Short Communication

Recombinant Caseous Lymphadenitis Vaccine with Palm Oil as Adjuvant Enhances the Humoral and Cell-Mediated Immune Responses in Rat Model

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Abstract | Caseous lymphadenitis (CLA) is a chronic contagious disease of sheep and goats worldwide. It is caused by a bacterium known as *Corynebacterium pseudotuberculosis* and vaccination is the most suitable method of control. This study measures the humoral and cell-mediated immune responses following vaccination with a recombinant CLA vaccine with palm-based oil as adjuvant. Twenty-five adult rats were divided into 5 groups. Group 1 was vaccinated intramuscularly with recombinant CLA vaccine without adjuvant, groups 2, 3 and 4 were vaccinated with the same vaccine containing 3%, 5% and 7% of palm-based oil, respectively while groups 5 was injected with PBS. The immunoglobulin and cell-mediated immune status were measured for a period of 10 weeks. Rats vaccinated with recombinant vaccine without adjuvant showed slightly high but short response of antibody, CD4+ and CD8+. Rats vaccinated with the recombinant vaccine containing 3% palm oil adjuvant showed significantly ($p<0.05$) highest and lasting antibody levels and percentages of CD4+ and CD8+ cells. Hence, it is concluded that recombinant CLA vaccine with 3% palm oil adjuvant has potential to be developed as a CLA vaccine.

Keywords | Caseous lymphadenitis, Palm oil, Recombinant vaccine

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Caseous lymphadenitis (CLA) is a chronic disease of sheep and goats worldwide (Abdullah et al., 2013) leading to disruption in trade of animals and animal products (Williamson, 2001). It is caused by *Corynebacterium pseudotuberculosis* that infects through non-intact skin such as open wound and abrasions (Adza et al., 2013). Abscesses that developed often ruptured, spreading the infection (Adza et al., 2013).

Vaccination is a suitable method of controlling this disease. Various attempts have been made to develop an efficient vaccine (Leamaster et al., 1987). Eventually, the toxoid phospholipase D (PLD) is most frequently used that resulted in partial protection. However, many undesirable side effects have been associated with the use of this exotoxin (Williamson et al., 2001). In this study, a newly developed recombinant CLA vaccine using different concentrations of palm oil as adjuvant was used to determine the humoral and cell-mediated immune responses in rat model.

Recombinant cells carrying the 40kDa outer membrane protein of *C. pseudotuberculosis* were prepared using pET-32 Ek/LIC vector and was confirmed by subjecting the pET32/LIC-Omp40 recombinant cells to SDS-PAGE (Puspitasari et al., 2012). To prepare the vaccine, the recombinant cells were grown, harvested and killed in 0.5% formalin-PBS overnight at 4°C. This was followed by washing three times in sterile PBS by centrifugation at 5,000 x g at 4°C to remove the formalin. Finally, the inactivated recombinant cells were re-suspended in sterile PBS to a final concentration of 1×10^6 cells/ml. Palm oil (Sime Darby, Malaysia) was used as adjuvant by mixing with the recombinant vaccine at 3%, 5% and 7%.

A total of 25 healthy female rats were selected and divided

into 5 equal groups. Rats of group 1 were vaccinated intramuscularly with the recombinant vaccine without adjuvant, group 2 with the recombinant vaccine containing 3% palm oil as adjuvant, group 3 with 5% palm oil and group 4 with 7% palm oil. Rats of group 5 were similarly injected with sterile PBS. Booster dose of the respective vaccine was administered two weeks after the first vaccination.

Whole blood was collected in heparin-containing Vacutainer tubes (BD Vacutainer, USA) and serum samples were collected in plain Vacutainer tubes (BD Vacutainer, USA) prior to vaccination and at weekly intervals post-vaccination throughout the 10-week study period. The whole blood samples were processed for lymphocyte isolation to determine the cell mediated immune status while the serum samples were subjected to indirect enzyme-linked immunosorbent assay to determine the immunoglobulin levels. Carbon dioxide was used to kill surviving rats at week 10. The Ethical Committee of Universiti Putra Malaysia approved the experimental protocol.

The whole blood samples were immediately processed for lymphocyte isolation using the protocol of Ficoll-Paque™ Plus (GE Healthcare, USA). The whole blood was diluted with sterile PBS pH 7.4 at the ratio of 1:1. Then, 6 mL of the blood were layered onto 4 ml of cold Ficoll Paque in a sterile 15 mL centrifuge tubes. The tubes were then centrifuged at 400 x g for 40 min at 4°C. The lymphocyte band, which appeared as cloudy was carefully pippeted into a clean centrifuge tube containing 3 mL PBS. The cells were re-suspended by gentle drawing in and out before being centrifuged twice at 400 x g for 10 min. The pellet was diluted in RPMI 1640 (Gibco, USA) and counted with hemocytometer before re-adjusted to a final concentration of 10^7 cells/mL (Shaqinah et al., 2012).

