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A comparison between IgG antibodies against *Eimeria acervulina*, *E. maxima*, and *E. tenella* and oocyst shedding in broiler-breeders vaccinated with live anticoccidial vaccines

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Abstract

The aim of this study was to compare the IgG response against *Eimeria acervulina*, *E. maxima*, and *E. tenella* and oocyst shedding in broiler-breeders vaccinated with live (virulent or attenuated) anticoccidial vaccines. Serum and faeces samples were collected from chickens before (4 days of age) and after (25 days of age) vaccination with virulent (group A) or attenuated (group B) vaccines and unvaccinated control birds (group C). Criteria for evaluation included the number of oocysts shed on the litter (McMaster technique), the levels of specific serum IgG (ELISA) and the frequencies of antigenic fractions recognised by IgG antibodies (immunoblotting). The results demonstrated that the virulent vaccine (A) presented an ideal association (low oocyst number and high IgG positive rate) for *E. acervulina* and *E. tenella* while the immunised flock with the attenuated vaccine (B) demonstrated this ideal association only for *E. maxima*. Both vaccinated flocks demonstrated a better association for three *Eimeria* spp. studied when compared to the control group, which presented a divergent association (high oocyst number and low IgG positive rate), particularly for *E. acervulina*. Immunoblotting assays revealed different antigenic fractions that may be considered as immunodominant antigens. The present study suggests that *E. acervulina* is critically present in the region studied and the use of live anticoccidial vaccines (virulent or attenuated) showed to be effective to control the clinical coccidiosis in vaccinated flocks.

Keywords: *E. acervulina*; *E. maxima*; *E. tenella*; Live anticoccidial vaccines; IgG antibodies; Oocyst shedding

1. Introduction

Avian coccidiosis caused by *Eimeria* spp. of protozoan parasites is responsible for important economic losses in the poultry industry world-wide. The disease causes intestinal epithelium lesions, reduction of body weight and several degrees of mortality according to the affected species. Seven *Eimeria* spp. have been recognised to infect chickens (*Gallus gallus*): *E. acervulina*, *E. maxima*, *E. tenella*, *E. brunetti*, *E. necatrix*, *E. mitis* and *E. praecox*. The occurrence of these species has been confirmed in countries where there have been intensive research efforts, such as the USA, UK, the Czech Republic, France, Sweden, Argentina, and Brazil [1,2]. The Brazilian poultry industry, considered the third largest producer of broilers in the world in 1997 [3], presents important economical losses due to the difficulties in controlling poultry coccidiosis.

Immunity to coccidiosis is largely dependent on cellular immunity and has been well documented in chickens [4]. Even though extensive work has failed to demonstrate any practical importance of circulating antibodies against *Eimeria* spp. in chickens [5], some investigators suggest that antibodies may play some role in providing protective immunity [6]. Immunodominant surface antigens of *E. acervulina* sporozoites have been identified by immunoblotting. It has been assumed that these markers are responsible for stimulating strong antibody response and also for generating a protective cellular immune response [7]. In addition, serum antibody titres are clear evidence of prior exposure to parasite and probably develop in parallel with cellular immunity [8].

The development of drug resistance represents a recurring problem that has led to the increased interest in the search for anticoccidial vaccines to control the disease. Live virulent

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strains comprise a variable number of wild type strains depending on their formulation and field application [9]. There are at least four types of live anticoccidial vaccines currently available for use in chickens, two of them are virulent and two are attenuated [10].

The present article describes the evaluation of live virulent and attenuated anticoccidial vaccines in an experimental farm. In addition, kinetics of IgG response in vaccinated birds with the virulent vaccine on a commercial farm is also studied. Criteria for evaluation included the number of oocysts shed on the litter, the presence of specific IgG antibodies in the serum and the frequencies of antigenic fractions recognised by IgG antibodies.

2. Materials and methods

2.1. Animals and vaccines

Two sets of experiments were carried out. In the first, day-old Hubbard Hi-Y (female) and Peterson PMM (male) chicks were received at the Experimental Farm School of Veterinary Medicine, Federal University of Uberlândia, Brazil. The chicks were allocated in flocks A, B and C, each flock consisting of 1100 birds (550 male and 550 female). All animals were given food and water without any anticoccidial drugs and kept in an area containing wood shaving litter at a density of 7.4 m^2 in flock A, 7.5 m^2 in flock B, 8.8 m^2 in flock C. Chickens of flock A were vaccinated with a live virulent anticoccidial vaccine Coccivac® (Schering-Plough Animal Health, USA). According to the manufacturer's technical manual, this vaccine consists of a stabilised suspension of sporulated oocysts of eight *Eimeria* spp.: *E. acervulina*, *E. maxima*, *E. tenella*, *E. necatrix*, *E. brunetti*, *E. praecox*, *E. hagani* and *E. mivati*. Chickens of flock B were vaccinated with a live attenuated vaccine Livacox® (Biopharm, Czech Republic) containing sporulated oocysts of *E. acervulina*, *E. maxima* and *E. tenella*. Flock C was comprised of non-vaccinated control birds. The floor pen chicks were vaccinated on the fourth day of age by administration in water, with recommended doses for both vaccines.

