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Research paper

Protective potential of diclazuril-treated oocysts against coccidiosis in layer chicks

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ABSTRACT

Diclazuril, which is widely used for the prevention of coccidiosis in chickens, has a lethal effect on asexual and sexual stages of *Eimeria* spp. However, little is known about its effect on the exogenous stages of *Eimeria* spp. In this study, we evaluated the effect of *in vitro* treatment with 0.2% diclazuril on unsporulated and sporulated oocysts of *Eimeria* spp. For this purpose, a total of 180 male layer chicks aged one day were randomly divided into 5 experimental groups. Each group was divided into 3 replicates of 12 chicks each. Group 1 (G1) and Group 2 (G2) were negative (non-immunized and non-challenged) and positive (non-immunized and challenged) controls, respectively. Group 3 (G3) was immunized per os with 1.0×10^4 non-diclazuril treated-sporulated oocysts. Groups 4 (G4) was immunized per os with 0.2% diclazuril treated-unsporulated oocysts (1.0×10^4) in which diclazzuril didn't affect sporulation. Group 5 (G5) was immunized per os with 0.2% diclazuril treated-sporulated oocysts (1.0×10^4). Chicks of G2, G3, G4, and G5 were challenged with 7.5×10^4 untreated sporulated oocysts at the age of 21 days, while the group 1 chicks remained unchallenged. G4 and G5 animals immunized with 0.2% diclazuril-treated oocysts showed a significant decrease in bloody diarrhea severity, lesion scores, and oocyst counts in comparison to those immunized with untreated oocysts. Furthermore, histopathologic findings showed a low number of parasitic stages in cecal tissues in G4 and G5. A significant increased body weight gain was observed in Gs 4 and 5 in comparison to G2. In addition, expression levels of IL-2, IL-12, and IFN-γ were significantly increased in G4 and G5. In conclusion, diclazuril is effective in attenuating *Eimeria* oocysts and thus provides an alternative approach for using diclazuril-treated oocysts to protect chicks against *Eimeria* challenge.

1. Introduction

Chemotherapy is the main strategy for coccidiosis control in poultry. Frequent and indiscriminate use of anticoccidial drugs has led to the development of resistance to anticoccidial drugs (Chapman, 1997; Ryley, 1980). Diclazuril, a benzene-acetonitrile derivative, possesses significant activity against different developmental stages of *E. tenella* and the asexual stages of *E. acervulina* (Conway et al., 2001; Maes et al., 1991). Diclazuril, a nucleoside analogue, affects mainly later phases of coccidial differentiation (Verheyen et al., 1988). Previous research has demonstrated that diclazuril disrupts the oocyst wall of *Eimeria* species (Verheyen et al., 1989). There are, however, negative views towards in-feed medication of livestock in general and fear of drug residues in foods and food additives. These provide a significant drive for focusing on the immunological control of avian coccidiosis (Shirley and Lillehoj, 2012). The vaccination strategy is an effective, practical, and an important alternative method for coccidiosis control (Dalloul and Lillehoj, 2006; Conway and McKenzie, 2007). Precocious lines are the first live attenuated vaccines that have been reported to protect poultry against coccidiosis (Shirley and Lillehoj, 2012). However, the production of live attenuated vaccines is a laborious and time-consuming process (Fetterer et al., 2014). New-generation molecular vaccines (Lillehoj et al., 2005) and novel adjuvants in combination with the recombinant *Eimeria* profilin subunit antigen (Jang et al., 2010) still

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need further development. Therefore, a new anticoccidial vaccine is urgently needed (Li et al., 2004; Shirley et al., 2005). Coccidian oocysts are highly resistant to environmental influences, including UV, ozone, and chlorine-based products (Dumetre et al., 2013). In a previous study, we found that 0.2% diclazuril caused a mild oocyst wall deformity at 48 h post-treatment and complete oocyst destruction after 72 h of treatment (Gadelhaq et al. (2017)). Therefore, the objective of this study was to investigate the effect of 0.2% diclazuril on the attenuation of *Eimeria* oocysts in experimentally infected chicks.

