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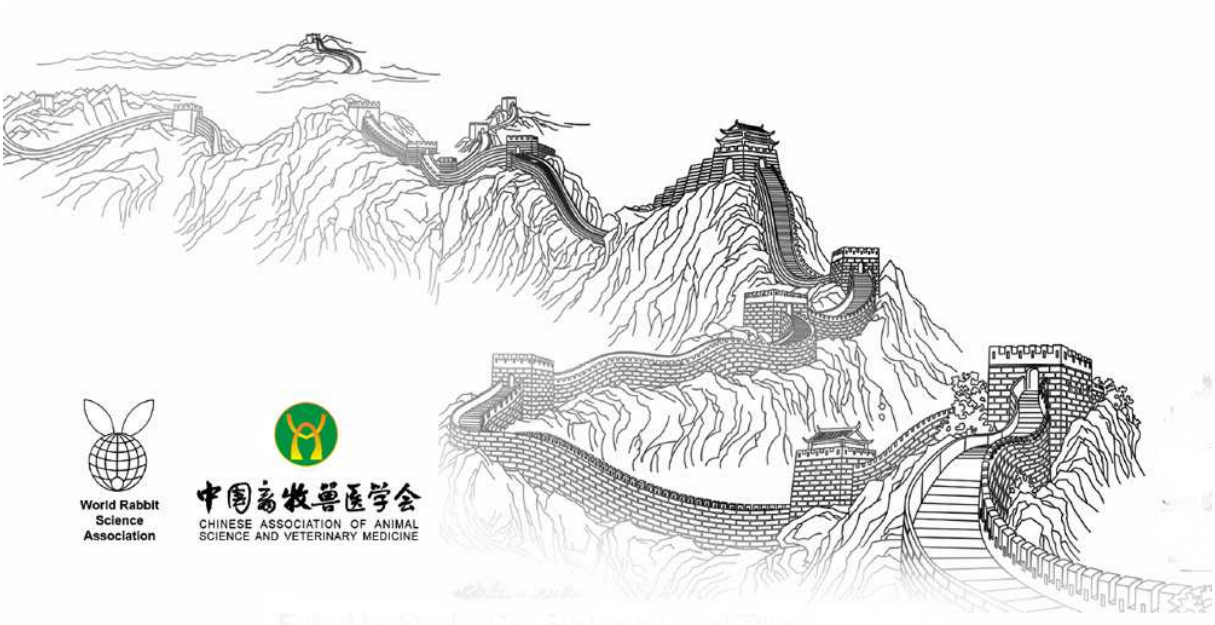

PROTECTION OF RABBITS AGAINST COCCIDIOSIS BY CO-INFECTION WITH *EIMERIA MAGNA, E. INTESTINALIS* AND *E. MEDIA*

Wang Y.[1], Tao G.[1], Li C.[1], Gu X., Suo X.[1], Liu X.[1]*

[1]College of Veterinary Medicine, China Agricultural University, Yuanmingyuan West Road, 2[#], 100193, Beijing, China
[*]Corresponding author: liuxianyong@cau.edu.cn

ABSTRACT

Rabbit Coccidiosis is caused by the infection of the eimerian parasites in the digestive system (liver and intestine). Vaccination with live oocysts, though demonstrating excellent performance against chicken coccidiosis, is not available for the rabbit industry. In this study, the immunogenicity of a single strain of *Eimeria intestinalis, E. magna* or *E. media* as well as the co-infection of these 3 strains has been tested. The administration of a single or 3 mixed species provided a good protection against homogenous or mixed challenge in terms of body weight gain and oocyst shedding reduction. This study showed that the 3 native isolates of rabbit coccidia could be selected for further studies on the development of live vaccines against rabbit coccidiosis.

Key words: Rabbit coccidiosis, Anticoccidial vaccine, Pathogenicity, Immunogenicity

INTRODUCTION

Coccidiosis, caused by protozoa of the genus *Eimeria*, has an economic impact for poultry and livestock, including rabbits (Chapman *et al*. 2002, Pakandl. 2009). The symptoms of the disease include anorexia, diarrhea, body weight loss, poor feed conversion and even death to weaning rabbits. In rabbitries, the administration of coccidiostats in feed is the most used method to obtain an efficacious prevention of the disease (Pakandl. 2009). A recent survey demonstrated that the coccidia infection rate in rabbits could exceed the 50% (Jing *et al*., 2011) in China. These data could be a "baseline" to address a correct strategy for the control of rabbit coccidiosis.