In the second set of experiments, newly hatched Cobb chickens placed in a commercial broiler-breeder farm in the region of Uberlândia, Brazil, were vaccinated with live virulent vaccine Coccivac® by aerosol on the first day of age at the hatchery. The flock consisting of 7071 chickens was kept in a building containing wood shaving litter at a density of 10 m^2 where food and water were given free of anticoccidial drugs.

2.2. Field samples

In the first set of experiments blood samples were collected from the wing vein of 25 chicks from each flock (A, B and C) at 4 days of age (pre-vaccination) and 25 days of age (21 days post-vaccination). In the second set of experiments, blood samples were collected from 40 chickens following 30, 60 and 90 days post-vaccination. Blood samples were centrifuged and sera stored at −20 °C until analysis. In parallel, in both experiments, the same weight of fresh faeces on the litter was also collected from different places in a poultry house of each flock at the same time where sera were collected. Oocysts in faeces were detected by means of flotation with saturated salt solution, counted and identified in McMaster chamber as described by Long et al. [11]. Counts were expressed as mean numbers of oocysts/g faeces.

2.3. Parasite and soluble antigens

Sporulated oocysts of *E. acervulina* ("Cu" strain), *E. maxima* ("I" strain) and *E. tenella* ("Pa" strain) were supplied by the Laboratory of Avian Coccidiosis, Universidade Estadual de Campinas (UNICAMP), Brazil. The soluble parasite antigens were obtained from suspension of sporulated oocysts in 0.01 M phosphate-buffered saline (PBS) pH 7.2 by disruption, freeze–thawing and sonication cycles [12]. After centrifugation at 12,000 × *g* for 15 min, the supernatants were collected, the protein concentration was determined [13], and antigen aliquots were stored at −20 °C.

2.4. ELISA

Optimal conditions for IgG-ELISA were established in preliminary experiments through block titration of the reagents, as described by Smith et al. [12] with modifications. Microtiter plates were coated overnight with *Eimeria* soluble antigens (10 μg/ml) and subsequently blocked with PBS plus 1% bovine serum albumin (BSA). Serum samples (1:50) were incubated for 1 h at 40 °C, following the biotinylated mouse anti-chicken IgG (1:500; prepared as described by Reading et al. [14]) for 1 h at room temperature (RT), and streptavidin-peroxidase (1:500; Sigma, USA) for 30 min at RT. Enzyme substrate (0.01 M ABTS and 0.03% H_2O_2) was added and the optical density (OD) was read at 405 nm (Titertek Multiskan, Flow Laboratories, USA). Positive control sera obtained from vaccinated chickens and negative control sera obtained from specific-pathogen-free (SPF) chickens (Laboratory of Avian Coccidiosis, UNICAMP, Brazil) were included in each assay. Cut-off was determined as the mean OD of negative control sera plus three standard deviations. Antibody titres were arbitrarily expressed as ELISA indexes (EIs) as follows: $EI = (OD_{sample}/OD_{cut\text{-}off})$, where values of $EI \geq 1.0$ were considered as positive [15].

2.5. SDS-PAGE and immunoblotting

Antigen solutions were mixed in sample buffer and separated by SDS-PAGE at 12% as previously described by Laemmli [16]. After electrophoresis, proteins were transferred to nitrocellulose membrane by using a

semidry transfer system (Multiphor II Electrophoresis Unit, Pharmacia-LKB, Sweden) as described by Towbin et al. [17]. Nitrocellulose strips were blocked with 0.05% Tween 20–PBS plus non-fat milk, and sequentially probed with chicken sera (1:50), biotinylated mouse anti-chicken IgG (1:250), and streptavidin-peroxidase (1:250; Sigma, USA). Finally, strips were revealed with 0.4% H_2O_2 and DAB (Sigma, USA).