2. Materials and methods

This study was conducted according to the ethical standards of Faculty of Veterinary Medicine, Beni-Suef University, Egypt and approved by the Institutional Animal care and Use Committee of Beni-Suef University (2018-BSUV12).

2.1. Parasites and diclazuril preparation

A mixed species of *E. tenella* (80%), *E. necatrix* (10%), *E. maxima* (5%), and *E. acervulina* (5%) oocysts were used. *Eimeria* spp. were derived from the field isolate and maintained by passaging in coccidia-free chicks. Sporulated oocysts were counted using McMaster counting chamber. Diclosol® 1% (diclazuril 10 mg/ml) was obtained from Pharma Swede Company (Egypt) and prepared at 0.2% concentrations from the stock diclazuril (a 20% (1:5) dilution of a 1% stock solution yields a 0.2% working solution).

2.2. Treatment of unsporulated and sporulated eimerian oocysts with 0.2% diclazuril

The 0.2% diclazuril preparation was applied to unsporulated (freshly excreted oosysts) and sporulated (each oocyst contains 4 sporocysts each with 2 sporozoites) oocysts (5×10^5), which were incubated in Petri dishes for 48 h at 25–29 °C and 80% relative humidity (RH). At the end of the incubation time, the numbers of sporulated oocysts were counted in case of unsporulated oocysts. The diclazuril-treated oocysts were washed with phosphate buffered saline (PBS) and stored at 4 °C until used.

2.3. Immunization and challenge of chicks with Eimeria oocysts

A total of 180 1-day-old Tetra male layer chicks were purchased from Cairo Co. Ltd., Egypt. Chicks were reared under standard management conditions and fed commercial withdrawal feed free from any anticoccidial drugs up to 28 days. At day 4 of age after acclimation, chicks were randomly divided into 5 groups. Each group was divided into 3 replicates of 12 chicks each. Each group was housed in a separate battery, with 4 vertical partitions. Each replicate was in a partition (40 cm × 50 cm), but in the same room. Group 1 (G1) was a negative control (non-immunized and non-challenged). Group 2 (G2) was a positive control (non-immunized and challenged). Group 3 (G3) was immunized per os with 1.0×10^4 non-diclazuril treated-sporulated oocysts. Groups 4 (G4) was immunized per os with 0.2% diclazuril treated-unsporulated oocysts (1.0×10^4). Group 5 (G5) was immunized per os with 0.2% diclazuril treated-sporulated oocysts (1.0×10^4). Chickens of G2, G3, G4, and G5 were challenged 17 days later by a single inoculum of 7.5×10^4 untreated sporulated oocysts per os, while G1 remained unchallenged (Table 1). Oocysts were administered orally *via* a rubber gastric tube. Seven days post-immunization, 3 chicks from each group were randomly selected and euthanized by cervical dislocation. Vaccine attenuation (*i.e.* treated oocysts) efficacy was evaluated by (i) clinical signs, (ii) oocysts shed per gram of feces (OPG), and (iii) cecal lesion scores (Johnson and Reid, 1970). Follow-up of the challenged chicks was continued until day 7 post-challenge. On the last day of the experiment, all chicks were euthanized and intestines were examined for lesions, and spleens were collected for further cytokine analysis *via* Quantitative RT-PCR (QPCR).

2.4. Evaluation of vaccine efficacy for protection of chicks against coccidiosis

2.4.1. Clinical signs of coccidiosis

During immunization and challenge experiments, clinical signs of *Eimeria* infection were observed. Specifically, bloody diarrhea scores were recorded for each group according to a previously published method (Du and Hu, 2004), and gross lesions were recorded and categorized according to a previously published procedure (Johnson and Reid, 1970) at day 7 post-immunization and post-challenge.

2.4.2. Parasitological findings

Fecal samples and cecal contents were examined for oocyst presence, and oocysts were counted at day 7 post-immunization and challenge by McMaster counting technique (Lillehoj and Ruff, 1987).

2.4.3. Body performance

All chicks of each group were weighed at days 0 and 7 post-challenge. Weight gain of chicks in each group was determined by subtracting the body weight of the chicks at the time of challenge from the body weight at the end of the experiment (Ma et al., 2011).