Live attenuated anticoccidial vaccines, including those formulated with precocious lines of *Eimeria* spp., exhibit, in experimental trials, excellent performance in preventing chicken coccidiosis (Dalloul *et al*., 2006; Licois *et al*., 1990; Licois *et al*., 1994; Pakandl *et al*., 1996; Drouet-Viard *et al*., 1997; Pakandl, 2005). However, strains of *Eimeria* from different geographic locations demonstrated distinctive pathogenicity and immunogenicity (Shi *et al*., 2013, Coudert *et al*., 1993). Therefore, comprehensive studies regarding the efficacy of native strains are required to develop new live anticoccidial vaccines for rabbits. In this study, the protective capacity of native strains of 3 common species, *E. magna*, *E. intestinalis* and *E. media*, has been evaluated.

MATERIALS AND METHODS

Animals and parasites

All coccidia-free New Zealand White rabbits used in this study were provided by CAU rabbit warren. To avoid coccidial contamination, all suckling rabbits were weaned at 18-20 day old and reared under coccidia-free condition. Rabbits were fed with a pellet feed supplemented with diclazuril (1 mg/kg) and monensin (50

mg/kg) till 5 days before the inoculation of protozoa; subsequently, not-supplemented pellet was provided. During the trails, all the rabbits have been housed individually, in wire cages.

The strains of *E. magna*, *E. media* and *E. intestinalis* used in this study were isolated from Hebei province, China and without attenuated selection. All the three species were propagated and kept in National Animal Protozoa Laboratory, China Agricultural University.

Experimental design
For immunogenicity test, 24 coccidia-free rabbits were randomly distributed into 4 groups. Five hundred or one thousand sporulated oocysts were given to rabbits in groups 1 and 2, respectively; while PBS was given to groups 3 and 4. Fourteen days later, 1×10^4 sporulated oocysts were inoculated into rabbits in groups 1 to 3 groups; while the 4th group was uninfected and unchallenged (UUC). Body weight of all rabbits was measured at 0, 3, 7, 10 and 14 days after the inoculation (dpi) and the challenge (dpc). Feces during the prepatent time (6.5 dpi for *E. magna*, 4.5 dpi for *E. media*, and 8.5 dpi for *E. intestinalis*) to 5 days later were collected after each inoculation. Total oocysts counts were carried out using modified McMaster counting chamber. The above trial was separately performed for each of the 4 isolates.

To test the efficacy of co-infection with 3 species, 5 groups of 6 rabbits each were used. Mixed population of 500, 1000 or 1500 sporulated oocysts of each species were given to each rabbit of groups 1 to 3, respectively; while group 4 was used as uninfected and unchallenged control (UUC). All animals except for the UUC group were challenged with 1×10^4 oocysts of each of the 3 species 14 days later. Individual body weight was measured at 0, 3, 7, 10 and 14 days after each inoculation. Feces between 4.5 days and 13.5 days post each infection were collected and oocysts output were counted.

Statistical Analysis
Statistical analysis was performed by one-way ANOVA of SPSS software (version 17.0). Data were expressed as mean ± standard deviation, and statistical significance was determined using Student's *t*-test; * indicates $p<0.05$, while ** indicates $p<0.01$.

RESULTS AND DISCUSSION

Strains of *E. magna, E. intestinalis* and *E. media* are of good immunogenicity
Body weight gain and oocyst reduction were used as the major criteria for the evaluation of the immunogenicity of *E. magna, E. intestinalis* and *E. media* after infection and homologous challenge with single species (Fig 1). The inoculation of each of the 3 species provided protective immunity against reinfection of the homologous species. After the challenge, the body weight gain of all infected rabbits showed no significant difference compared with that of the rabbits in the UUC group. For rabbits infected with *E. magna*, the oocyst output was under the detection threshold, while oocysts of more than 1×10^6 per rabbit were detected from the other 2 groups, immunized with *E. intestinalis* and *E. media*, respectively. These data seem to suggest that all these 3 isolates possess the potential immunogenicity to be formulated in the live vaccine against rabbit coccidiosis.

