by isotyping Elisa also cellular immune response IL-2 and IFN- γ by indirect Elisa. Second was conducted vaccination to 30 goats by LPS vaccine was divided 3 groups. First and second groups were inoculated LPS vaccine 50 µg/ml and 100µg/ml respectively. The third group was inoculated with aquadest as a control. Booster vaccination conducted two times every 2 weeks. Serum was collected after 2 weeks First and second booster vaccination and to determine titer IgG, IL-4 and IL-12 by indirect Elisa. (Shafee, M., M. Rabbani, M.U.D. Ahmad, K.Muhammad, A.A. Shiekh, M.A.Awan, and M.Z. Shabbir. 2012).

3. Result

Humoral response in mice showed both vaccine LPS and Brucella abortus S-19 can induce antibody but differentiate antibody titer. LPS of *B. abortus* S-19 confer 800 until 3200 while inoculation Active vaccine showed 400 until 800, that the result about humoral antibody can see on figure 1. Cellular immunity response in mice showed that both there vaccines were induced but the titer of IFN-γ and IL-2 response difference. Response IFN-γ showed 926 pg/ml on active vaccine *B. abortus* S-19 but on LPS of *B. abortus* S-19 was 82 pg/ml. Response to IL-2 on Active *B. abortus* S-19 vaccine confer 233 pg/ml but inoculation LPS of B.abortus vaccine showed 31 pg/ml this result can see on table 1. Based on the result showed humoral antibody response after inoculation LPS of *B. abortus* vaccine confer higher titer than active vaccine of whole *B. abortus* S-19 but inverse to response cellular immunity. Humoral sub class antibody response by EILSA isotyping in mice showed both vaccines can induce IgG1, IgG2a, IgG2b and IgM,.

Antibody response in goat showed that on two weeks after first vaccination for control group showed 0 (zero titer), by LPS of

B. abortus S-19, dose 50 µg failed or zero titer to induce antibody but vaccination by LPS of

B. abortus S-19, dose 100 µg was 500. Antibody response two weeks after second vaccination showed that for control group showed 0 (zero titer), vaccination by LPS of

B. abortus S-19, dose 50 µg was 340 but by dose 100 µg was 960. Response cellular immunity to IL-12 showed that for control group was 15.33 pg/ml but by LPS of *B. abortus* S-19 with dose 50 µg was 22.88 pg/ml but by 100 µg confer 62.15 pg/ml on two weeks after first vaccination. Response to IL-12 showed that vaccination for control group was 18.63 pg/ml by LPS of *B. abortus* S-19 with dose 50 µg was 12.04 pg/ml but by 100 µg was 130.88 pg/ml on two weeks after second vaccination. Cellular immunity response to IL-4 on two weeks after first vaccination for control was 17.03 pg/ml by LPS of *B. abortus* S-19, dose 50 µg showed was 55.57 pg/ml while by dose 100 µg was 49.35 pg/ml. IL-4 cellular responses on two weeks after second vaccination for control group was 22.97 pg/ml by LPS of *B. abortus* S-19, dose 50 µg showed was 22.17 pg/ml but by 100 µg was 143.89 pg/ml. Response humoral antibody can see on table 2, while cellular immune response can see on table 3 and table 4

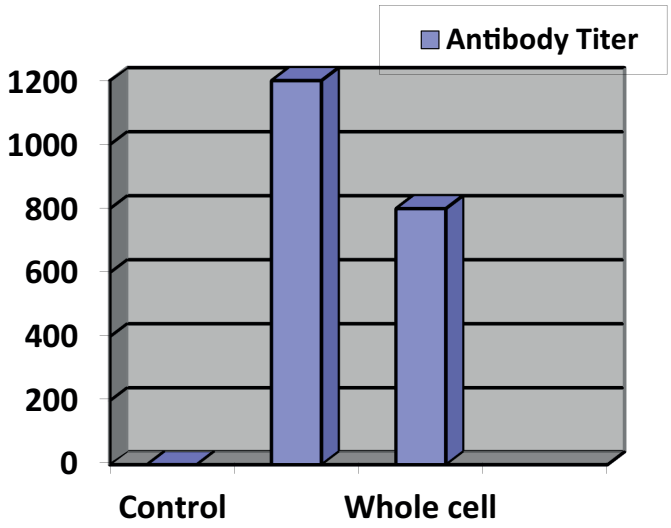

Table 1: Celular immune IFN-γ and IL-2 response by vaccination *B. abortus* S-19 on two weeks after last vaccination.

Treatment	Titer of IFN-γ (pg/ml)	Titer of IL-2(pg/ml)
Po (Control)	22	6
LPS as an inactive vaccine	82	31
Whole Cells as active vaccine	926	233

Table 2: Result of response humoral antibody on two weeks after first and second vaccination.

Treatment	Titer of antibody after first vaccination	Titer antibody after second vaccination
Po (Control)	0	0
LPS 50µg	0	340
LPS 100 µg	500	960

Table 3: Cellular IL-12 response on two weeks after first and second booster vaccination.

Treatment	Titer of antibody after first vaccination	Titer antibody after second vaccination
Po (Control)	15.33	18.63
LPS 50µg	22.88	12.04
LPS 100 µg	62.15	130.88

Table 4: Cellular IL-4 response on two weeks after first and second booster vaccination.

Treatment	Titer of antibody after first vaccination	Titer antibody after second vaccination
Po (Control)	17.03	22.97
LPS 50µg	55.57	22.17
LPS 100 µg	49.35	143.89

4. Discussion

Based on result response humoral antibody in mice showed vaccination by LPS higher titer than whole cell of *B. abortus* S-19 also by aqua-dest, because LPS as inactive vaccine can direct to induced lymphocyte B to produce antibody but whole cell as active vaccine first time to phagocyte cells and multiply in this cells so required time to produce antibody. LPS of *B. abortus* can induce Lymphocyte B to produce IgM for long time, so LPS tendency to produce IgM but whole Cell *B. abortus* S-19 tendency to produce IgG1, IgG2a and IgG2b.

Response cellular IFN-γ and IL-2 titer showed higher by whole cells than LPS of *B. abortus* S-19 also by aqudest. There is whole cells active vaccine can multiply in phagocyte cells and then to produce cytokines including IFN-γ and IL-2.

Based data on vaccination in goat, vaccination by LPS of *B. abortus* S-19 used 50 µg lower to produce antibody also cellular IL-12 and IL-4 response compared by using 100 µg on two weeks after first or second booster vaccination. Because humoral antibody also cellular IL-12 and IL- 4 response was influenced dose of vaccination, vaccination by 50 µg not enough to induce humoral and cellular antibody.

5. Conclusion

Vaccination in mice by LPS of *B. abortus* S-19 as an inactive vaccine confer higher antibody titer than whole cells as active vaccine on two weeks after last vaccination, but inverse to response cellular IFN-γ and IL-2 titer. Sub class antibody response vaccination by LPS tendency to induce IgM but whole cells tendency to induce IgG1, IgG2a and IgG2b.

Vaccination in goat by LPS of *B. abortus* S-19 by dose 100 µg can induce antibody also IL-4 and IL12 higher titer than by dose 50 µg.

Suggestion

LPS of *B. abortus* S-19 could be used as sub unit alternative vaccine for animals but needed convert dose depend of animals.

Develop research LPS of isolate local *B. abortus* will be used as sub unit vaccine.

Potency LPS as sub unit vaccine was applied to other animals.

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