2.6. Statistical analysis

Statistical analysis consisted of determinations of geometric means with 95% confidence intervals. The differences between the means were analysed by the unpaired Student *t*-test, and values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Eimeria-specific IgG response and oocyst shedding

Chicken flocks were monitored for *Eimeria*-specific IgG antibody response and oocyst shedding. In the first set of experiments, IgG levels to *E. acervulina*, *E. maxima* and *E. tenella* expressed as EI obtained for chickens at 4 days of age (pre-vaccination) and 25 days of age (post-vaccination) from flock A (virulent vaccine), flock B (attenuated vaccine), and flock C (unvaccinated birds) are demonstrated in Fig. 1. Variations on the levels of maternal-derived antibodies against three species of *Eimeria* were found as demonstrated by different positivity rates (EI > 1) at 4 days of age among the different flocks. Both vaccinated flocks (A and B) showed significantly higher positivity rates than the flock C (control group) at 25 days of age for *Eimeria* species analysed ($P < 0.05$). However, only for *E. maxima*, IgG positivity rate was significantly higher in flock B (96%) than flock A (32%) ($P < 0.05$).

Analyses of specific IgG antibody levels for *E. acervulina* from flocks A, B and C at 4 days of age showed EI mean values with 95% confidence intervals of 2.4 (2.2–2.7), 1.9 (1.8–2.2), and 1.2 (1.1–1.5), respectively, which were significantly higher than those obtained at 25 days of age [1.1 (1.0–1.2), 1.6 (1.5–1.7), 0.8 (0.8–0.9), respectively] (Fig. 1A). As shown in Fig. 1B, only in flock A, IgG levels against *E. maxima* had EI values significantly higher at 4 days of age [1.6 (1.4–2.0)] as compared at 25 days of age [0.9 (0.9–1.1)]. In contrast, IgG anti-*E. tenella* levels showed EI values significantly higher at 25 days of age [2.2 (2.0–2.3)] than at 4 days of age [1.2 (1.1–1.6)] only in flock B (Fig. 1C). Sera collected post-vaccination from flocks A and B had significantly higher EI mean values for all *Eimeria* spp. when compared with flock C, whereas the highest antibody levels were found in flock B ($P < 0.001$).

Oocysts of *E. acervulina*, *E. maxima* and *E. tenella* were found in litter samples from all vaccinated flocks, when chickens were 25 days of age. Although the oocyst number varied among the different flocks, a higher *Eimeria* total oocyst was present in litter samples from flock B as compared to flock A, approximately 20-fold for *E. acervulina*, 1.5-fold for *E. maxima*, and 4-fold for *E. tenella* (Fig. 1A–C, respectively). On the other hand, chickens from unvaccinated flock C showed higher *E. acervulina* oocyst shedding (60.2×10^6/g faeces) than vaccinated birds from flock A (0.7×10^6/g faeces) and flock B (16.3×10^6/g faeces), approximately 60- and 4-fold higher, respectively (Fig. 1A). In contrast, *E. maxima* and *E. tenella* oocyst numbers detected in litter sample from flock C showed very low oocyst number (0.1×10^6/g and none, respectively), as shown in Fig. 1B and C. No oocysts were found in the litter sample from birds at 4 days of age, except flock B for *E. maxima* (0.5×10^6/g faeces).

3.2. Kinetics of IgG response to Eimeria and oocyst shedding in vaccinated birds

In the second set of experiments, kinetics of *Eimeria*-specific IgG response and oocyst number in chickens vaccinated with live virulent vaccine are shown in Fig. 2. IgG levels against *E. acervulina*, *E. maxima* and *E. tenella* were quantified at 30, 60 and 90 days post-vaccination. High positivity rates (96–100%) were found for all species of *Eimeria* throughout kinetics, except for *E. acervulina* at 30 days post-vaccination (53%). Increasing IgG levels were also showed for all *Eimeria* species reaching EI mean values with 95% confidence intervals of 3.2 (3.1–3.4) for *E. acervulina* (Fig. 2A), 2.9 (2.7–3.0) for *E. maxima*, (Fig. 2B), and 3.4 (3.3–3.5) for *E. tenella* (Fig. 2C) at 90 days post-vaccination. Oocyst numbers of *E. acervulina* in litter samples collected at 60 days of age (2.8×10^6/g faeces) were higher than those collected at 30 and 90 days of age, about 14- and 9-fold higher, respectively. In contrast, the highest *E. maxima* and *E. tenella* oocyst numbers were detected in litter samples collected at 30 days of age (1.4 and 0.6×10^6/g faeces, respectively), approximately 7- and 6-fold higher than oocyst numbers collected at 60 days of age, respectively.

3.3. SDS-PAGE and immunoblotting

Electrophoretic profiles (SDS-PAGE) of soluble antigens from *E. acervulina*, *E. maxima* and *E. tenella* are shown in Fig. 3, whereas representative immunoblots of the first experiment are shown in Fig. 4. Only the major antigenic bands recognised by chicken sera are demonstrated for *E. acervulina*, *E. maxima*, and *E. tenella*.

The frequencies (%) and the approximate sizes (kDa) of more prominent antigenic fractions of *E. acervulina*, *E. maxima* and *E. tenella* recognised by specific IgG antibodies in chicken sera of the first experiment are summarised in Table 1.