After the primary infection, all infected rabbits showed loss of body weight gain, indicating that the wild strains were pathogenic in some degree. Among the 3 species, *E. magna* has a relatively high pathogenicity, evidenced by the obvious clinical signs and body weight loss in rabbits inoculated with 1×10^3 oocysts. This result may indicate that the strain used in this study was more pathogenic than other strains (Licois *et al.* 1995). *E. intestinalis* and *E. media* strains used in this study exhibited moderate or low pathogenicity. Reduction of appetite and loss of body weight gain were detected in all infected rabbits. However, the visible clinical signs, including anorexia, diarrhea and exfoliated mucosa, could only be seen in rabbits inoculated with high doses of oocysts.

Co-infection with *E. magna, E. intestinalis* and *E. media* were protective against challenge with oocysts of 3 species

In order to test the feasibility of co-infection, oocysts of 3 species were inoculated into rabbits simultaneously. In UCC group, diarrhea was observed and mortality was 50% (3/6); meanwhile, the body weight gain decreased dramatically during 4 dpc to 10 dpc after the challenge. In contrast, infected rabbits did not show any clinical sign, and no significant difference in body weight was found when compared to the UUC group (Fig 2). The only difference among the 3 infected-groups was the oocyst shedding after the challenge. The oocyst output decreased along with the increased infection dosages.

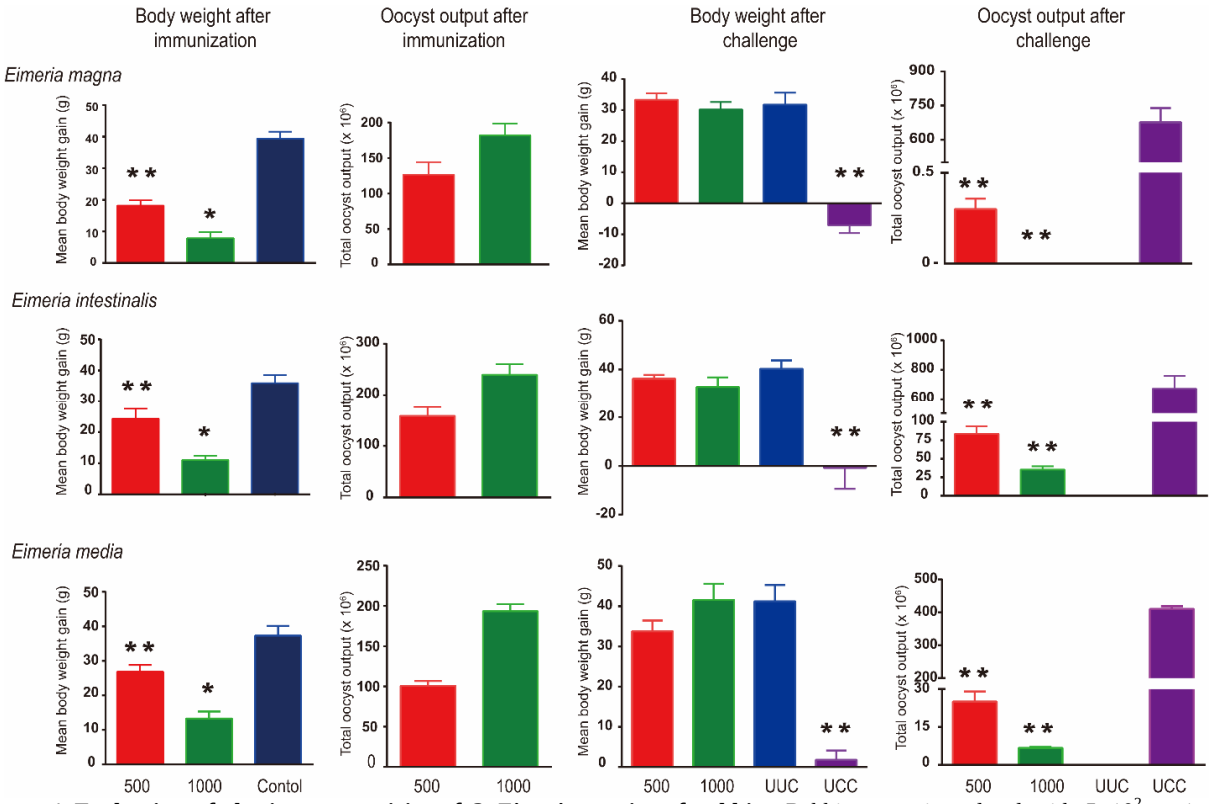

Figure 1 Evaluation of the immunogenicity of 3 *Eimeria* species of rabbits. Rabbits were inoculated with 5×10^2 or 1×10^3 sporulated oocysts of each species, or PBS (UUC and UCC), respectively. 14 days later, a challenge of 1×10^4 sporulated oocysts homologous species were performed. Statistical significance was determined using Student's *t*-test; *p<0.05, **p<0.01.

