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Vaccine of live attenuated *Eimeria coecicola* boosts immunity against coccidiosis for sustainable rabbit production in Yogyakarta, Indonesia

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Vaccine of live attenuated *Eimeria coecicola* boosts immunity against coccidiosis for sustainable rabbit production in Yogyakarta, Indonesia

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Abstract. Coccidiosis is one of the prominent problems in the rabbit industry. Control of coccidiosis is mainly used chemical coccidiostat as drug or as substances in feed which induce resistance development and antibiotic contamination. To date, there is no commercially available vaccine to prevent rabbit coccidiosis cases. We used live-attenuated *Eimeria coecicola* to induce protective immunity against rabbit coccidiosis in Yogyakarta. Pressure selection was performed to attenuate wild-type *E. coecicola* with lower pathogenicity but possessing the ability to induce an immune response to coccidia infection. The precocious line had reduced the prepatent period to 165 hours and 65% less oocyst production compared to wildtype. The group vaccinated with the precocious line exhibited significantly reduced total oocyst excretion compared to the non-vaccinated group ($P < 0.0001$) when challenged with homolog infection. Our trial showed no mortality rate and without detrimental responses of vaccinated rabbits ($P < 0.0001$). The excreted oocysts in post-vaccinated rabbits were found since the dosage of 5 x 10^2 which was presenting fecundity and the ability of *E. coecicola* precocious line to recirculate. Later, the recirculate oocysts may provoke a continuous flock immunity. The vaccine candidate is useful as the more environmentally friendly approach and disease prevention management for sustainable rabbit production.

1. Introduction

Rabbit farming increases as a potential source of animal protein worldwide. Rabbit industrial-scale production previously concentrated in European countries expanded to Asia, America, and Africa (1, 2). The rabbit becomes a very interesting species after in 1970 the zootechnical sector developed a more profitable rearing method that led to the expansion of rabbit farms. In Asia, the growth of the rabbit industry arose from small and medium small-sized farms to profitable modern agriculture after recognizing its large potential on productivity, low energy consumption, and high efficiency (2, 3). Rabbits kept for reproduction to convert 20% of their protein intake into meat, represents its high-efficiency reproduction compared to chicken, sheep, and cattle (1).

Among many diseases threat in rabbit, husbandry is coccidiosis caused by *Eimeria* spp. There are two coccidiosis patterns in rabbits, namely hepatic coccidiosis caused by *E. stiedai* dan intestinal form caused by 10 different *Eimeria* species (4). *Eimeria* spp. infecting rabbits caused different clinical signs of disease from subclinical forms to severe diarrhoea which leads to a high mortality rate especially to the younger kit (4). *Eimeria* cases are reported from traditional to intensive farms in Asia (5, 6), Europa America (7, 8), and Africa (9).

Curative treatment of rabbit coccidiosis still showed high efficacy by using sulpha-based compounds mainly for poultry, salinomycin, robenidine, and diclazuril (4). However, resistance development to the antibiotic can be generated after long exposure. Furthermore, sporadic treatment is inefficient due to difficulties to remove infectious oocyst from the environment and later reduce disease transmission. Coccidiosis problems are considered to be the costliest diseases in the rabbit industry. Several intensive researches have been performed to prevent coccidiosis since rabbit kit.

There are concerns about developing coccidiosis vaccine nowadays by using precocious lines of several *Eimeria* spp. The vaccination was using single species of *Eimeria* spp. which is attenuated by precociousness (10-13). Vaccination strategy promising to produce immune protection against coccidiosis in the laboratory-scale experiment. Our study was designed to use live attenuated *E. coecicola* to induce immune protection of local rabbits in Yogyakarta, Indonesia. *E. coecicola* was obtained from our previously published work (5) which is a dominant species that circulate in the area. In this paper, the ability to induce protective immunity against wild-type infection is described.

2. Material and methods

This study design was approved by ethical clearance no. 00047/04/LPPT/IV/2017 issued by LPPT Universitas Gadjah Mada, Indonesia. All of the experiments were using New Zealand rabbits in the practical animal laboratory, Veterinary Medicine, Universitas Gadjah Mada. The rabbits were in a closed-house and breed to obtain the uniform age of kits for pressure selection and immunogenicity tests. The parent rabbits were treated with diclazuril before breeding (10) and faecal samples were tested periodically for coccidia infection. The kits with the variation of ages of 28-35 days old were used for infection. The kits were also treated with chemotherapeutic method two weeks before the experiment and tested free from coccidia infection. All pellet food was without any coccidiostat during coccidia infection.

