Antibody response in cattle after local isolate SE vaccine administration

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Antibody response in cattle after local isolate SE vaccine administration

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Abstract. Septicemic Epizootica (SE) in livestock caused by bacterial infection of *Pasteurella multocida*. The serotype of *P. multocida* in Indonesia is the Asian serotype B:2. The objective of this study is to determine antibody responses of cattle after vaccinating with local isolates Local isolate SE Vaccine. Ten female cattle aged around one year used in this experiment. Cattle divided into 3 groups, Group A (n=4) vaccinated with local isolates SE vaccines, Group B (n=4) vaccinated with commercial SE vaccines and Group C (n=2) unvaccinated. The concentration of SE vaccine is 2mg, and the vaccine was administrated 3ml subcutaneously. Antibody responses measured before and after vaccination using indirect-ELISA. The result of antibody titre of cattle vaccinated with local isolate SE vaccine in seppic-montanide formulation showed higher results compared to SE commercial vaccine and unvaccinated group. This local isolate SE vaccine has good potential as a candidate for SE vaccine in cattle.

1. Introduction

The control of animal diseases in livestock is very important to be implemented in order to achieve beef self-sufficiency in Indonesia by 2026 as it is the most efficient way of managing disease spreading in livestock populations [1]. Septicemia epizooticae (SE) is one of the most common bacterial diseases that sporadically appears as the case of SE outbreaks in Southeast Asia including Indonesia. This disease was first reported in Indonesia in 1885 and causes significant economic losses if not handled carefully [2].

Septicemia Epizootica is caused by *Pasteurella multocida* with a very wide distribution of the P. multocida B: 2 serotypes in most Asian countries [3–5]. Serotype of *P. multocida* that infects cattle and buffalo in Indonesia is Asian serotypes B: 2 [6]. Predisposition factors such as poor quality feed, overcrowded, tiring transport conditions, chills can lead to infection [7]. Clinical symptom of this disease is fever accompanied by respiratory disturbances and oedema in the submandibular, neck and chest area, and bacteremia occurs after 12 hours infection [8]. Cattle become infected by ingestion or inhalation of causative agent, and in endemic area up to 5% of cattle normally be carrier [9].

This disease is economically very detrimental due to decreased productivity of livestock, loss of labor, and the high cost of overcoming it [10]. The prevalence of *P.multocida* isolated from nasal and mouth swabs in Bali cattle was 5% [11]. Therefore, SE is classified into 22 types of strategic infectious diseases in Indonesia whose control coordination is carried out at the central level.

The control and eradication program of SE in Indonesia in general focuses on prevention of outbreaks through mass vaccination in SE endemic areas. The SE vaccine used in Indonesia made of Katha strain from Burma, while most other countries in Asia use indigenous strain (local isolate), such

as Malaysia and India. Currently, SE vaccine using local isolate of *P. multocida* has been developed and already tested in New-Zealand rabbits with a good result protection against pathogenic *P. multocida*. The aims of the present study were to determine the antibody response of cattle vaccinated with local isolate SE vaccine compared to commercial SE vaccine.

2. Methodology

2.1. Ethical approval

These experiments performed on cattle were approved by the Animal Care and Use Committee of Indonesian Agency of Agricultural Research and Development. All cattle were kept in animal facilities at Indonesian Research Centre for Veterinary Science and received feed and water ad libitum.

2.2. Preparation vaccine

Pure culture was grown on Blood Agar media, then single colony sub-cultured on CSY broth, and distributed to CSY agar medium + 5% cow blood. Plate incubated for 24 hours at 37^0 C. Cultures were harvested with 0.3% formal saline buffer and store in refrigerator 40^0 C for 48 hours. Seed vaccine then was formulated in Mountenaid I SA 70. For checking vaccine sterility, vaccine was grown on BA, DSA, BHI broth and RCMM media. The vaccine safety test was carried out by inoculated mice with 0.2 ml of the vaccine intramuscularly and observed for 5 days. The result showed that there was no growth of bacteria or fungi throughout the medium. Safety of SE vaccine showed that mice were kept alive for 5-7 days of observation.

2.3. Immunization of cattle

Ten healthy beef cattle of one year old were divided into 3 groups: Group A (4), Group B (4), and Group C (2). All experimental cattle were checked for absence of natural anti *P.multocida* antibody by indirect-ELISA before conducting experiments. The cattle were maintained under ideal conditions of feeding and management. Group A was immunized with 3 ml of local isolate SE vaccine, Group B was immunized with 3 ml of commercial SE vaccine, and Group C as a control was unvaccinated through intramuscular route. A booster SE vaccine were given in 3 months (Group A and B). All cattle from each group were bled through jugular vein on day 0, 28, 56, 84, 112, 140, 196, 224, 252, and 280. Sera were separated and stored at −20°C until further used.