These results together demonstrated that a controlled infection with *E. magna*, *E. intestinalis* and *E. media* could be useful against a mixed infection of homologous coccidia .Furthermore, depression of body weight gain after administration of virulent field strains, strongly suggests that alternative strategies, for instance the employ of precocious lines, should be a better solution in the control of rabbit coccidia.

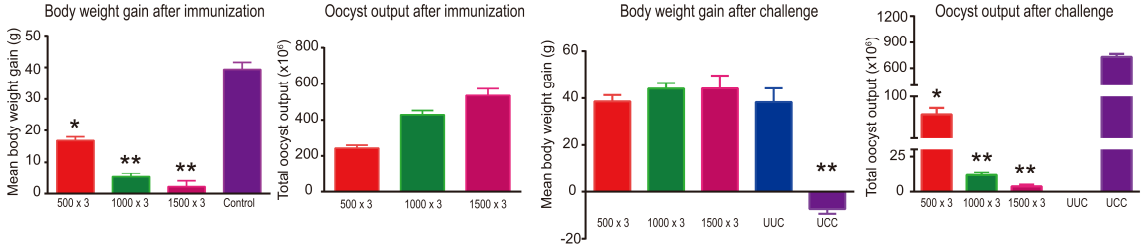

Figure 2 Evaluation of the co-immunogenicity of 3 *Eimeria* species of rabbits. Three groups of rabbits were infected with three different doses of sporulated oocysts (500, 1000, 1500 of each species), respectively. 14 days later, a challenge of 1×10^4 oocysts of each of the 3 species was performed. Statistical significance was determined using Student's *t*-test; *p<0.05, **p<0.01.

CONCLUSION

Three isolates of *E. magna, E. intestinalis* and *E. media* seemed to show a good immunogenicity, though *E. magna* showed a relatively high pathogenicity. Co-administration of these 3 species could protect rabbits against challenge with mixed oocysts of them, indicating that an anticoccidial vaccine based on virulent strains of *Eimeria* could be feasible. However, further work, such as testing the minimum dosage for each species formulated into the vaccine, should be conducted.

ACKNOWLEDGEMENTS

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VACCINATION OF RABBITS AGAINST COCCIDIOSIS WITH 3 SPECIES OF RABBIT COCCIDIA

Wang Y., Tao G., Li C., Suo X., Liu X.*

National Animal Protozoa Laboratory & College of Veterinary Medicine,
China Agricultural University, Beijing 100193, China
* Corresponding author: liuxianyong@cau.edu.cn

INTRODUCTION

Coccidiosis caused by infection of parasites in the genus *Eimeria* is a disease of economic importance for livestock and poultry, including rabbits (Chapman et al. 2002, Pakandl. 2009). The symptoms of this disease include anorexia, diarrhea, body weight loss, poor feed conversion and even death to weaning rabbits. Administration with coccidiostats is the only efficient method to prevent rabbit coccidiosis (Pakandl. 2009). Although, a recent survey demonstrated that coccidia infection rate in rabbits was more than 50% (Jing et al., 2011), indicating that more strategies should be addressed to be explored for the control of rabbit coccidiosis. Even though several precocious lines have been successfully selected in previous studies (Licois et al., 1990; Licois et al., 1994; Pakandl et al., 1996; Drouet-Viard et al., 1997; Pakandl, 2005), there is no commercial vaccine for rabbit coccidiosis. As strains from different geographic locations demonstrated distinctive pathogenicity and immunogenicity on rabbit coccidia (Shi et al., 2013, Coudert et al., 1993), comprehensive studies covering Chinese local strains are required for the development of live anticoccidial vaccines for rabbits. In this study, the immunogenicity of local strains of the 3 most common species, *E. magna*, *E. intestinalis* and *E. media* was evaluated.

MATERIALS AND METHODS

Animals and parasites

All coccidia-free New Zealand White rabbits used in this study were provided by CAU rabbit warren.

The strains of *E. magna*, *E. media* and *E. intestinalis* used in this study were isolated from Hebei province, China.