Flourescein isothiocyanate (FITC) anti-rat CD4+ and CD8+ monoclonal antibodies were used for analysis of lymphocyte subpopulations (Puspitasari et al., 2012). Twenty µL of the lymphocyte cell suspension was dropped onto glass slides, transferred into a humid chamber and incubated at 37°C for 1 h. Then, the slides were fixed by cold acetone for 10 min, washed 3 times by immersion in PBS pH 7.4 and transferred into humid chamber. Twenty µL of FITC anti-rat CD4+ and FITC anti-rat CD8+ diluted 1:20 in PBS pH7.4 and 1% FCS were dropped onto the slides before incubation at 37°C for 1 h. The slides were then washed 3 times in PBS pH 7.4. A drop of PBS/glycerol (10:90) (Johnson et al., 1982) was added and a cover slip was applied. The slides were examined using flourescent microscope at 40x magnification. The lymphocytes that positive for a particular marker appeared as green flourescence ring. The positive results were expressed as a percentage of the total lymphocytes (Lovat et al., 1987).

Whole cell *C. pseudotuberculosis* suspension were prepared in carbonate-bicarbonate buffer, pH 9.6 to give a final concentration of 10^6 cfu/ml (Puspitasari et al., 2012). Each well of the microtitre plate was filled with 50 µL of the cell suspension and incubated overnight at 4°C. The plates were then washed three times with PBS-T (0.05% (v/v) Tween 20 in PBS) followed by incubation for 1 h at 37°C with 200 µL of 0.1% (w/v) blocking buffer. Following further washing with PBS-T, 100 µL of 1:300 dilution of test serum samples were added into each well and incubated at 37°C for 1 h and washed three times with PBS-T. To determine the IgG levels, 100 µL of the goat anti-rat IgG horseradish peroxidase conjugate (Santa Cruz Biotechnology, USA), diluted at 1:8000 was added into each well and incubated for further 1 h. After a final three-wash step with PBS-T, bound conjugate was detected using 100 µL of TMB Onse Solution Substrate (Calbiochem, USA) per well and incubated for 30 min. The reaction was stopped by adding 50 µL of 2.5M sulphuric acid per well. Optical density values were measured at 450nm wavelength in a microplate reader (WHYM201).

All data were analysed statiscally using univariate analysis of variance (ANOVA) and Post Hoc (Turkey test) in statistical package SPSS software version 16. The data were considered significant at $p<0.05$.

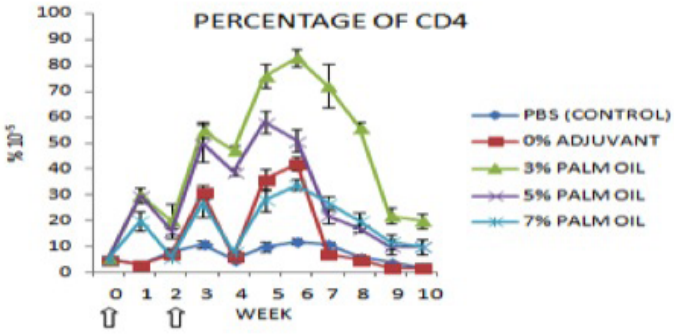

Figure 1: The response of CD4+ T cells in rats following vaccination with recombinant vaccine containing different concentrations of palm oil as adjuvant
The arrows indicate time of vaccination

Figure 1 shows the response by systemic lymphocyte subset CD4+ during the 10-week study period. Prior to vaccination, all groups showed no significant difference ($p>0.05$) in the percentage of CD4+. Following vaccination, group 5 showed significantly lower ($p<0.05$) percentage of CD4+ cells while group 2 showed gradual and significant ($p<0.05$) increased that reached peak at week 6 before started to decline gradually between weeks 7 and 10 post-vaccination. The remaining groups showed no significant differences ($p>0.05$) with each other but remained significantly ($p<0.05$) higher than group 5 (Figure 1).

Figure 2 shows the response by systemic lymphocyte subset

CD8+ during the 10-week study period. Prior to vaccination, all groups showed no significant difference ($p>0.05$) in the percentage of CD8+ cells. Following vaccination, groups 2 and 3 showed gradual and significant ($p<0.05$) increased in the percentage of CD8+ T-cells. However, between weeks 3 and 9, group 2 showed significantly higher ($p<0.05$) percentages of CD8+ than group 3. Groups 1 and 4 were significantly ($p<0.05$) low but remained significantly ($p<0.05$) higher than the control unvaccinated group 5 (Figure 2).