E. coecicola was isolated from Yogyakarta origin from our previously published work (5). The isolate was kept in 2.5% kalium dichromate and conditioned at 4°C until use. The oocyst species was passaged with pressure selection (14). The oocysts were isolated in each passage until a reduced prepatent period was obtained in the 18^{th} passage. The stable isolate was collected from the faecal sample dropped individually on the rabbit cage. Faecal samples were sedimented by short centrifugation (400x g, 10 min). After sedimentation, the water was discharged, and saturated sugar was added to float the oocysts. Parasitological objects were observed microscopically under 400 x magnification. Oocysts per gram of faeces (OPG) were counted for samples using the McMaster technique. Oocysts were counted by multiplying the total number of oocysts by 50.

To analyse immune protection of live-attenuated *E. coecicola* (EC), five groups of rabbits containing 4 each were infected with 1×10^2, 2×10^2, 4×10^2, 5×10^2, 1×10^3, and one control group (C). Oocyst count was obtained by using a Neubauer chamber followed by serial dilution. On day 14 after vaccination, the groups were challenged with 5×10^5 wild-type (WT) isolate. Faecal samples were collected from day 3 to day 12 after challenge infection and summarized in total number from each group presented in Fig. 1. Graphical presentation and data processing for significance analysis was performed by using Graph Pad Prism.

3. Results and discussion

Coccidiosis led to multiple effects on rabbit husbandry. From death due to severe clinical manifestation to subclinical forms causing lower efficiency on food conversion ratio. Since the infectious forms of coccidiosis mostly occurred in the young rabbit after 4 weeks old, the effect is more likely due to the mortality of the young rabbit. Instead, kits survive from the diseases often incapable to pass the transition from suckling to weaning period generate the possibility to grow in suboptimal or rabbits will remain infectious as the carrier to another healthy animal. Different from poultry coccidiosis with a very well-developed vaccination for prevention, commercially available vaccines for rabbit coccidiosis are not yet available. Since the high cost of disease treatment, vaccination becomes very strategically important to reduce the risk and therefore maintain the maximum potential of rabbit husbandry to become profitable.

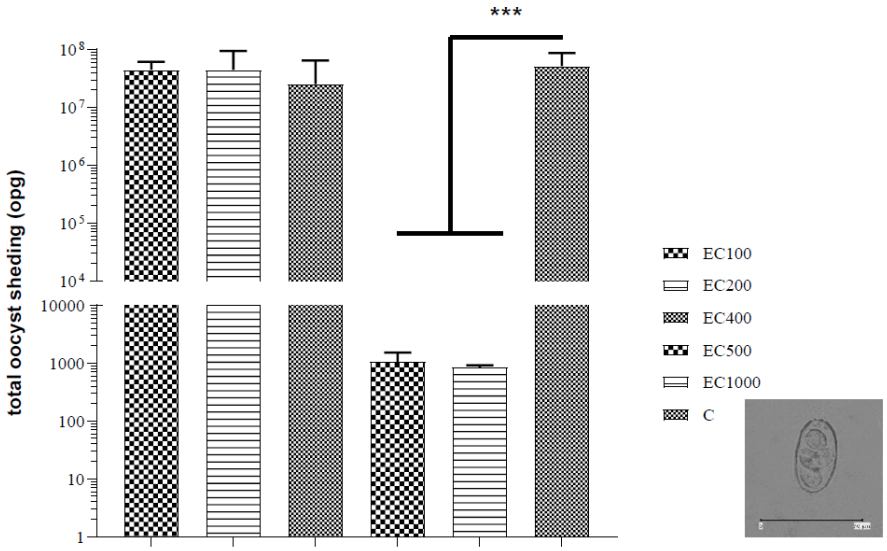

Fig. 1. Oocyst's reduction significantly was observed from the vaccinated groups starting from dosage 5 x 10^2 live attenuated *E. coecicola*

The oocysts of wild-type and processed for precociousness selection were our isolate previously isolated (5). The precocious line of *E. coecicola* in this experiment was obtained by 18 times passages with a reduction of the prepatent period to 145 hours. Protective immunity was observed significantly from oocyst excretions between EC-vaccinated groups compared to control (C) when challenged with 5 x 10^5 WT (Fig. 1). Total oocysts excreted were significantly lower ($P \leq 0.001$) from the vaccinated group with 5 x 10^2 and 1 x 10^3 EC dosages. Whilst, vaccination with lower dosages i.e., 1 x 10^2, 2 x 10^2, and 4 x 10^2 were not enough to induce immunity. Rabbit infected with 5 x 10^5 WT oocysts without vaccination (C group) showed mild diarrhoea, reduced appetite, and lethargy during 5 to 8 days post-challenge infection. No clinical manifestations were observed in the vaccinated groups after challenge infection.