Similar to ELISA, immunoblotting results demonstrated high individual variation. Sera collected at 4 days of age

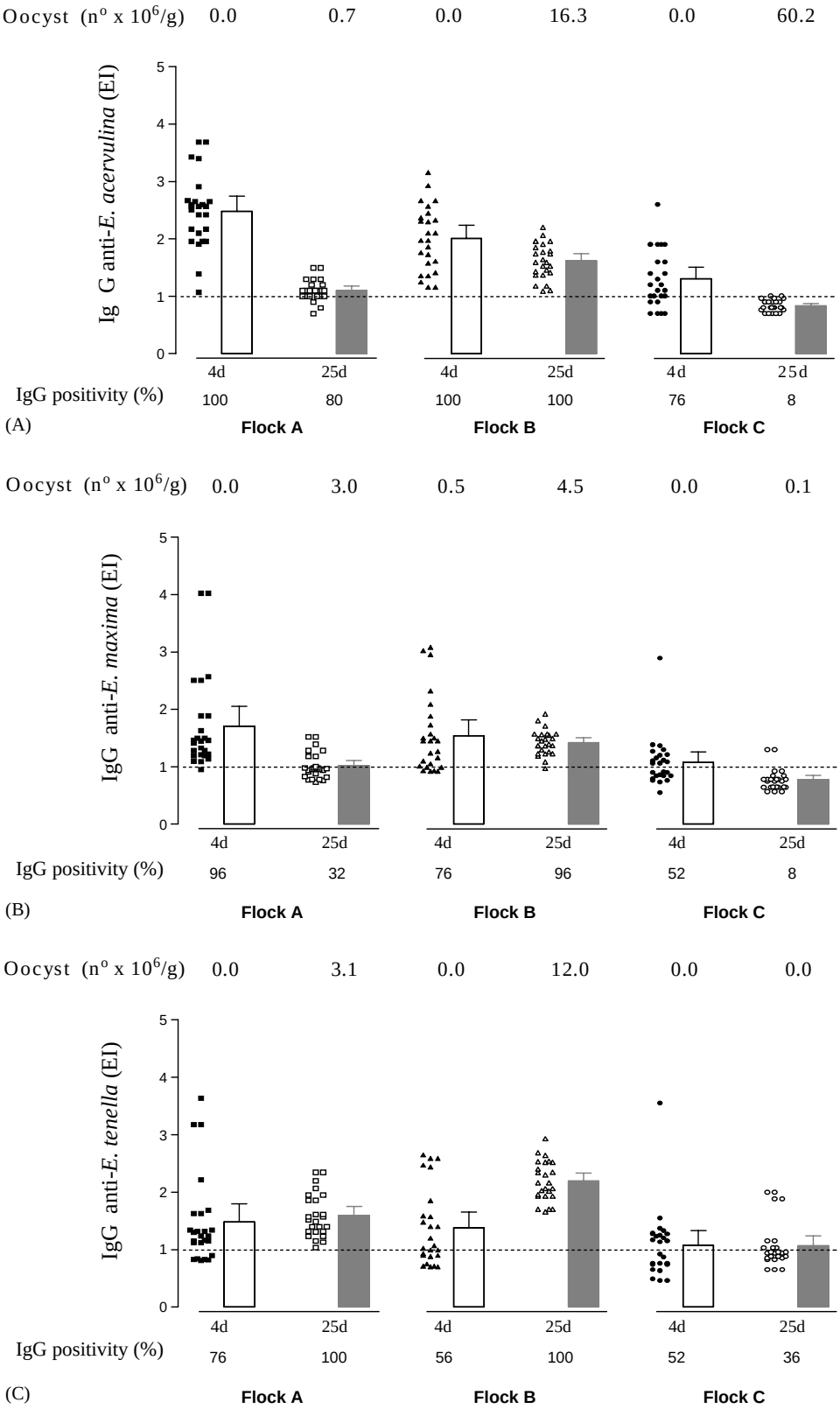

Fig. 1. IgG levels to *E. acervulina* (A), *E. maxima* (B) and *E. tenella* (C) determined by ELISA index (EI) for the first set of experiments. Flock A: field samples collected at 4 days of age (pre-vaccination) and 25 days of age (21 days post-vaccination with live virulent anticoccidial vaccine). Flock B: field samples collected at 4 days of age (pre-vaccination) and 25 days of age (21 days post-vaccination with live attenuated anticoccidial vaccine). Flock C: field samples collected from non-vaccinated control birds. The dashed lines indicate the cut off (EI > 1) and bars indicate the geometric means plus standard deviations obtained for each flock. Oocyst shedding (number × 10^6/g faeces) and specific IgG positivity rate (%) are also demonstrated.