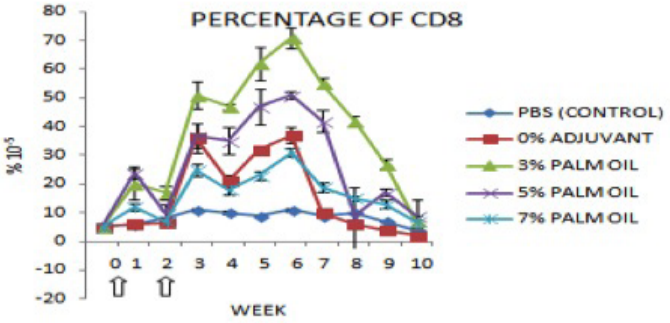

Figure 2: The response of CD8+ T cells in rats following vaccination with recombinant vaccine containing different concentrations of palm oil as adjuvant
The arrows indicate time of vaccination

At the start of the experiment, all groups showed low antibody levels (Figure 3). Following vaccination, the antibody levels of all groups showed gradual increase with group 2 showing significantly ($p<0.05$) highest level at week 1 post-vaccination. The remaining groups showed no significant ($p>0.05$) differences but significantly ($p<0.05$) higher than group 5. Similarly following booster dose, group 2 showed the highest antibody level by week 4 but declined sharply on week 5 although remained significantly ($p<0.05$) higher than the level prior to vaccination (Figure 3). Generally, only group 2 showed significantly ($p<0.05$) high antibody levels.

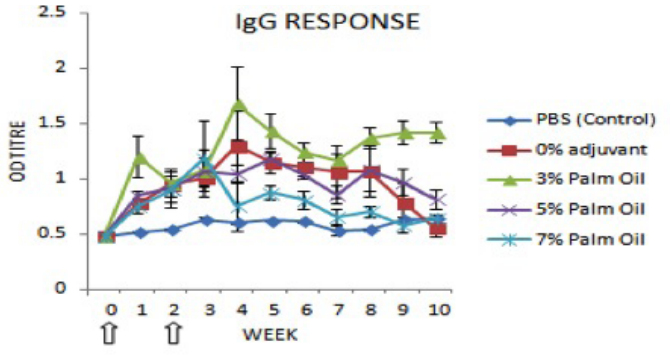

Figure 3: The IgG response in rats following exposure to the vaccine containing different concentrations of palm oil adjuvant
The arrows indicate time of vaccination

Recombinant proteins or synthetic peptides are generally safer than crude inactivated microorganism but they are less immunogenic. Therefore, co-administration of an adjuvant with recombinant protein is important to produce a high immune response as recombinant proteins are generally poor immunogens when administered alone. However, the first strategy is to identify the best concentration of adjuvant to be used that gives the best immune responses (Aucouturier et al., 2001).

In this study, two potential adjuvants were used at different concentrations. It was found that recombinant CLA vaccine with 3% palm oil as adjuvant produced significantly ($p<0.05$) higher antibody levels. This is in agreement with Aucouturier et al. (2001) who concluded that water-in-oil emulsions produce higher IgG2a antibody levels compared to other types of emulsion. Similarly, Mufti (2011) concluded that palm oil as adjuvant enhances the immunogenicity of *Pasteurella multocida* antigen and stimulates higher antibody production while Wanasawaeng et al. (2009) successfully used palm oil in improving the Newcastle disease vaccine. This is because crude palm oil contains between 600 and 1000 ppm of tocopherol/tocotrienol (Hafid et al., 2010). Nevertheless, the antibody response following vaccination with oil adjuvant CLA vaccine in this study was quite late, which was observed at week 5 and rapidly decreased thereafter.

Since *C. pseudotuberculosis* is an intracellular organism (Simmons et al., 1997), systemic cellular immune response plays significant role in protection. Heddens et al. (1986) revealed that goats infected with *C. pseudotuberculosis* have compromised cell-mediated immunity while Hodgson et al. (1999) revealed that vaccinating goats with vaccine containing phospholipase D (PLD) exotoxin does not stimulate the cell-mediated immunity. In this study, rats vaccinated with recombinant CLA vaccine containing 3% palm oil showed significantly ($p<0.05$) higher CD4+ and CD8+ percentages, the two important cells in the cell-mediated immunity. Furthermore, the responses were observed as early as week 3 post-vaccination. Mahan et al. (1998) have demonstrated that sheep vaccinated with inactivated *C. ruminantium* in Complete Freud Adjuvant generated peripheral blood monocyte due to the increase of CD4+ and CD8+ cell populations. Thus, oil based adjuvant, particularly the 3% palm oil has potential to induce cell-mediated immune response against *C. pseudotuberculosis* and possible protect the goats.

