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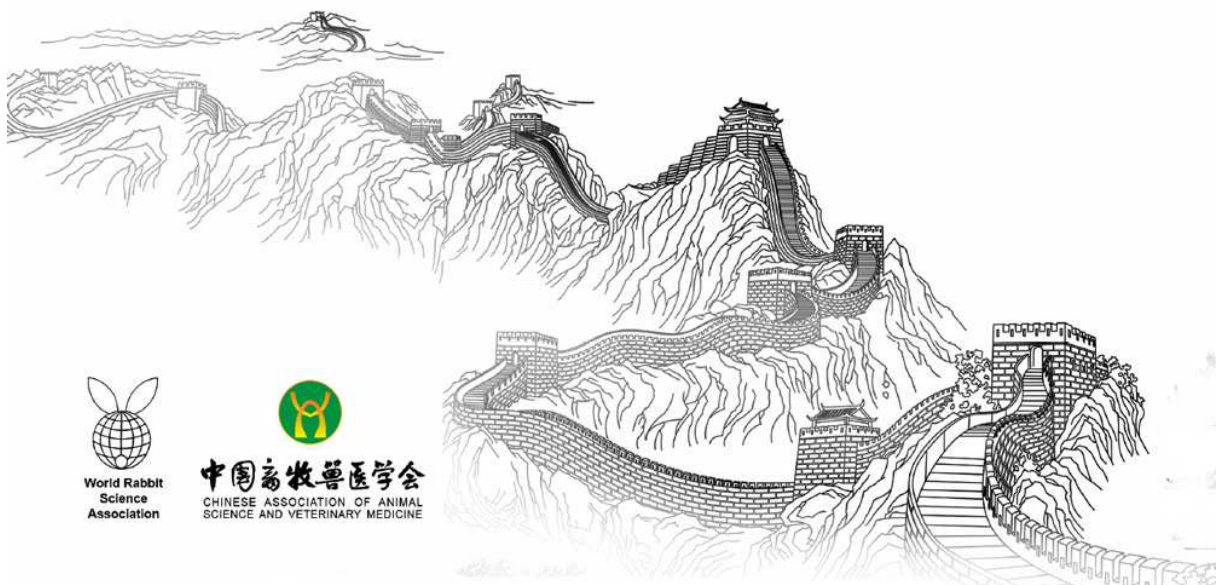
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ATTENUATION OF *EIMERIA INTESTINALIS* THROUGH SELECTION OF A PRECOCIOUS LINE

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ABSTRACT

Vaccination with live attenuated oocysts is the most promising method for control of coccidiosis in poultry and livestock. In this study, we selected a precocious line of *Eimeria intestinalis* (PEI8) from an original strain (OEI) by collecting the first excreted oocysts during 8 successive propagations. The prepatent period of PEI8 was shortened from 204 h to 132 h. The peak of oocyst shedding of PEI8 occurred 3 days earlier than that of the parental strain (OEI). Meanwhile, the multiplication rate of PEI8 was only 0.1~1% of that of OEI. The pathogenicity test showed that PEI8 was much less virulent than OEI. Rabbits immunized with PEI8 or OEI were both protected against challenge with the parental strain in terms of weight gain and oocyst output. These results indicate that the PEI8 was less pathogenic but maintained its immunogenicity. Further study will be performed to test PEI8 as a component of live attenuated vaccine for rabbit coccidiosis.

Key words: Precocious selection, *Eimeria intestinalis*, Pathogenicity, immunogenicity

INTRODUCTION

Rabbit coccidiosis is a disease caused by *Eimeria* species with high pathogenicity, such as *E. magna*, *E. intestinalis* and *E. flavescens*. *E. intestinalis* is highly prevalent in rabbit farms (Jing *et al.*, 2012). Infection with small number of *E. intestinalis* oocysts could induce severe symptoms in weaning rabbits: depress, body weight loss, diarrhea and even death (Coudert *et al.*, 1993). On the other hand, it was proved to be one of the most immunogenic indicating the feasibility of vaccination to protect rabbits against reinfection (Licois and Coudert 1980). Vaccination with live attenuated oocysts has been proved an effective strategy for the control of coccidiosis in chickens (Long and Rose, 1982). One way to get live attenuated strain is to select a precocious line by selecting the first excreted oocysts during successive passages (Jeffers, 1975; Shirely *et al.*, 1983; Shirely and Bellatti, 1984; Johnson *et al.*, 1986; McDonald *et al.*, 1982, 1986; Licois *et al.*, 1990, 1994, 1995; Pakandl, M., 2005, 2006).