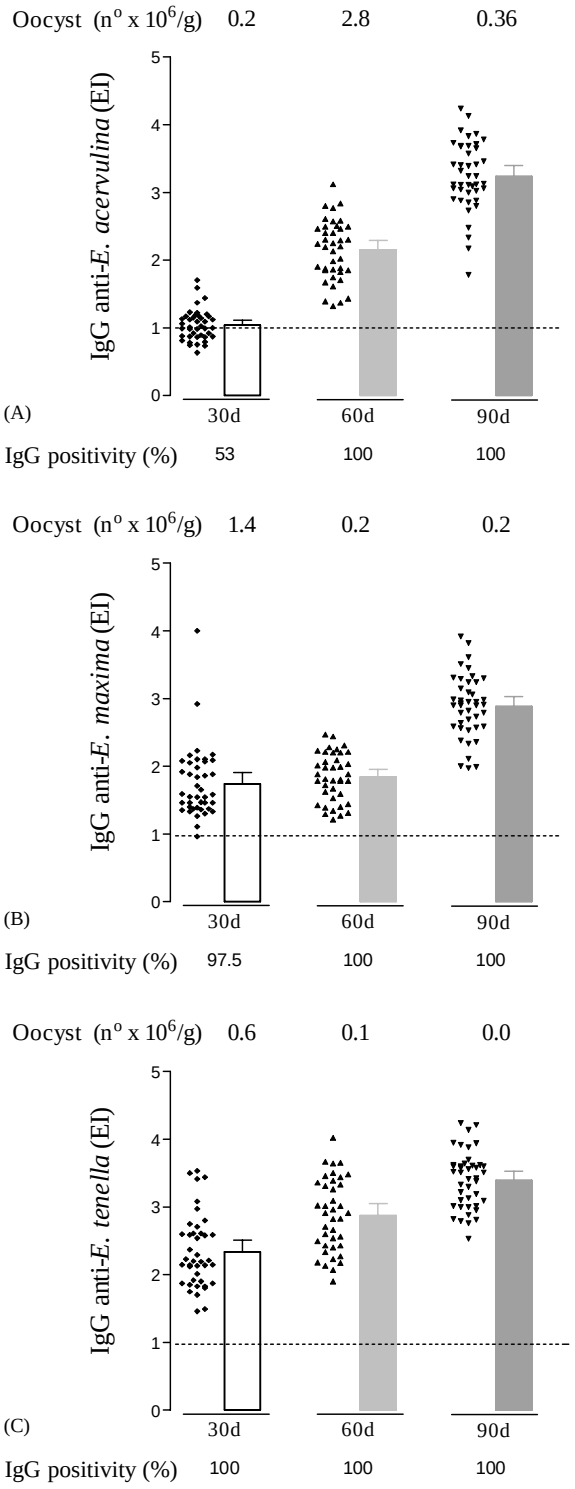

Fig. 2. Kinetics of IgG response to *E. acervulina* (A), *E. maxima* (B) and *E. tenella* (C) determined by ELISA index (EI) in vaccinated birds for the second set of experiments. Field samples were collected at 30, 60 and 90 days of age, following vaccination with virulent anticoccidial vaccine by aerosol at first day of age. The dashed lines indicate the cut-off (EI > 1) and bars indicate the geometric means plus standard deviations obtained for each period of time. Oocyst shedding (number × 10^6/g faeces) and specific IgG positivity rate (%) are also demonstrated.

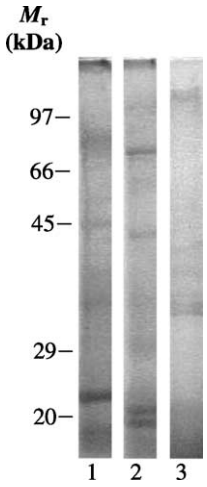

Fig. 3. Electrophoretic profile (SDS-PAGE) of soluble antigens from *E. acervulina* (lane 1), *E. maxima* (lane 2) and *E. tenella* (lane 3). Polyacrylamide gels at 12% were stained with Coomassie brilliant blue. Relative molecular masses (Mr) in kDa are shown on the left.

from all flocks recognised immunodominant bands of *E. acervulina* (frequency higher than 70%) with molecular mass ranging from 217–220, 75–81 to 70 kDa. In addition, a lower molecular weight band (18 kDa) was recognised

Table 1
Frequencies (%) of *E. acervulina*, *E. maxima* and *E. tenella* major antigenic fractions recognised by specific IgG in chicken sera in the first set of experiments