2.4.4. Evaluation/Examination of histopathological sections

Histopathological examination of cecal tissues was done according to a previously described method (Bancroft et al., 2012). Small pieces of the cecal tissue were collected in 10% buffered formalin for histopathology. The fixed tissues were washed in running tap water overnight, dehydrated and infiltrated by paraffin wax. Serial paraffin sections (5 μm thickness) were obtained, and the sections were deparaffinized in three, consecutive washings in xylol for 5 min, and rehydrated with five, successive washings with alcohol in descending order of 100, 95, 80, 70, and 50% in deionized water. The histological sections were then subjected to conventional Hematoxylin and Eosin (H and E) staining procedure.

2.4.5. Quantitative RT-PCR experiments for the detection of cytokine expression levels

Randomly selected spleen samples, which were aseptically obtained from 3 chicks per group at day 7 post-immunization and 6 chicks per group at day 7 post-challenge, were preserved at −20 °C until further use.

Total RNA was extracted from the tissue samples using the RNeasy mini kit (QiagenRNeasy Mini Kit, Germany) according to the kit manufacturer's instructions. Purified RNA was eluted in 50 μl RNase-free water and stored at −70 °C. The primer and probe sequences of IFN- γ, IL-2, IL-12, and 28S rRNA are shown in Table 2. TaqMan probes were labeled with the fluorescent reporter dye FAM (6-Carboxyfluorescein) at the 5′end and with the fluorescent quencher dye TAMRA (6-Carboxytetramethylrhodamine) at the 3′end. QPCR was performed using the QuantiTect probe RT-PCR kit (Applied Bio-systems, Qiagen, Germany). Amplification and detection of specific products were performed using the Stratagene MX3005 P Real-Time PCR System (Applied Bio-systems) (Hong et al., 2007).

3. Statistical analysis

Data were analyzed using ANOVA and a Tukey multiple range test to determine differences between groups. Results are expressed as means ± SD. Probability values of less than 0.05 ($P < 0.05$) were considered significant.

Table 1
Experimental design to study the protective potential of diclazuril treated- oocysts against challenge infection in chickens.

Group	Immunization dose (4-day-old chicks)	Challenge dose (21-day-old chicks)	Follow-up and sample collection (at day 7 post-challenge)
Negative control (non-immunized, non-challenged) (G1)	–	–	All chicks were sacrificed humanely
Positive control (non-immunized, challenged) (G2)	PBS (1000 µl)	7.5×10^4 sporulated oocysts	All chicks were sacrificed humanely
Non-diclazuril treated-sporulated oocysts* (G3)	1×10^4 non-diclazuril treated-sporulated oocyst	7.5×10^4 sporulated oocysts	All chicks were sacrificed humanely
Diclazuril treated-unsporulated oocysts** (G4)	1×10^4 diclazuril treated-unsporulated oocysts	7.5×10^4 sporulated oocysts	All chicks were sacrificed humanely
Diclazuril treated-sporulated oocysts (G5)	1×10^4 diclazuril treated-sporulated oocysts	7.5×10^4 sporulated oocyts	All chicks were sacrificed humanely

* Sporulated oocyst means oocyst containing 4 sporocysts each contains 2 sporozoites.
** Unsporulated oocysts mean freshly excreted oocysts.

Table 2
Real-time PCR primer and GenBank accession numbers.

Target gene		Primer and probe sequences (5′–3′)	Gene bank accession numbers	Reference
28SrRNA	F	GGCGAAGCCAGAGGAAACT	X59733	Balu et al., 2011
	R	GACGACCGATTTGCACGTC		
	P	(FAM)AGGACCGCTACGGACCTCCACCA(TAMRA)		
IL- 2	F	TTGGAAAATATCAAGAACAAGATTCATC	AJ009800	Kaiser et al., 2000
	R	TCCCAGGTAACACTGCAGAGTTT		
	P	(FAM)-ACTGAGACCCAGGAGTGCACCCAGC(TAMRA)		
IFN- γ	F	GTGAAGAAGGTGAAAGATATCATGGA	Y07922	Kaiser et al., 2000
	R	GCTTTGCGCTGGATTCTCA		
	P	(FAM)-TGGCCAAGCTCCCGATGAACGA-(TAMRA)		
IL-12p35	F	TGGCCGCTGCAAACG	NM213588.1	Balu et al., 2011
	R	ACCTCTTCAAGGGTGCACTCA		
	P	(FAM)CCAGCGTCCTCTGCTTCTGCACCTT(TAMRA)		