2.4. Determine antibody response

Determining the humoral immune response of experimental cattle carried out by indirect-ELISA using sonicated antigen of *P. multocida*. Wells of microplate (u bottom) were coated with antigen of *P. multocida* in carbonate bicarbonate buffer and left overnight at room temperature. Following overnight the plates were washed 3 times with PBST. Blocking buffer was added to all the wells and incubated at 37 incubations at 4º C for 45 minutes and repeated the washing step with PBS-T. Serum sample diluted to 1: 400 in serum diluent was added to all the wells and incubated at 37ºC for 1 hr. Each plate had antigen, conjugate, serum substrate and both known positive and negative controls. After washing the plate 3 times with PBST, 100µl of 1: 5000 dilution of conjugate was added to all the wells and incubated for 1 hour. 100µl of freshly prepared OPD substrate in citrate buffer was added to each well. Covered the plate and kept for 5-10 minutes to visualize colour development. Reaction was stopped by adding 50µl of 1N H2SO to all the wells and read the plate at 492 nm in an ELISA reader.

2.5. Statistical analysis of data

Data antibody titers of each Group were statistically analyzed by analysis of variance and t-test.

3. Result and discussion

Septicemia epizootica is endemic in several regions of Indonesia such as South Sulawesi, East Nusa Tenggara, and Aceh. This disease often outbreaks, especially in the rainy season which results in a high morbidity and mortality rate. Treatment SE in cattle with antibiotics is less effective and requires

considerable cost because the bacterium may persevere in the tonsils of carriers for several months and appear actively in the nasopharynx from where it may shed intermittently in the nasal secretions [12]. Hence, vaccination program is the only approach to prevent SE at present [13]. By vaccination of animals is not only protects susceptible population from the infection but also developed herd immunity that confers indirect protection to the unvaccinated population. Increased level of humoral immunity or antibody response to the immunization prevent circulation of infectious agent in susceptible populations [14].

Killed vaccines usually used to prevent SE disease in cattle, and generally, most Asian countries use killed vaccines contain *P. multocida* serotype B:2 from local strain to control the disease while Indonesia using commercial killed SE vaccine made of Katha strain of *P. multocida* from Burma. Instead of using SE commercial vaccine, we have developed SE vaccine using *P. multocida* local isolate that isolated from spleen of infected cattle from East Nusa Tenggara. This vaccine and has tested in rabbits with a good protection results against pathogenic *P. multocida*. In this study, the SE vaccine was tested in cattle to determine the antibody responses after vaccination compared to commercial one. For the evaluation of immune response by each vaccine, indirect ELISA was used.

Figure 1 shows the antibody titre of cattle after giving immunization that were observed for a year. Based on the graph, throughout the period, group A (cattle vaccinated with local isolate SE vaccine) and B (cattle vaccinated with commercial SE vaccine) started to experience the increased in OD value on day 28post-vaccination while Group C (unvaccinated) did not face this sudden increase in the whole period thus, stay stable until day 336 post-vaccination. Group A has higher OD value when compared to Group B even though both increased around the same time and decreased after 252 post-vaccinations. There was a significantly different of antibody responses in cattle among those Group. The antibody titres of cattle in Group A were significantly higher ($P<0.05$) at 56 to 336 days post immunization than those cattle in Group B on the same day. Humoral immunity plays an important role in protection against the disease. Based on the results study, it seems likely that the high antibody response of cattle after being vaccinated with local isolate SE vaccine can prevent SE disease because Humoral immunity plays an important role in protection against the disease [15].

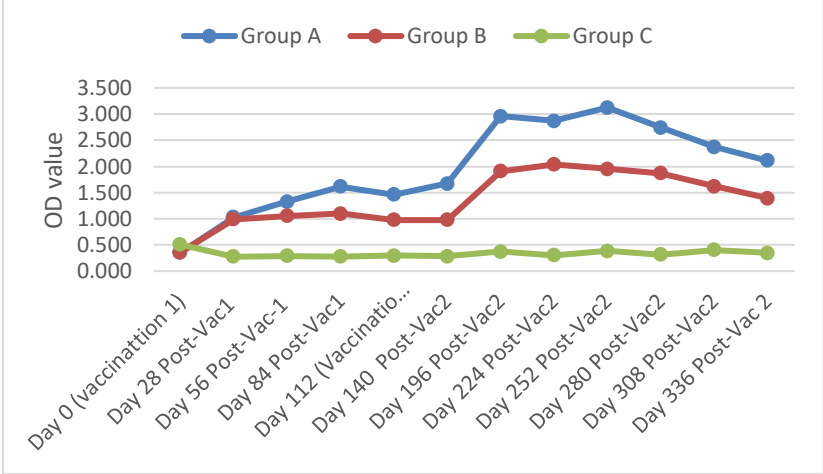

Figure 1. Figure with short caption (caption centred)

In this experiment, local isolate SE vaccine was formulated in seppic-montanide adjuvant, because in the previous study showed that the use of seppic-montanide adjuvant had a good efficacy to induce antibody responses against *P. multocida* in rabbits compare to oil-adjuvant and aluminium hydroxyl gel. Another reason is the use of the oil-adjuvant in the vaccine is unpopular in the field because of its viscosity and difficulty of administration and has side effect in the site of injection. Vaccine

formulation in oil adjuvant produces high viscosity, so it is not preferred for field [16] and resulting reactogenicity, as seen in guinea pigs where swelling was seen around the injection area.

4. Conclusion

The SE vaccine made of *P. multocida* local isolates in cattle showed a higher in increasing antibody response than those SE vaccine made of the katha strain. The antibody titer increases significantly at all observation time.

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