Antigenic fractions (kDa)	Frequency (%)					
	Flock A		Flock B		Flock C	
	4 days	25 days	4 days	25 days	4 days	25 days
E. acervulina						
217–220	100	57	100	37*	100	14*
75–81	86	100	86	100	100	29*
70	86	29	86	50	71	0*
45–50	20	14	20	75	14	43
18	80	0*	80	0*	14	14
E. maxima						
219	71	100	71	100	14	71
93–96	14	57	14	100*	43	0
56	0	57*	0	71*	57	0*
51	0	14	0	86*	0	0
42	0	14	0	57*	28	0
27	0	0	0	57*	0	0
18	71	0*	71	57	28	0
E. tenella						
90–93	57	100	57	57	0	0
81–84	100	100	100	100	86	0*
27	14	71*	14	0	0	0
18	0	100*	0	14	0	0

Flock A: sera were collected at 4 days of age (pre-vaccination) and 25 days of age (21 days post-vaccination with a live virulent anticoccidial vaccine). Flock B: sera were collected at 4 days of age (pre-vaccination) and 25 days of age (21 days post-vaccination with a live attenuated anticoccidial vaccine). Flock C: sera of non-vaccinated birds (control group).

* $P < 0.05$.

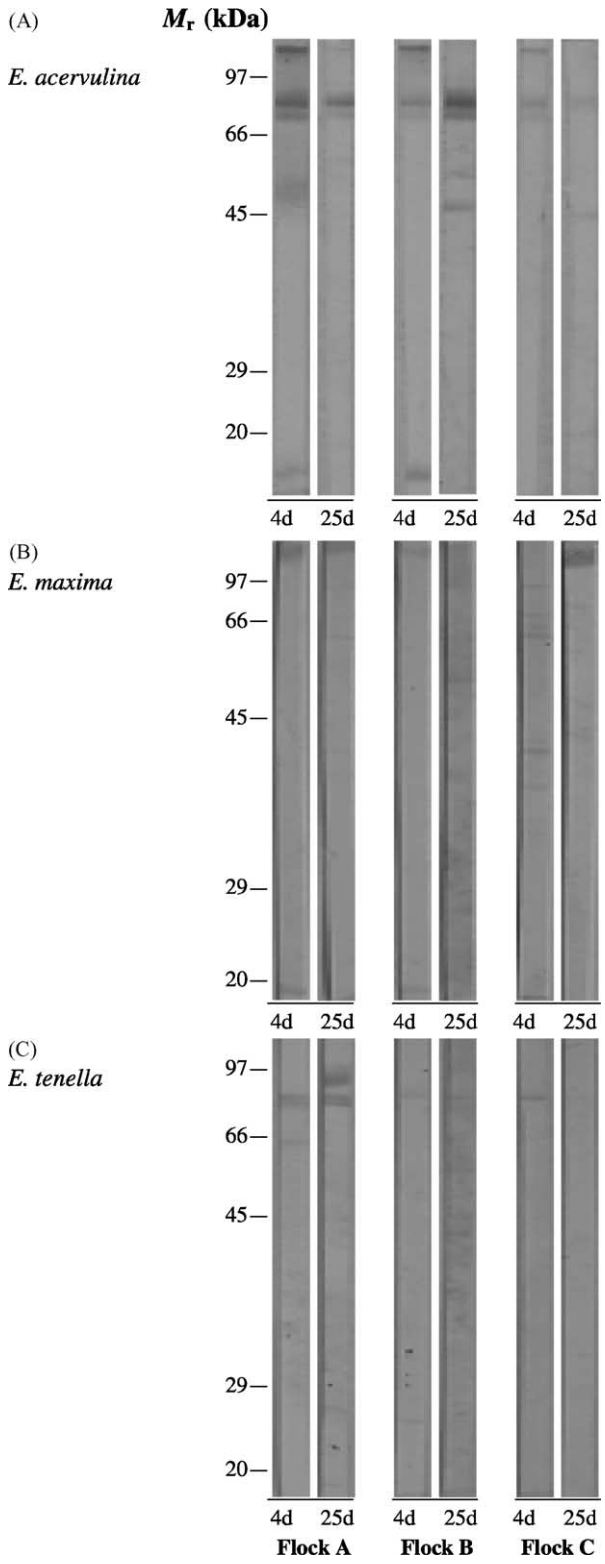

Fig. 4. Representative immunoblots showing the major antigenic fractions of *E. acervulina* (A), *E. maxima* (B) and *E. tenella* (C) recognised by chicken sera in the first set of experiments. Chicken sera were collected at 4 days of age (pre-vaccination) and 25 days of age (21 days post-vaccination) after live virulent (flock A) and attenuated (flock B) anticoccidial vaccines. Sera were also collected from non-vaccinated control birds (flock C). Relative molecular mass (Mr) in kDa are shown on the left.