Experimental design

For immunogenicity test of each species, 24 coccidia-free rabbits were randomly distributed into 4 groups and 1 rabbit per cage. Five hundred or one thousand sporulated oocysts or PBS were given to rabbits, respectively. Fourteen days later, 1×10^4 sporulated oocysts were inoculated except the unimmunized and unchallenged group (UUC). Body weight of all rabbits was measured at 0, 3, 7, 10 and 14 days post prime inoculation (dpi) and challenge (dpc). Feces from the prepatent time (6.5 dpi for *E. magna*, 4.5 dpi for *E. media*, and 8.5 dpi for *E. intestinalis*) to 5 days later were collected after each inoculation. Total oocysts counts were carried out using modified McMaster counting chamber.

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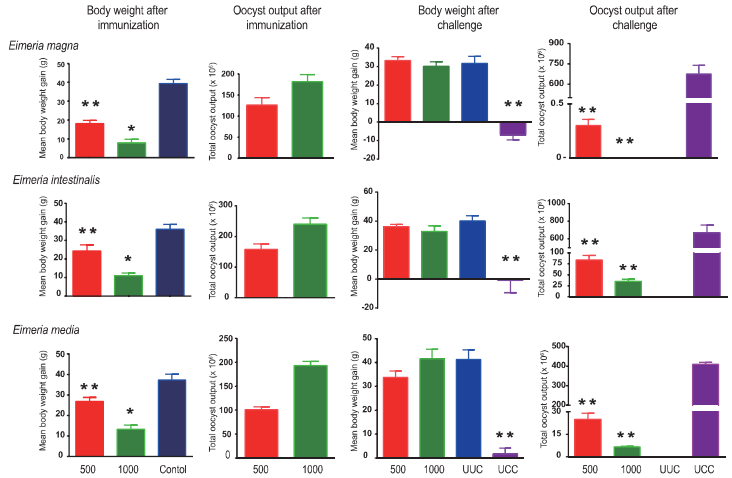

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After the primary immunization, all immunized rabbits showed loss of body weight gain, indicating that the wild strains were pathogenic in some degree. Among the 3 species, *E. magna* has a relatively high pathogenicity, evidenced by the obvious clinical signs and body weight loss in rabbits inoculated with 1×10^3 oocysts. This result may indicate that the strain used in this study was more pathogenic than described before (Licois et al. 1995). *E. intestinalis* and *E. media* strains used in this study exhibited moderate or low pathogenicity. In addition, although the visible clinical signs, including anorexia, diarrhea and exfoliated mucosa, could only be seen when high doses of oocysts were inoculated, reduction of appetite and loss of body weight gain were detected in all infected rabbits. This phenomenon implied the significance of subclinical coccidiosis in the field, a very common infection situation of rabbit coccidia might cause huge losses in rabbit farms (Pakandl et al. 2009).

Co-immunization with *E. magna*, *E. intestinalis* and *E. media* were protective against challenge with oocysts of 3 species

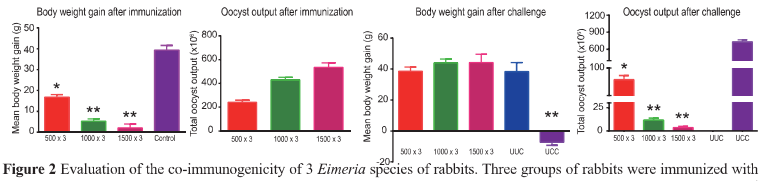

Figure 2 Evaluation of the co-immunogenicity of 3 *Eimeria* species of rabbits. Three groups of rabbits were immunized with three different doses of sporulated oocysts (500, 1000, 1500 of each species), respectively. 14 days later, a challenge of 1×10^4 oocysts of each of the 3 species was performed. Statistical significance was determined using Student's t-test; *, $p<0.05$, **, $p<0.01$.

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These results demonstrated that co-immunization with the 3 species were feasible and reliable against mixed species challenge. In addition, *E. magna, E. intestinalis* and *E. media* could develop specific immunity rapidly and thus could provide enough protection against challenge. Furthermore, rabbits immunized with virulent field strains results in depression of body weight gain after immunization indicating that live attenuated strains, likely precocious lines, must be better vaccine candidates against rabbit coccidia.

CONCLUSION

Three isolates of *E. magna*, *E. intestinalis* and *E. media* were of good immunogenicity, though *E. magna* showed a relatively high pathogenicity. Co-immunization with these 3 species could protect rabbits against challenge with mixed oocysts of them, indicating that the anticoccidial vaccine based on virulent strains of *Eimeria* is feasible. However, further work is needed to test the minimum dosage for each species formulated into the vaccine.

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