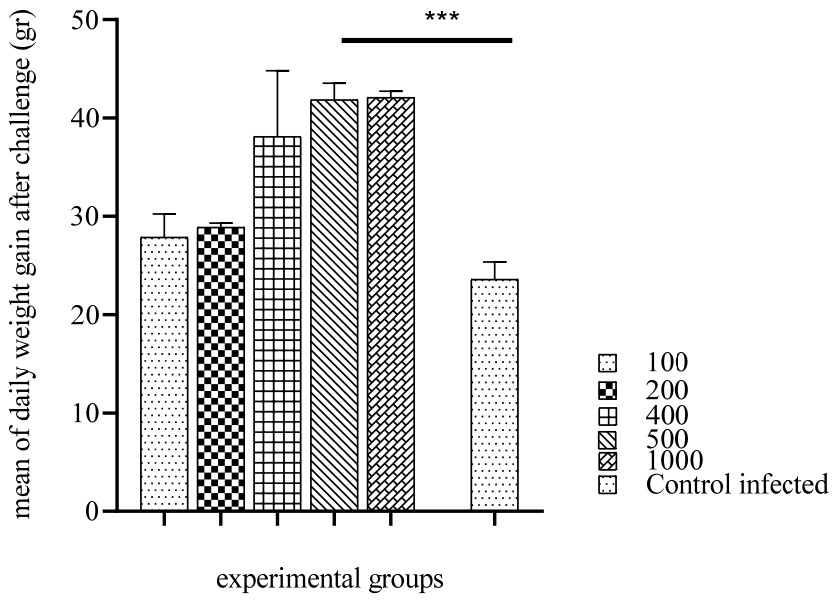

Fig. 2. Mean of daily weight gain of vaccinated groups compared to unvaccinated group ($P \leq 0.0001$)

Rabbit mortalities were not observed from all vaccinated and unvaccinated groups until the end of the experiment. Reduced appetite after vaccination was not significantly observed after vaccination of 1 x 10^2, 2 x 10^2, 4 x 10^2, and 5 x 10^2 but occurred in rabbits vaccinated with 1 x 10^3 EC. Rabbits vaccinated with 5 x 10^2 and 1 x 10^3 EC dosages gained daily weight significantly different ($P \leq 0.0001$) compared to unvaccinated group after challenge infection (Fig. 2). These dosages provided protective immunity against challenge infection.

Vaccination strategies by using a selection of precocious lines have been reported in *E. media (10)*, *E. magna (15)*, *E. intestinalis (16)*, *E. piriformis (12)*, *E. coecicola* (14) and *E. flavescens (17)*. In our experiment, protection of the EC was observed significantly from 5 x 10^2 and 1 x 10^3 oocyst vaccinated orally. It is comparable with several types of research that previously showed immune induction by the different number of oral immunizations i.e.: 1 x 10^2 (10), 1. 5 x 10^3 trivalent mixed species (18), 1 x 10^2 (16). However, reduced appetite occurred in rabbits vaccinated with 1 x 10^3 EC on days 5 to 7 after vaccination. Therefore, 5 x 10^2 oocysts were the recommended dosage for vaccination which can induce immunity without detrimental effect showed.

The EC in our experiment also showed fecundity from the dosage of 5 x 10^2 infection. EC oocysts shedding in the environment showed enrichment ability and indicating that the attenuate-oocyst may recirculate again to infect the other rabbit further induce a flock immunity. Coccidiosis mostly affects young rabbits just after weaning (5- to 6-week-old animals) which are not protected from the immunity acquired by their mother (4). The vaccination since around thirty days old is quite important to prevent coccidiosis and thus avoiding usage of continuous coccidiostat. Vaccination strategy with live rabbit *E. coecicola* in this paper showed protective immunity against clinical coccidiosis.

4. Conclusion

Live-attenuated of *E. coecicola* oocyst yields immune protection against coccidiosis infection. Immunity is provided by a single dosage of vaccination starting from 5 x 10^2 live attenuated of *E. coecicola*. *E. coecicola* may act as vaccine candidate for prevention of rabbit coccidiosis. Further studies on field trials with different rabbit breeds can provide more information on the wide application of vaccine and it's future prospect.

Competing Interests

The authors declare that they have no competing interests.

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