predominantly by sera from flocks A and B. Comparing pre- and post-vaccination sera, three immunodominant antigenic fractions (217–220, 75–81 and 70 kDa) showed a significant reduction on their frequencies only in flock C ($P < 0.05$). Additionally, the 217–220 and 70 kDa antigenic fractions had also decreased frequencies in both flocks A and B though significantly only in flock B ($P < 0.05$) while the 75–81 kDa band maintained with high frequency. Sera of vaccinated birds at 25 days of age showed no reactivity to the 18 kDa band in comparison to its high frequency (80%) found at 4 days of age. Another antigenic fraction (45–50 kDa) showed increased frequency, although non-significant ($P = 0.06$) in post-vaccinated sera only from flock B.

Immunodominant antigenic fractions of *E. maxima* with molecular mass of 219 kDa and 18 kDa were recognised by bird sera at 4 days of age in all flocks, particularly in flocks A and B, exhibiting frequency over 70%. When analysing these antigenic bands in post-vaccination sera, only p219 was recognised in all flocks, while p18 maintained its frequency only in flock B. In contrast, several non-recognised immunodominant antigenic fractions by pre-vaccination sera (p93–96, p56, p51, p42 and p27) showed significantly increased frequency (over 50%) in post-vaccinated sera from flock B ($P < 0.05$). From these, only p56 was recognised in post-vaccination sera from flock A ($P = 0.03$). In flock C, only p219 was recognised (71%) by bird sera at 25 days of age.

For *E. tenella* antigenic fractions, only the 81–84 kDa bands were immunodominant (over 80%) at 4 days of age for all flocks, maintaining with high frequency in post-vaccinated sera from flocks A and B. Two antigenic bands, p27 and p18, showed significantly increased frequency in post-vaccinated sera only from flock A ($P < 0.01$). Another band (p90–93) presented frequency over 50% in both pre- and post-vaccination sera from flocks A and B, and were not recognised by sera from flock C, at any time. In addition, no bands were detected in bird sera at 25 days of age from flock C.

4. Discussion

Immunity to coccidiosis is T-cell dependent [18] and, although cell-mediated immune responses are of paramount importance, antibodies participate in protection per se and, possibly, as modulators of cellular responses [19]. It is well established that infection with eimerian coccidia, which invade the intestinal epithelial cells of their hosts induces the production of specific antibodies [20]. Lillehoj and Lillehoj [21] have pointed out the potential role of *Eimeria*-specific antibodies to inhibit indirectly the parasite invasiveness and/or development and may offer an additional method of controlling coccidiosis.

The presence of oocysts in the vaccinated birds is expected as a consequence of parasite development within the intestine cell, necessary for the induction of protective immune

response against later challenges. Thus, constant re-infection with small number of oocysts would be expected to maintain high level of protecting immunity stimulated initially by vaccine [22]. High levels of anti-sporozoite serum IgG probably indicate multiple exposures to the parasite and sera with low titres of sporozoite-specific IgG may represent chickens not exposed to sufficient antigenic challenge to induce an antibody response [23]. Thus, low numbers of oocysts and high IgG positivity rate would represent a highly favourable association to rear broiler-breeders in the field.

In the present work, two live anticoccidial vaccines were evaluated, one virulent and another attenuated, by associating the number of *E. acervulina*, *E. maxima* and *E. tenella* oocysts detected in litter samples and IgG positivity rate against three species of *Eimeria*. The results obtained in the first set of experiments demonstrated that the virulent vaccine (A) presented the ideal association mentioned above for *E. acervulina* and *E. tenella* while the immunised flock with the attenuated vaccine (B) demonstrated an ideal association only for *E. maxima*. According to these results two factors may be considered: (1) initial oocyst number per dose in different vaccines; and (2) distinct degrees of pathogenicity conferred by vaccine strains. Therefore, hypothetically, the number of oocysts per dose of vaccine A should be lower when compared to the number of attenuated oocysts in vaccine B, since the virulent strain may trigger more easily the disease. This is in agreement with our results that showed lower presence of oocysts in the litter of flock A than in the litter of flock B. However, the analysis of parasitological and humoral responses of the host for vaccine A failed to establish the ideal association for *E. maxima*, inducing a deficient antibody response in this flock. In contrast, higher number of oocysts was found in flock B for *E. acervulina* and *E. tenella* than in flock A, although the chickens in flock B did not show symptoms of clinical coccidiosis latter on (data not shown).

