Short communication

Co-infection of chickens with *Eimeria praecox* and *Eimeria maxima* does not prevent development of immunity to *Eimeria maxima*

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1. Introduction

One consistent finding in studies of avian coccidiosis is the species-specific immunity that develops after a primary infection with *Eimeria* oocysts (Allen and Fetterer, 2002; Shirley et al., 2007). The absence of appreciable cross-immunity between species is the basis for incorporating different *Eimeria* species in live oocyst vaccines. In fact, most live oocyst vaccines for use in broilers are composed of only three *Eimeria* species, namely *E. acervulina*, *E. maxima*, and *E. tenella*, because these are the predominant and/or the most pathogenic species in poultry operations. Recent studies by our group and others have found that, irrespective of whether drugs or vaccines are used to control coccidiosis, *E. praecox* is present on a high percentage of commercial poultry farms (Jenkins et al., 2006; Morris et al., 2007; Haug et al., 2008). In a recent study, *E. praecox* was shown to reduce the clinical effects of *E. maxima* infection when chickens were infected at the same time with both species (Jenkins et al., 2008). This work suggests that co-infection of chickens with two *Eimeria* species that infect similar regions of the gut, one species non-pathogenic (*E. praecox*), the other species pathogenic (*E. maxima*), elicits non-specific immunity against both *E. praecox* and *E. maxima*, thereby reducing clinical signs of coccidiosis. The purpose of the present study was to determine if *E. praecox*, in addition to its ameliorating effect on *E. maxima* infection, inhibits the development of immunity against *E. maxima* or *E. praecox* challenge.

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2. Materials and methods

2.1. Parasites

_Eimeria maxima_ (strain Arkansas 1) and _E. praecox_ (strain North Carolina 2) were derived from field samples, single oocyst-isolated, and propagated every 2–3 months in susceptible chickens using standard procedures (Ryley et al., 1976). The purity of both strains was confirmed by species-specific polymerase chain reaction (PCR) based on ITS1 rDNA sequence (Jenkins et al., 2006).

2.2. Cross-immunity studies

The effect of _E. praecox_ on development of immunity to _E. maxima_ infection was carried out by infecting susceptible 1-day-old male Sexsal chickens (3 groups/treatment, 5 chickens/group, 15 chickens total/treatment) with either \(10^3\) _E. praecox_ (Ep) oocysts, or \(10^3\) _E. maxima_ (Emax) oocysts, or a mixture of _E. praecox_ and _E. maxima_ (Ep + Emax) oocysts at the above dose levels. At 28 days, chickens were challenged with either \(5 \times 10^4\) _E. praecox_ oocysts or \(5 \times 10^3\) _E. maxima_ oocysts. The timing of primary and challenge infections was based on current strategies to vaccinate chickens against coccidiosis using live oocyst vaccines and to allow sufficient time for immunity to develop (Allen et al., 2005; Chapman et al., 2005; Chapman and Rayavarapu, 2007). Controls included non-immunized and non-infected chickens (NINC), and non-immunized chickens that were challenged with either \(5 \times 10^4\) _E. praecox_ oocysts or \(5 \times 10^3\) _E. maxima_ oocysts (NIC). On day 7 post-challenge, all chickens were killed by cervical dislocation and necropsied for determining upper-middle intestinal lesion scores using standard procedures (Johnson and Reid, 1970). Body weights were obtained for all individual chickens to allow calculation of weight gain during the experimental period. Feed conversion ratio was calculated for each replicate of treatments by dividing total feed consumed by total weight gain during the experimental period. The entire study was repeated twice for a total of three studies.

2.3. Statistical analysis

Body weight gain, intestinal lesion scores, and feed conversion ratios were compared between treatment groups using Duncan’s Multi-Range Comparison Test (SAS Institute, Inc., Cary, NC). Results were expressed as mean ± S.E.M. of three independent trials. Significant differences between groups were noted if \(P \leq 0.05\).

3. Results

3.1. Effect of _E. praecox_ infection on immunity to _E. maxima_

3.1.1. Weight gain depression

Control non-immunized groups (NIC) that were challenged with _E. maxima_ oocysts displayed a significant reduction (\(P < 0.05\)) in average weight gain (Fig. 1A). Average weight gain was reduced by an equal amount (\(P < 0.05\)) in chickens immunized with _E. praecox_ (Ep) alone and challenged with _E. maxima_ (Fig. 1A). In contrast, complete protection against reduced weight gain was observed in chickens immunized with either _E. maxima_ (Emax) alone or a mixture of _E. praecox_ and _E. maxima_ (Ep + Emax) showing no significant difference from non-challenged controls (NINC, \(P > 0.05\), Fig. 1A).

