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Partial protection against four species of chicken coccidia induced by multivalent subunit vaccine

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ABSTRACT

In this study, a multivalent subunit vaccine was designed to protect chickens against simultaneous infection by several *Eimeria* species. This vaccine contains recombinant proteins from four *Eimeria* species – *E. tenella, E. necatrix, E. acervulina* and *E. maxima* – and was evaluated for efficacy in animals. To produce this vaccine, candidate antigens from each *Eimeria* species were first screened in chickens via intramuscular inoculation and subsequent challenge. Antigens tested include recombinant proteins TA4 and SO7 from *E. tenella*, NA4 and NPmz19 from *E. necatrix*, LDH, 3-1E and MIF from *E. acervulina*, and Em6 and Em8 (two portions of EmTFP250) from *E. maxima.* A homologous challenge was then performed to identify which antigen from each species conferred the best protection. The antigens identified as most protective against its species were then challenged by heterologous species. Finally, the selected recombinant proteins from each of the four respective species were mixed with the final concentration of 400 μg/ml (100 μg of each protein/ml) to form the multivalent subunit vaccine, which was tested for efficacy in animals. The results indicated that TA4 from *E. tenella*, NA4 from *E. necatrix*, LDH from *E. acervulina*, and Em8 from *E. maxima* each induced the most effective protection from homologous challenge. Cross-protection results showed that TA4 provided partial cross-protection against *E. necatrix*, NA4 provided partial cross-protection against *E. tenella* and *E. acervulina*, LDH provided partial cross-protection against *E. tenella* and *E. necatrix*, and Em8 provided partial cross-protection against *E. tenella* and *E. acervulina.* The multivalent subunit vaccine provided partial protection against *E. tenella*, *E. necatrix*, *E. acervulina* and *E. maxima* challenge, and resulted in ACIs of more than 170. These results suggest that our candidate multivalent vaccine could protect chickens against simultaneous infection by several *Eimeria* species.

1. Introduction

Coccidiosis in chickens, one of the most serious diseases facing the poultry industry worldwide, is a parasitic infection caused by seven species of *Eimeria-E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. mitis* and *E. praecox* (Sharman et al., 2010; Blake and Tomley, 2014; Witcombe and Smith, 2014). The species *E. tenella, E. necatrix, E. maxima,* and *E. acervulina* are the most important in terms of global disease burden and economic impact (Blake and Tomley, 2014; Reid et al., 2014). The primary strategy to control avian coccidiosis relies on supplementing feed with coccidiostats; however, with the rise of drug resistance, the costly process of drug discovery, legislative restrictions on in-feed drugs, and public demands for chemical free meat, there is growing demand for alternative control strategies (Blake and Tomely, 2014; Witcombe and Smith, 2014 Witcombe and Smith, 2014). Live vaccines containing virulent or attenuated strains of *Eimeria* are available (William, 2002; Witcombe and Smith, 2014). However, live vaccines have inherent production limitations, risk of vaccinal pathogenicity as well as the potential reversion to a pathogenic form, and cost issues (Dalloul and Lillehoj, 2006; Sharman et al., 2010; Blake and Tomely, 2014; Witcombe and Smith, 2014 Witcombe and Smith, 2014). Consequently, anticoccidial subunit vaccines composed of protective antigens, either native or recombinant, have been pursued as an alternative strategy (Jenkins, 2001; Talebi and Mulcahy, 2005; Sharman et al., 2010). CoxAbic®, the first commercial subunit anticoccidial vaccine, has been successfully commercialized and has been reported to be effective in the field (Sharman et al., 2010; Blake and Tomely, 2014; Witcombe and Smith, 2014 Witcombe and Smith, 2014).

Under natural conditions, avian coccidiosis is often caused by simultaneous infections of several *Eimeria* species (del Cacho et al., 2012). Furthermore, protective immunity induced by a given *Eimeria* species is extremely specific (Dalloul and Lillehoj, 2006).

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Table 1
Characteristics of the nine protein expression plasmids.

Plasmids	*Eimeria* species	Predicted molecular weight of recombinant protein(kDa)	GenBank accession number of the inserted gene
pET28bk-TA4	*E. tenella*	25	M21088.1
pET32a-SO7	*E. tenella*	35	X15898.1
pET28a-NA4	*E. necatrix*	29.7	EU523548
pET32a-NPmz19	*E. necatrix*	55	EU795423
pET28a-LDH	*E. acervulina*	41	FJ617009
pET28a-3-1E	*E. acervulina*	22.3	AF113613
pET28a-MIF	*E. acervulina*	12.5	EU598448
pET32a-Em6	*E. maxima*	68	AY239227 (4916–5882 of EmTFP250)
pET32a-Em8	*E. maxima*	71	AY239227 (6242–7310 of EmTFP250)

Therefore, an ideal vaccine should contain antigens from multiple *Eimeria* species to provide effective protection against simultaneous infections of economically important species of *Eimeria* (Talebi and Mulcahy, 2005; del Cacho et al., 2012). In our previous studies, nine genes from four *Eimeria* species have been cloned, namely TA4 and SO7 from *E. tenella*; NA4 and NPmz19 from *E. necatrix*; LDH, 3-1E and MIF from *E. acervulina*; and Em6 and Em8 (two portions of EmTFP250) from *E. maxima*. Encouragingly, these genes have shown promise as DNA vaccines by inducing protection against homologous *Eimeria* species challenge (Xu et al., 2008; Song et al., 2010, 2013). In this study, the protective efficacies of these nine proteins were compared and the most effective recombinant protein from each of the four *Eimeria* species was determined. These four determined proteins were then evaluated for protection against heterologous species challenge. Finally, a multivalent subunit vaccine was formed by mixing the selected recombinant proteins of each species and its protective efficacy was evaluated by challenge with *E. tenella*, *E. necatrix*, *E. acervulina* and *E. maxima.*