4. Results

4.1. In vitro result

The treatment of unsporulated oocysts by 0.2% diclazuril led to a non-significant effect on sporulation percent, and 90% of treated oocysts were sporulated with a mild deformity of the oocyst wall (Supplementary Fig. 1).

4.2. Post-immunization findings

Chicks immunized with diclazuril-treated sporulated oocysts (G5) had no blood in the feces at day 7 post-immunization. The chicks of G4 showed mild bleeding in the feces (score 1). Chicks of G3 (immunized with untreated oocysts) showed severe bloody diarrhea. Concerning the lesion scores, G5 showed few scattered petechiae on the cecal wall, with normal cecal wall and content. G4 chicks had more numerous lesions, with noticeable blood in the cecal contents, and cecal walls are somewhat thickened and only one of the euthanized chicks showed unilateral cecal content tinged with blood. Contrary, all 5 chicks of G3 displayed bilateral bloody cecal core.

4.3. Post-challenge findings

4.3.1. Clinical and parasitological findings

Bloody diarrhea was obvious and severe in the control group (G2) while the other immunized groups (3, 4, and 5) showed diarrhea without blood in feces. In addition, cecal lesions were severe (score 3–4) in G2. However, in group 5, 30% of chicks had no lesions, and 70% developed mild lesions (score 1). G4 (diclazuril-treated unsporulated occysts) had 70% of chicks with no lesions; however, the remaining 30% developed mild lesions (score1). In addition, group 3 showed 50% of chicks with no lesions, 40% with mild lesions (score 1), and 10% with severe lesions (score 3) (Fig. 1). The OPG at day 7 post-challenge in G3, G4 and G5 was significantly lower ($P < 0.001$) than in G2 birds (Fig. 2).

4.3.2. Body performance

Body weight and body weight gain post-challenge were significantly higher ($P < 0.05$) in all the immunized birds (G3, G4, and G5) and the negative control group (G1) than in the non-immunized challenged control group (G2). However, there was no significant difference between the immunized group (G5) and the negative control group (G1) ($P < 0.001$). In addition, a significant increase ($P < 0.05$) in body weight gain was observed in G5 as compared to G3 and G4 (Fig. 3).

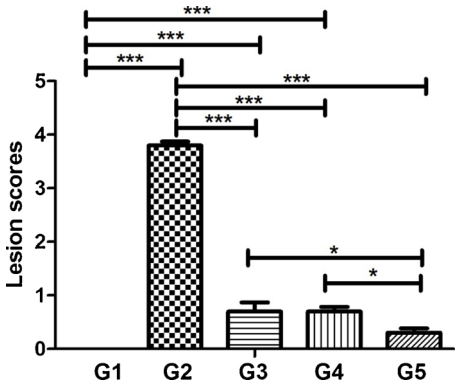

Fig. 1. Cecal lesion scores at day 7 post-challenge in the different groups. G1was a negative control (non-immunized, non-challenged), G2 was a positive control (non-immunized, challenged), G3 was non-diclazuril treated-sporulated oocysts, G4 was diclazuril treated-unsporulated oocysts, and G5 was diclazuril treated-sporulated oocysts (*$P < 0.05$; ***$P < 0.001$).