In this study, we obtained a precocious line of *E. intestinalis* and further evaluated its potential capacity as a vaccine candidate.

MATERIALS AND METHODS

Selection for the precocious line

A strain of *E. intestinalis* isolated from a field sample from Hebei province in China, referred as OEI, was used for the precocious selection. Collection of the first excreted oocysts in faeces was conducted for 8

generations of propagation. This selection followed the previously described protocols (Jeffers, 1975; Licois and Coudert 1980).

Pathogenicity and immunogenicity test

Freshly sporulated oocysts of OEI and PEI8 were used as inocula in the pathogenicity and immunogenicity tests. Ten groups of 4 rabbits were used. Rabbits of 4 groups were inoculated with 1×10^2 , 1×10^3 , 1×10^4 , 1×10^5 oocysts of OEI; while the other 4 groups with the same dosages of PEI8. The other 2 groups were unimmunized and unchallenged (UUC) control and unimmunized and challenged (UCC) control. All animals except for the UUC group were challenged with 1×10^5 OEI oocysts 14 days post first inoculation. Rabbits were weighed twice a week. To investigate the reproductivity of the PEI8, daily oocyst output during 5 to 14 days in the first 8 groups were counted using a McMaster chamber. To explore the immunogenicity of the PEI8, total oocyst output after challenged was also detected.

Statistical Analysis

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

RESULTS AND DISCUSSION

Selection of the *E. intestinalis* precocious line

The prepatent time of the precocious line PEI8 was reduced by 72 h in total, from 204 h to 132 h after 8 consecutive passages. The prepatent time gradually decreased from 204 h to 190 h after 5 passages of selection (Table 1). Between the 6th and 8th passages, the prepatent period was shortened by about 20 h each passage. The selection process of this *E. intestinalis* precocious line was similar with the results of a previous study, where rapid decreases of prepatent period were also found in the last 3 or 4 passages (Licois et al., 1990). The peak of PEI8 oocyst excretion occurred at 3 days earlier than that of OEI, also similar with the previous report (Licois et al., 1990).

Table 1 Precocious Selection for *E. intestinalis* (PEI8)

Strain inoculated	Number of rabbits	Prepatent time (h)	Dosage for inoculation	Oocyst output	Strain obtained
P0	2	204	5×10^4	6.8×10^7	P1
P1	2	199	7×10^4	6118	P2
P2	2	205	7×10^4	1.5×10^6	P3
P3	3	197.5	1×10^5	9.251×10^5	P4
P4	2	190	1×10^5	2.8×10^5	P5
P5	3	174	1×10^5	4×10^6	P6
P6	3	154	1.5×10^5	4×10^5	P7
P7	3	132	1×10^5	1.5×10^5	P8 (PEI8)

Oocyst excretion curve and fecundity of *E. intestinalis* precocious line

Table 2 Comparison of total oocyst output between *E. intestinalis* strains OIE and PEI8

No. of oocysts inoculated		10^2	10^3	10^4	10^5
Oocyst output ($\times 10^6$)	OIE	$74 \pm 2.4^{**}$	$110 \pm 7.3^{**}$	$1708 \pm 4.7^{**}$	350 ± 11.4
	PEI8	0.47 ± 0.02	2.5 ± 0.13	15 ± 0.51	130 ± 2.1

Note: Statistical significance was determined using Student's *t*-test; * $p < 0.05$, ** $p < 0.01$.

