Eimeria piriformis live-attenuated vaccine is successfully lower clinical coccidiosis of rabbits raised in tropic

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Abstract. Livestock can sustain expected levels of productivity if the disease is controlled successfully. Rabbit coccidiosis is the major disease-causing high mortality and morbidity. Since no vaccine is available, the eradication is primarily based on careful management combined with medication in feed or water. Chemical coccidiostat is not only raising antibiotic resistance. It also adds environmental contamination since antibiotic is secreted onto land and water. In this report, we attenuate Eimeria piriformis and used it as the vaccine candidate for New Zealand rabbits in Yogyakarta, Indonesia. 90% of rabbits immunized with live attenuated E. piriformis showed no severe signs when challenged with wild type containing 5 x 10^5 oocyst. The use of live-attenuated E. piriformis is promising as coccidiosis prevention and control program. The strategy would reduce the continuous usage of chemoprophylactic substances in rabbit husbandry and therefore contribute in reducing environmental contamination of antibiotics

1. Introduction

The livestock industry consumes more than half of antibiotics in the total usage in the world which is predicted to reach 200,000 tons in 2030 [1]. Antibiotic usage is found in common as feed additives and chemoprophylactic which is not used as therapeutic of disease. The gastrointestinal microbiota is then also being affected and indeed result in growing development of resistant induced strains. When the gastrointestinal secretion is come out to the surrounding, the resistant strains also contaminate land and water in the nearby environment. The zoonotic strains may infect humans and other susceptible animals which cannot be cured again with normal dosage and type of antibiotics [2, 3]. Nevertheless, health management is the key role in maximizing food animal production. Beside of the usage of antibiotics, vaccination is an efficient way to control and prevent animal diseases during productive stage [4].

On the rabbit meat husbandry coccidiosis remain a threat for efficient production system. Preventive strategy mainly use antibiotic in feed and water and no vaccine commercially available until now. The cost of rabbit coccidia is not only due to high mortality of the kits, but subclinical animal become unprofitable. The recovered animal may act as carrier, low absorption of nutrition and therefore more cost are needed for suffering animals [5, 6]. On the other hand, oocyst eradication in tropical climate is quite challenging. The warm and humidity support sporulation of oocyst in the environment after secreted starting an efficient non-host cycle of parasite.

Vaccination strategy may provide protection to animal earlier for their expected performance during productive phase. In this report, we use precocious line of E. piriformis from our previous work [7] to immunized rabbit in several area in Yogyakarta.

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2. Material and methods

The location of test was in Bantul, Kulonprogo and Sleman districts. The area located in 7°47.44 south latitude and 110°8.24 eastern longitude ordinates. This study involving animals was assigned by ethical committee of LPPT Universitas Gadjah Mada, Indonesia. Farmers contributed to this report were informed consent and available to collaborate with our work.

*E. piriformis* were originated from Yogyakarta and from our previously published work [7]. Oocyst isolation with selection pressure were generated by method previously described [8]. After our optimization in laboratory condition, the precocious line was applied to rabbit farmer located in three different districts of Yogyakarta. Total of 433 rabbits were used in this study. There are four different rabbits breed for test i.e: Rex, New Zealand White, Dutch and Bligon. Bligon is a term used in local farmers for mixed breed. Vaccination is single delivery and the dosage is $10^3$ oocysts. Challenge infection dosage was $5 \times 10^5$ wild type oocysts on day 14 after immunization of precocious line. Clinical manifestations were evaluated including lethargy, reduced appetite and faecal sample consistency. Data processing and presentation were performed by using Graph Pad Prism.

3. Results and discussion

All rabbit vaccinated were successfully survive supported by the commitment of each farmer until the end day of observation i.e., 14 days post infection. All intake supply and environments were occurred naturally according to farmers habits.

![Variation of rabbit breed for experiment](image)

**Figure 1.** Variation of rabbit breed for experiment

The mortality rates were 0%. Decrease of appetite were observed in some rabbit mainly in day 6-8 post vaccination. Watery cecotrops as indication of diarrhoea also reported from 44 rabbits occurring mostly on day 8 to 11 post challenge infection. No medication support was given during observation when diarrhoea was reported to us. All rabbits exhibited higher weight after 14 days of infections, with $390 \pm 150$ grams addition in total. Given that the food supply was quite vary in mark and composition, we used net weight obtained during observation time. For the weight gain evaluation, we did not use small breed for quantification.

The variation of breed during field trial represents the situation in Yogyakarta. Rex’s breed is quite common since represent dual role as fattening and pet rabbit. The pet rabbit mainly is Dutch breed. Whilst, New Zealand White is also a common fattening rabbit in the population. The “Bligon” rabbit represent crossbreed among different breeds according to the farmers explanation.

Clinical signs due to intestinal injuries after vaccinations were not observed. Although decrease of appetite were observed during 6-8 days of infection in some rabbits, they able to survive and gain weight as indicated in the increase of total weight after 14 days post infection (Table 1.).
Table 1. Parameters for field trial observation involving traditional farmers

| Parameters                  | Value                                                                 |
|-----------------------------|----------------------------------------------------------------------|
| Mortality                   | 0% from 433 rabbits                                                  |
| Decrease of appetite        | 32% during 6-8 days post vaccination                                |
| Presence of diarrhoea       | 10% watery-very soft cecotropes during 8-11 days post challenge infection |
| Lethargy                    | 0%                                                                  |
| Final weight gain           | $390 \pm 150$ grams*                                                 |
| Positive samples            | 67% on day 14 post infection                                         |
|                            | 250-500 OPG                                                          |

Challenge by specific pathogens naturally in the field trial sometimes cannot be achieved since the research timeframe is limited [9]. Therefore, the observation from natural coccidia in this report were not performed. We evaluate only the presence of oocysts in 14 days post infection which represented the disease onset from species contained in the mixture immunized *Eimeria* spp. The positive samples from 340 rabbits were mainly ranging from 5-10 per microscopic view or represent 250-500 OPG. In the laboratory condition (data will be published elsewhere), we also found 50-400 OPG observed in the day 12 post infection. These vaccinated rabbits showed a complete defence against $5 \times 10^5$ wild-type isolate.

Besides, this comparable result also indicated that our vaccine candidate was able to reproduce and may provoke reinfection to other rabbit for inducing cell-mediated immunity against coccidiosis. Since the oocysts were attenuated before, the ingested oocyst is supposedly not harmful to any other rabbit under healthy condition. Rabbits survive were hopefully have developed cell mediated immunity mediated by CD8+ and CD4+ from T cell [6]. Immune reaction mediated by payer patches of ileal intestinal were quite important to induce immune response [5]. However, the rabbits don’t have immunity against coccidiosis from maternal source [6] compared to chicken or laying hens [10]. Additionally, normal developmental stage of coccidia is happened after days age. Less than four weeks of age the sporozoite of rabbit coccidia were not able to infect and developed in the epithelial cell of intestine [10].

4. Conclusion
Live-attenuated of *E. piriformis* oocysts yields immune protection against coccidiosis infection. Immunity is provided by a single dosage vaccination of live attenuated *E. piriformis*. *E. piriformis* used here may act as vaccine candidate for prevention of rabbit coccidiosis. The excreted oocysts in the vaccinated rabbits may also provoke recirculation for a herd immunity if ingested by other. We conclude in this report that the vaccine candidate was without detrimental effect to rabbit under field condition during the onset of coccidiosis i.e., until 14 days of infection.

Competing interests
All of the author approved manuscript and declare without any competing interests in this paper.

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