On the other hand, both vaccinated flocks demonstrated a better association for three *Eimeria* species studied when compared to the control group, except for *E. acervulina* (flock C at 25 days old) that presented a divergent association demonstrating the highest number of oocysts in the litter and the lowest specific IgG positivity rate. These results disagree with earlier studies [24] that showed a direct relation between the number of oocysts in the litter and IgG serum levels, being associated to the severity of infection. The time of this oocyst exposure, insufficient to stimulate a serum IgG response, could be a possible explanation. Accordingly, Lillehoj and Ruff [25] reported that parasite reactive serum IgY (IgG) and biliary sIgA antibodies reach maximum levels 8–14 days later after oral infection. In addition, latter observations revealed that only flock C presented clinical and pathological manifestations due to *E. acervulina* infection (data not shown). Although few epidemiological studies on the prevalence of these species have been developed in Brazil [26], the present investigation suggests that *E. acervulina* is critically present in the studied region. Therefore, the use of live polyvalent anticoccidial vaccines (virulent or attenuated) showed to be effective to control the clinical coccidiosis in vaccinated flocks, even in the presence of high number of *E. acervulina* oocysts in the environment.

The presence of maternally transferred IgG antibodies to three species of *Eimeria* analysed was found in all flocks at 4 days of age, as reported by Smith et al. [27] for *E. maxima*. These authors showed that breeding hens infected with *E. maxima* produced parasite-specific antibodies that were transferred to eggs. Such transmission-blocking immunity is able to reduce oocyst shedding and is comparable to the most effective anticoccidial drugs, and may be sufficient to control coccidiosis in the field [28].

Transmission-blocking immunity by antibodies may be achieved by using *Eimeria*-specific monoclonal antibodies against the homologous parasite [29,30]. Our results showed that non-vaccinated chickens demonstrated a sharp decrease in maternally derived antibody levels at 25 days of age. This rapid decline on IgG levels emphasises the importance of vaccination to prevent clinical coccidiosis, since only non-vaccinated control chickens developed clinical disease as mentioned previously.

Due to the fact that vaccine A is more frequently used in the regional poultry industries, this vaccine was evaluated in the field for a longer period in the second set of experiments. Kinetics of IgG antibodies pointed out a high rate of IgG positivity which remained constant and a strong association with the number of oocysts, reflecting the immunity acquired by the chickens. The oocyst number maintained a decreasing profile with tendency to reach a baseline number of oocysts in the litter. However, the presence of a greater number of *E. acervulina* oocysts lately was verified in association with an increasing IgG response. A reasonable explanation for this fact could be related to a greater prevalence of this species in the region.

In electrophoresis studies, *E. acervulina* immune sera recognised 10 dominant bands ranging from 21 to 110 kDa [7]. In our immunoblotting analysis, two *E. acervulina* antigenic fractions (75–81 and 70 kDa) demonstrated reactivity in vaccinated chicken sera. However, non-vaccinated chickens at 25 days old presented low or no reactivity to these bands, suggesting that such fractions may represent potentially immunodominant antigens to induce a protective immunity, since flock C was more susceptible to infection.

In sporulated oocysts and merozoites of *E. maxima*, the protein band consistently recognised by maternally derived protective IgG is a 230 kDa protein which appears to be the most abundant protein [27]. In the present study, the 219 kDa band of *E. maxima* was often detected in serum samples from 4-day-old chickens and later was also found in serum samples of vaccinated chickens from flocks A and B. Thus, this band seems to represent an important immunodominant component for triggering a protective immune response. On the other hand, the control group at 25 days old did not show any reactivity to the antigenic fractions between 40 and 100 kDa, while sera from vaccinated chickens showed

wide reactivity to these bands. These results suggest that antigenic fractions of lower molecular masses may also be considered as immunodominant antigens.

Karkhanis et al. [31] identified a 26 kDa polypeptide with protective activity purified from *E. tenella*-sporulated oocysts using SDS-PAGE electrophoresis. Observations in the present study revealed that the 27 kDa antigenic fraction presented reactivity in serum samples of vaccinated chicken from flock A, while this reactivity was not seen in sera from flocks B and C. On the other hand, 81–84 kDa fractions were very frequent in serum samples at 4 days of age as well as in vaccinated chickens. Therefore, these fractions might be reasonable candidates as vaccine antigens for the development of protective immune response since the control group did not present any reactivity to these antigens.

The present study did not aim to define the best vaccine categorically since other criteria and other commercial vaccine studies in addition to those used in this study would be necessary. The observed variability in the performance of different strains after vaccination may be due to the flock management, poultry genetic background, adequate cycle of the parasite strains contained in the vaccine to stimulate immunity, exposure to bacterial and viral infections that may be immunossuppressing and environmental factors. Such studies should include a survey on the prevalence of species and the best response to drugs or vaccines obtained in poultry production. Further studies on the role of anticoccidial vaccines in the field should be explored in order to better understand the immune response mechanism developed by chickens in management situations in poultry farms.

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