The first excreted oocysts of PEI8 and OEI in feces were detected at 132 h and 204 h, respectively (Table 1 and Fig. 1A-D). The peak of oocyst output occurred at day 7 for PEI8 and day 10 for the OEI. The total oocyst output of PEI8 reduced to only about 1/3 to 1/100 of that of OEI (Table 2). The maximal oocyst output of PEI8 was 1.3×10^8 when the rabbits were inoculated with 1×10^5 oocysts; while that for OEI was 1.7×10^9 with an inocula of 1×10^4 .

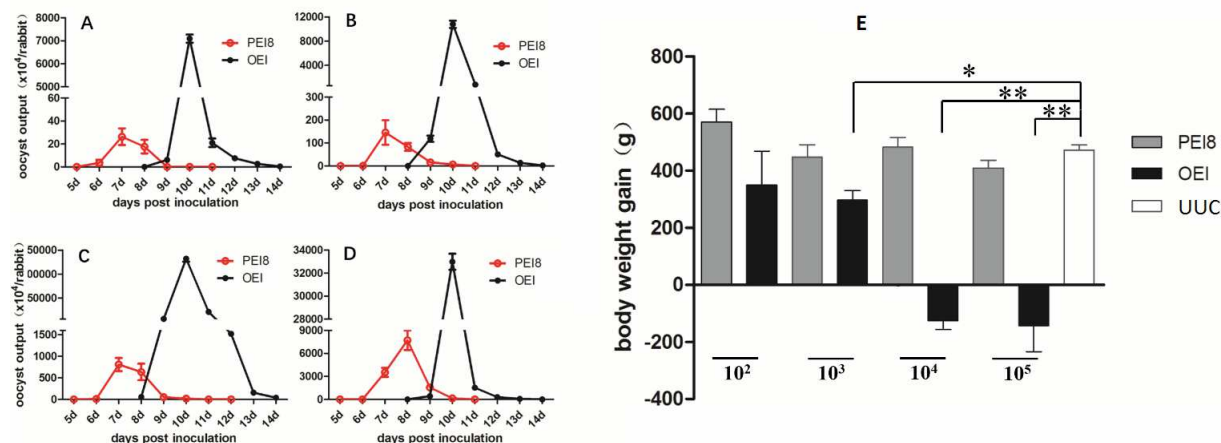


Figure 1: Oocyst output (A-D) and body weight gain (E) of rabbits (n=4) inoculated with different doses of PEI8 or OEI. A, 1×10^2 ; B, 1×10^3 ; C, 1×10^4 ; D, 1×10^5 . Statistical significance was determined using Student's *t*-test; * $p < 0.05$, ** $p < 0.01$.

Pathogenicity and immunogenicity of *E. intestinalis* precocious line

No rabbit died except one in the UCC group inoculated with 1×10^5 OEI oocysts. Diarrhoea only occurred in animals inoculated with 1×10^4 and 1×10^5 OEI oocysts. No significant decrease of weight gain occurred in rabbits inoculated with PEI8 oocysts compared that of the UUC group. In contrast, inoculation with 1×10^3 OEI oocysts was enough to induce decrease in weight gain. Significant difference was found between rabbits in UUC group and animals inoculated with 1×10^3 ($P < 0.05$), 1×10^4 ($P < 0.01$) and 1×10^5 ($P < 0.01$) OEI oocysts (Fig. 1). This result indicate that the pathogenicity of PEI8 declined greatly compared with OEI.

The body weight gain per day of UCC group decreased drastically between 7-14 days post challenge compared with that of UUC group ($P < 0.01$). There was no significant difference between the body weight gain of immunized animals with either PEI or OEI oocysts and the UUC group ($P > 0.05$) (Fig. 2).