2. Materials and methods

2.1. Parasites, birds and plasmids

Before the challenge infection, sporulated oocysts of *E. tenella*, *E. necatrix*, *E. acervulina* and *E. maxima* were passed through chickens and collected. Newly-hatched Lohman chickens were raised in a sterilized room under coccidia-free conditions until the end of the experiment. Food and water without anti-coccidia drugs were available ad libitum. All experiments were approved according to the Animal Care and Use Committee of the Jiangsu Province Animal Care Ethics Committee. Protein expression plasmids were provided by the Laboratory of Veterinary Molecular and Immunological Parasitology, Nanjing Agricultural University, China. Characteristics of the nine protein expression plasmids were shown in Table 1.

2.2. Preparation of the recombinant proteins

The nine protein expression plasmids (Table 1) were transformed into *E. coli* BL21 (DE3). The recombinant proteins were expressed and purified with His-Bind Purification Kit (Novagen) following the instructions of the kit. The purities of protein were determined by SDS-PAGE and protein concentrations were estimated using Bicinchoninic Acid kit (Sigma–Aldrich, USA). The recombinant proteins were diluted in PBS buffer with a concentration of 400 μg/ml. Stocks proteins were prepared and stored at −80 °C until further use.

2.3. Protective efficacy comparison of the recombinant proteins in homologous and heterologous challenge experiments

Animal experiments were carried out to compare protective efficacies of the recombinant proteins of each species following the animal experimental design (Table 2) and evaluation described in 2.5 and 2.6. The most protective proteins from each species were chosen to test for heterologous protection (Table 3) and to form the multivalent subunit vaccine against four species of *Eimeria.*

2.4. Protective efficacy of the multivalent subunit vaccine

The four recombinant proteins from each species selected in 2.3 were mixed with the final concentration of 400 μg/ml (100 μg of

Table 2
Protective efficacy comparison of the recombinant proteins from each species in homologous challenge experiments.

Groups	Challenge with *Eimeria* spp.	Average body weight gain (g)	Relative body weight gain (%)	Mean lesion scores	Oocyst output ($\times 10^5$)/OPG	Oocyst decrease ratio (%)	ACI
Unchallenged control	PBS	49.31 ± 5.51^{a}	100	0.00 ± 0^{a}	0 ± 0^{a}	100	200
Challenged control	*E. tenella*	28.50 ± 7.66^{b}	57.80	2.62 ± 0.92^{b}	44.8 ± 2.58^{e}	0	121.50
Recombinant TA4 protein	*E. tenella*	43.78 ± 5.70^{d}	88.79	1.30 ± 0.65^{c}	6.9 ± 0.59^{d}	84.60	174.79
Recombinant SO7 protein	*E. tenella*	42.70 ± 8.24^{c}	86.60	1.38 ± 0.68^{c}	7.4 ± 0.68^{d}	83.48	171.81
Challenged control	*E. necatrix*	20.14 ± 8.89^{b}	40.84	2.62 ± 0.94^{b}	2.27 ± 1.86^{b}	0	104.63
Recombinant NA4 protein	*E. necatrix*	43.38 ± 11.68^{c}	87.97	1.53 ± 0.90^{c}	0.43 ± 0.37^{c}	81.06	171.64
Recombinant NPmz19 protein	*E. necatrix*	42.73 ± 16.35^{c}	86.66	1.58 ± 0.91^{c}	0.59 ± 0.42^{c}	74.01	169.80
Challenged control	*E. acervulina*	22.52 ± 9.21^{b}	45.67	2.52 ± 0.86^{b}	1.73 ± 1.12^{b}	0	80.45
Recombinant LDH protein	*E. acervulina*	42.83 ± 10.89^{c}	86.86	1.37 ± 0.89^{c}	0.31 ± 0.12^{c}	82.08	172.19
Recombinant 3-1E protein	*E. acervulina*	42.56 ± 6.93^{c}	86.31	1.38 ± 0.70^{c}	0.32 ± 0.26^{c}	81.50	171.46
Recombinant MIF protein	*E. acervulina*	40.82 ± 9.39^{c}	82.78	1.44 ± 0.96^{c}	0.43 ± 0.35^{c}	75.14	167.38
Challenged control	*E. maxima*	18.67 ± 6.73^{b}	37.86	2.63 ± 0.84^{b}	1.99 ± 1.55^{b}	0	71.56
Recombinant Em6 protein	*E. maxima*	19.17 ± 5.51^{b}	38.88	2.24 ± 1.01^{b}	1.23 ± 0.98^{b}	38.19	96.48
Recombinant Em8 protein	*E. maxima*	41.94 ± 8.84^{c}	85.05	1.31 ± 0.81^{c}	0.49 ± 0.34^{c}	75.38	170.95

Note: significant difference ($P < 0.05$) between numbers with different letters. No significant difference ($P > 0.05$) between numbers with the same letter in columns.