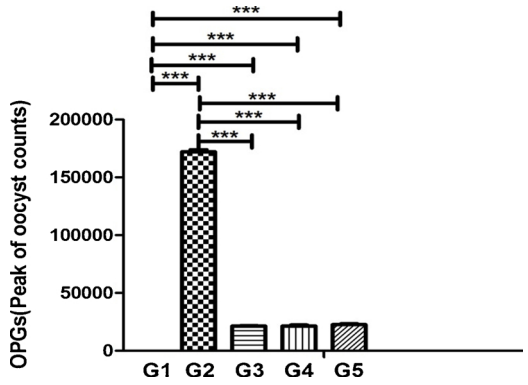

Fig. 2. Oocyst counts per gram feces (OPG) in the different groups at day 7 post-challenge. G1was a negative control (non-immunized, non-challenged), G2 was a positive control (non-immunized, challenged), G3 was non-diclazuril treated-sporulated oocysts, G4 was diclazuril treated-unsporulated oocysts, and G5 was diclazuril treated-sporulated oocysts (***$P < 0.001$).

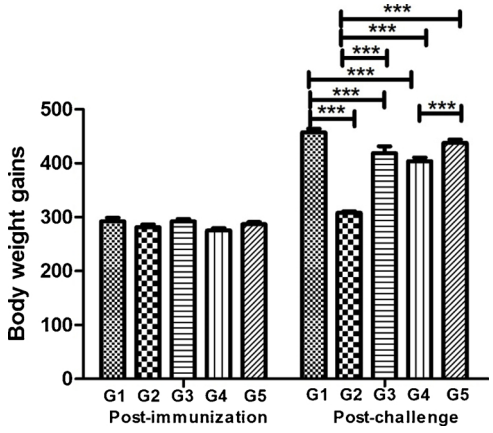

Fig. 3. Body weight gains (in grams) at day 7 post-immunization and post-challenge in different groups. G1was a negative control (non-immunized, non-challenged), G2 was a positive control (non-immunized, challenged), G3 was non-diclazuril treated-sporulated oocysts, G4 was diclazuril treated-unsporulated oocysts, and G5 was diclazuril treated-sporulated oocysts (***$P < 0.001$).

4.3.3. Cytokine level post-immunization and post-challenge

The expression of cytokines, including IL-2, IL-12 and INF-γ showed a significant increase ($P < 0.001$) in the immunized groups (3, 4 and 5) compared to the uninfected control group (G1) at day 7 post-immunization (Fig. 4A). Similarly, cytokine gene expression levels in chickens, which were immunized with the untreated oocysts (G3), were significantly higher in comparison to the other immunized groups (Fig. 4A). Furthermore, at day 7 post-challenge, cytokine gene expression levels were significantly higher ($P < 0.001$) in the immunized groups compared to the controls (G1and G2) (Fig. 4B). Additionally, G4 and G5 had cytokine gene expression levels higher than G3 (Fig. 4B).

4.3.4. Histopathological findings

Moderate destruction and sloughing of the cecal mucosa with the proliferation of the cecal lymphoreticular tissue were observed in the submucosa of G4 chicks post-immunization. Furthermore, different endogenous stages of *Eimeria* spp. (schizonts, gametocytes, and oocysts) were localized in the cecal core and wall (Fig. 5A, B & C). In addition, normal lining of cecal epithilium with hyperplasia of the cecal crypts and high proliferation of cecal tonsils in the submucosa was observed in G4 chicks post-challenge (Fig. 5D). The cecal wall in G5 had mild mucosal damages with bleeding post-immunization (Fig. 6A). Furthermore, post-challenge findings were characterized by an intact cecal mucosa with crypt hyperplasia, proliferation of cecal tonsils, and cecal glands (Fig. 6B).

5. Discussion

The overuse and misuse of anticoccidial drugs have led to the emergence of a drug-resistant *Eimeria* species (Ruff and Danforth, 1996). Furthermore, its residues in poultry products pose adverse side effects to the consumer (McDougald, 2003). Bans or limitations on the use of medication before slaughtering were disclosed in poultry industry to avoid the potential threat to human health (Vermeulen et al., 2001). Vaccination presents an appealing alternative to anticoccidial drugs in reducing the impact of *Eimeria* spp.