In conclusion, this study revealed that the recombinant CLA vaccine without adjuvant failed to stimulate quick antibody and cell-mediated immunities. However, adding 3% palm oil as adjuvant enhanced the antibody and cell-mediated immune responses, highlighting the potential use of palm oil as adjuvant in vaccine preparation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTION

This work was a part of Master of Science research of the first author Roslindawani. Zamri-Saad was the supervisor while Effendy was the co-supervisor. Other authors helped in writing and revision of this manuscript.

REFERENCES

- Abdullah FFJ, Osman AY, Adamu L Azri NA, Haron AW, Saad MZ, Omar AR, Saharee AA (2013). Caseous lymphadenitis in a goat. South Asian J. Life Sci. 1(1): 19-20.
- Adza RMN, Zamri–Saad M, Jesse FFA, Saharee AA, Haron AW, Shahirudin S (2013). Clinical and pathological changes in goats inoculated with *Corynebacterium pseudotuberculosis* by intradermal, intranasal and oral routes. Online J. Vet. Res. 17: 73–81.
- Aucouturier J, Dupuis L, Ganne V (2001). Adjuvant designed for veterinary and human vaccines. Vaccine. 19: 2666-2672.
- Hafid SRA, Radhakrishnan AK, Nesaretnam K (2010). Tocotrienols are good adjuvants for developing cancer vaccines. BMC Cancer. 10: 5. http://dx.doi.org/10.1016/S0264-410X(00)00498-9
- Hedden JA, Thomson CM, Songer JG, Olson GB (1986). Characterization of lectin-binding lymphocytes in goats with caseous lymphadenitis. Am. J. Vet. Res. 47(6): 1265-1267. http://dx.doi.org/10.1186/1471-2407-10-5
- Hodgson AL, Carter K, Tachedjian M, Krywult J, Corner LA, McColl M, Cameron A (1999). Efficacy of an ovine caseous lymphadenitis vaccine formulated using a genetically inactive form of the *Corynebacterium pseudotuberculosis* phospholipase D. Vaccine. 17(7): 802-808. http://dx.doi.org/10.1016/S0264-410X(98)00264-3
- Johnson GD, Davidson RS, McNamee KC, Russel G, Goodwin D, Hallbrow EJ (1982). Fading of immunoflourescence during microscopy: a study of the phenomenon and its remedy. J. Immunol. Methods. 55: 231-242. http://dx.doi.org/10.1016/0022-1759(82)90035-7
- Leamaster BR, Shen DT, Gorman JR, Leather CW, Wells HD (1987). Efficacy of *Corynebacterium pseudotuberculosis* bacterin for the immunologic protection of sheep against development of caseous lymphadenitis. Am. J. Vet. Res. 48(5): 869-872.
- Lovat PE, Hannam-Harris AC, Watson JG (1987). Enumeration of lymphocyte subpopulations by immunoflorescent staining of whole smears. J. Immunol. Methods. 97: 37-40. http://dx.doi.org/10.1016/0022-1759(87)90102-5
- Mahan SM, Kumbula D, Burridge MJ, Barbet AF (1998). The inactivated *Cowdria ruminatium* vaccine for heartwater protects against heterologous strains and against laboratory and field tick challenge. Vaccine. 16(11): 1203-1211. http://dx.doi.org/10.1016/S0264-410X(98)80120-5
- Mufti AR (2011). Development of a new stable formulation of *Pasteurella multocida* nano vaccine by using a mixture of palm oil and coconut oil as adjuvant. 7th IndoChina Conference on Pharmaceutical Sciences. 14-16 December 2011, Bangkok, Thailand.
- Puspitasari Y, Zamri-Saad M, Sabri MY, Zuki AB (2012). Humoral and cellular immune responses in goats exposed to recombinant cells expressing Omp 34kDa *Brucella melitensis* gene. Online J. Vet Res. 16(1): 16-25.
- Shaqinah N, Mazlina M, Zamri-Saad M, Hazilawati H, Jasni S (2012). *In vitro* penetration and survival of *Brecella melitensis* in lymphocytic cells of goats. Online. Vet. Res. 16(3): 104-110
- Simmons CP, Hodgson AL, Strugnell RA (1997). Attenuation and vaccine potential of *aro*Q mutants of *Corynebacterium pseudotuberculosis*. Infect. Immun. 65(8): 3048-3056.
- Wanasawaeng W, Tawatsin A, Sasipreeyajun J, Poomvises P, Chansripornchai N (2009). Development of inactivated Newcastle disease vaccine using palm oil as an adjuvant. Thai J. Vet. Med. 39(1): 9-16.
- Williamson LH (2001). Caseous lymphadenitis in small ruminants. Vet. Clin. North Am. Food Anim. Prac. 17: 359–71. http://dx.doi.org/10.1016/S0749-0720(15)30033-5