3.1.2. Feed conversion ratios (FCR)

The average feed conversion ratio (FCR) in non-challenged controls (NINC) was 2.40 ± 0.09, whereas average FCR in non-immunized groups that were challenged with _E. maxima_ oocysts (NIC) was 3.16 ± 0.36 (Fig. 1B), representing a significant increase over controls (\(P < 0.05\)). A similar increase in average FCR was observed in chickens immunized with _E. praecox_ (Ep) alone and challenged with _E. maxima_ (Fig. 1B). However, average FCR in _E. maxima_-challenged
chickens that had been immunized at 1 day of age with either *E. maxima* (Emax, FCR = 2.34 ± 0.12) or with a combination of *E. praecox* and *E. maxima* (Ep + Emax, FCR = 2.53 ± 0.33) was similar to non-challenged controls (NINC, Fig. 1B).

### 3.1.3. Intestinal lesion scores

The average intestinal lesion score in non-immunized chickens (NIC) that were challenged at 4 weeks of age with *E. maxima* oocysts was 1.9 ± 0.1 (Fig. 1C). A similar average lesion score was observed in *E. praecox*-immunized chickens (Ep) that had been challenged with *E. maxima* oocysts (2.1 ± 0.6). However, intestinal lesions were greatly reduced (*P < 0.05*) in *E. maxima*-challenged chickens that had been immunized with either *E. maxima* (Emax, 0.2 ± 0.1) or with a combination of *E. praecox* and *E. maxima* (0.1 ± 0.1) (Ep + Emax, Fig. 1C).

### 3.2. Effect of *E. maxima* infection on immunity to *E. praecox*

#### 3.2.1. Weight gain depression

Control non-immunized groups that were challenged with *E. praecox* oocysts displayed lower, yet insignificant (*P > 0.05*), average weight gain compared to non-challenged controls (NINC, Fig. 2A). Weight gain was also reduced in chickens immunized with *E. maxima* alone and challenged with *E. praecox* (Emax, Fig. 2A). Average weight gain in *E. praecox*-challenged chickens that had been immunized by oral inoculation with *E. praecox* (Ep) alone or with a combination of *E. praecox* and *E. maxima* (Ep + Emax) was greater than non-immunized, *E. praecox*-challenged controls (Fig. 2A).

#### 3.2.2. Feed conversion ratios (FCR) and intestinal lesions

No significant differences were observed in FCR between non-immunized *E. praecox*-challenged (NIC) and non-challenged control groups (NINC, *P > 0.05*, Fig. 2B) reflecting the low pathogenicity of *E. praecox*. Also, regardless of prior exposure to *E. praecox* and/or *E. maxima*, no significant increase in average FCR was observed in any group (*P > 0.05*). Similarly, negligible lesions were observed in *E. praecox*-infected groups, regardless of whether chickens were not immunized or were immunized with *E. praecox* and/or *E. maxima* (data not shown).

### 4. Discussion

The present study demonstrated that immunity to *E. praecox* or *E. maxima* challenge develops in chickens that were infected at 1 day of age with both *Eimeria* species. Although *E. praecox* can ameliorate clinical signs associated with *E. maxima* (Jenkins et al., 2008), it does not appear to interfere with development of immunity to *E. maxima*. While *E. praecox* appears to be less pathogenic than other *Eimeria* species, it can affect weight gain at high challenge doses, and immunity to *E. praecox* readily develops in chickens (Gore and Long, 1982). This study provides evidence that acquired resistance to *E. praecox* challenge is similar to immunity against *E. maxima*. That is, co-infection of chickens at 1 day of age with *E. maxima* and *E. praecox* does not prevent development of immunity to *E. praecox*.

The practical implications of this study and previous work showing an ameliorating effect of *E. praecox* on clinical signs associated with *E. maxima* are several-fold. One is that including *E. praecox* in a live oocyst vaccine may not only induce immunity to *E. praecox*, but also lessen the negative effects caused by vaccine strains of *E. maxima* (Williams, 1998). Second, the high prevalence of *E. praecox* in the field (Jenkins et al., 2006; Morris et al., 2007; Haug et al., 2008) may reflect persistent drug-resistance in this species, thus alternative control measures, such as vaccination, may be warranted. It remains unknown what impact *E. praecox* has on broiler productivity relative to other more pathogenic *Eimeria* species. A number of authors have shown that at high oocyst doses *E. praecox* has significant impact on weight gain and feed conversion efficiency (Long, 1968; Gore and Long, 1982; Jorgensen et al., 1997; Williams, 1998). Whether chickens are exposed to these levels of *E. praecox* in the field is unknown.

These findings may also provide some insight on the nature of immunity to *E. maxima*, and possibly other *Eimeria* as well, and indicate potential ways to reduce clinical effects of coccidiosis during a primary infection. Our research findings suggest that inducing a local nonspecific immune response in a region of the chicken intestine invaded by a particular *Eimeria* species may reduce the clinical signs of coccidiosis, but still allow for limited replication of the parasite, and development of protective immunity to challenge infection.
References