In the present study, 0.2% diclazuril was used to attenuate *Eimeria* oocysts *in vitro*. The 4-day-old chicks were immunized with diclazuril-treated oocysts and challenged with 7.5×10^4 sporulated oocysts of *Eimeria* species at the age of 21 days. The immunized chicks showed mild lesion scores with less bloody diarrhea and low oocyst counts in feces. In addition, histopathological findings revealed low numbers of parasitic stages in the groups immunized with diclazuril-treated oocysts compared to chicks immunized with the untreated oocysts. Chicks immunized with the untreated oocysts manifested signs of severe coccidiosis, with bloody diarrhea, a high number of oocysts, and a severe destruction of the cecal mucosa. Attenuation of *Eimeria* oocysts by diclazuril was confirmed post-immunization in chicks. This was evidenced by mild diarrhea and lesion score in chicks immunized with diclazuril-treated oocysts. Diclazuril acts on the sexual and asexual

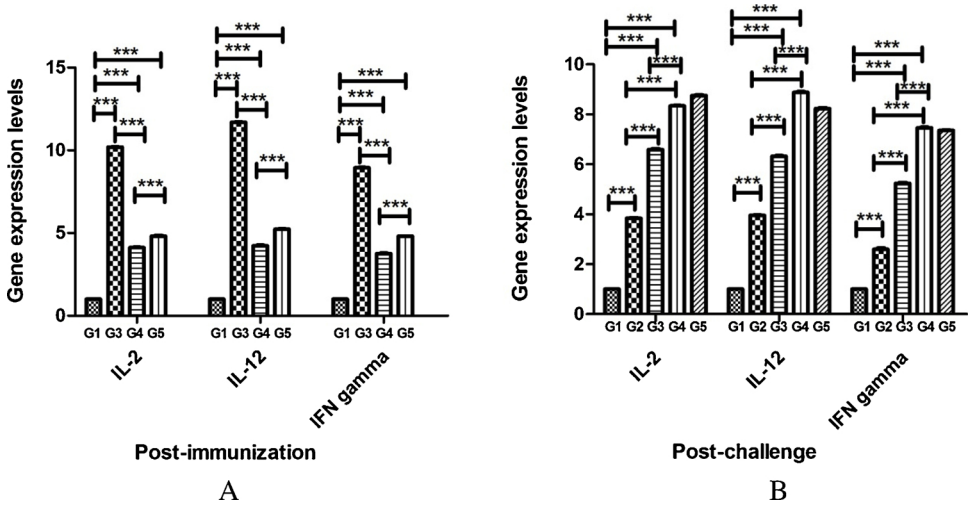

Fig. 4. IL-2, Il-12, and INF-γ expression levels at day 7 post-imunization (A) and post-challenge (B). G1was a negative control (non-immunized, non-challenged), G2 was a positive control (non-immunized, challenged), G3 was non-diclazuril treated-sporulated oocysts, G4 was diclazuril treated-unsporulated oocysts, and G5 was diclazuril treated-sporulated oocysts (***$P < 0.001$).