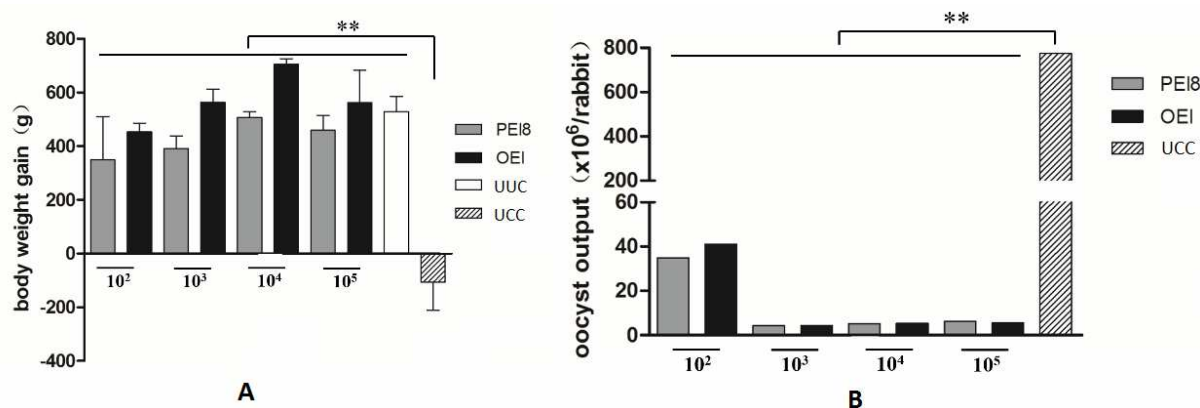


Figure 2 Body weight gain (A) and oocyst production (B) of rabbits challenged with 1×10^5 OEI oocysts. Statistical significance was determined using Student's *t*-test; * $p < 0.05$, ** $p < 0.01$.

After challenge, OEI or PEI8 immunized rabbits (with dosages 1×10^3 , 1×10^4 and 1×10^5) shed less than 1% of those of the UCC group ($P < 0.01$). The pathogenicity test data indicate the virulence of PEI8 declined significantly similar to reports on avian coccidia (Shirley et al, 1984).

Little is known about the mechanism of the precociousness of eimerian parasites, though shortened generations of schizogony and accelerated development of asexual stages were observed in several lines selected for precociousness (Jeffers, 1975; Licois et al., 1990). Our next effect will be made towards the genetic mechanisms of precocious selection and also the formulation of selected PEI8 into an anticoccidial vaccines for rabbits.

CONCLUSIONS

The precocious line produced significantly fewer oocysts during its shorter production period than the parental strain and showed lower pathogenicity. Meanwhile, the immunogenicity of PEI8 was as excellent as its parental strain. Therefore, it should be a favourable candidate for a live attenuated vaccine for rabbit coccidiosis.

ACKNOWLEDGEMENTS

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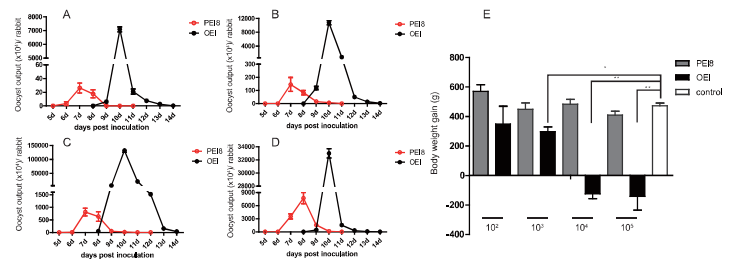


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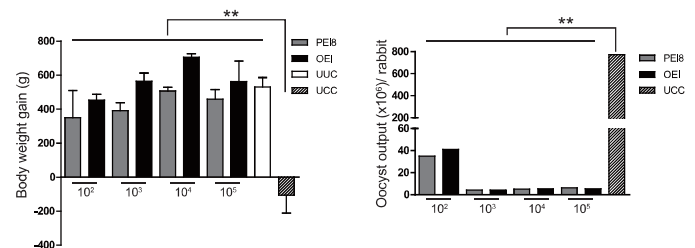


Figure 2 Body weight gain (A) and oocyst production (B) of rabbits challenged with 1×10^5 OEI oocysts. Statistical significance was determined using Student's t-test; * $p < 0.05$, ** $p < 0.01$.

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