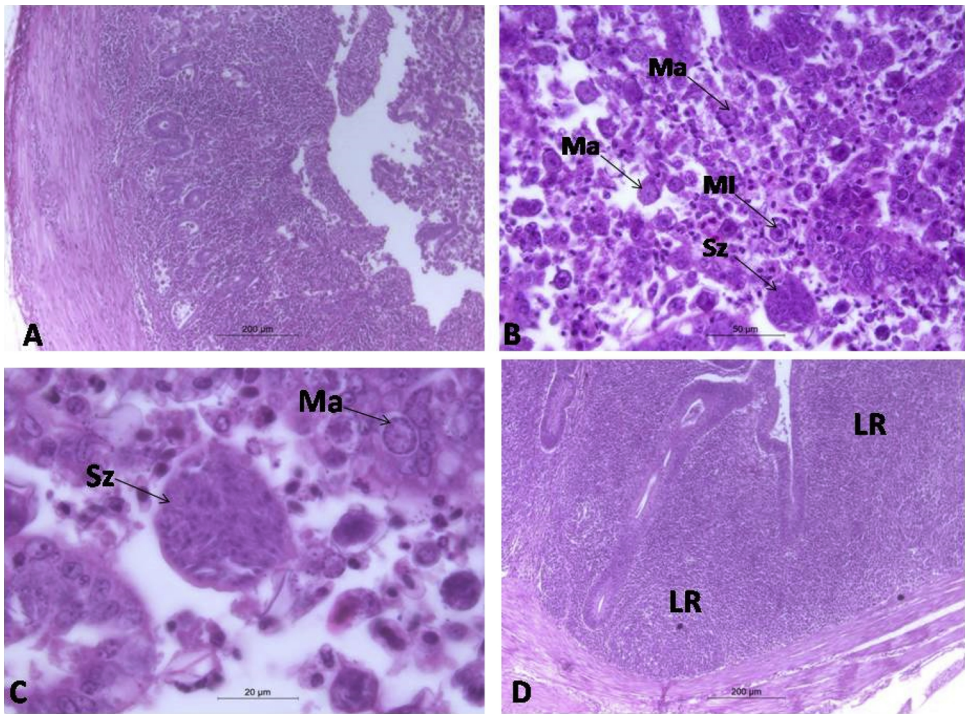

Fig. 5. Histopathological pictures in G4 birds (diclazuril-treated sporulating oocysts).
A. Cecum in G4 birds (post-immunization) showed a moderate damage and sloughing of the cecal mucosa with proliferation of the cecal lymphoreticular tissue in the submucosa and macro and microgametocytes with schizonts of *Eimeria* spp. localized in the cecal core and wall (H&E, x100).
B. Different endogenous stages of *Eimeria* spp. in the cecum at a higher magnification (Arrows; Ma = Macrogametocyte, Mi = microgametocyte, Sz = schizont) (H&E, x1000).
C. Cecum showed a cecal core containing blood cells, with damaged epithelial cells and endogenous stages of *Eimeria* spp. at higher magnification (Arrows; Ma = Macrogametocyte, Sz = schizont) (H&E, x1000).
D. Cecum in G4 birds (post-challenge) displayed a normal lining epithelium with hyperplasia in the cecal crypt epithelia and highly proliferating cecal tonsils (LR = lymphoreticular) (H&E, x100).

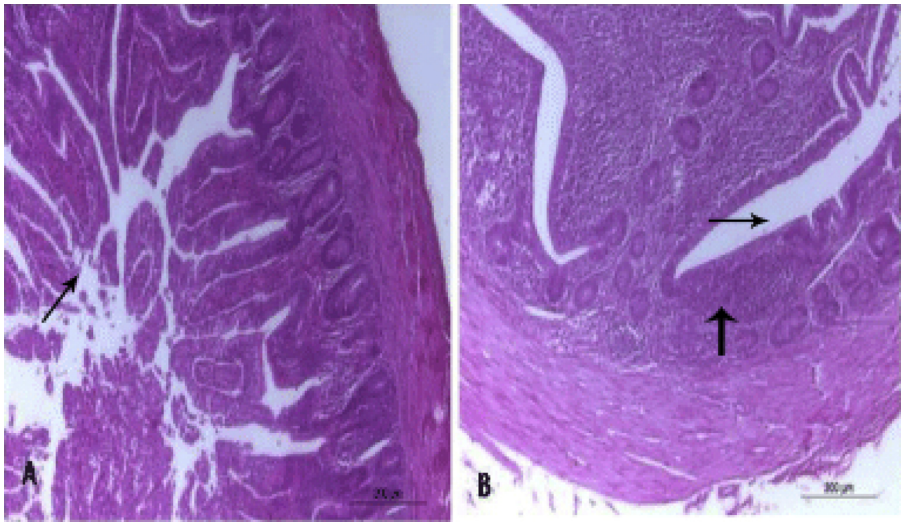

Fig. 6. Histopathological picture of cecum in G5 birds (diclazuril-treated sporulated oocysts).
A. Cecum in G5 birds (post-immunization) expressed mild mucosal damages with bleeding (arrow) (H&E, x100).
B. Cecum in G5 birds (post-challenge) showed an intact cecal mucosa with crypt hyperplasia (horizontal arrow), proliferating cecal tonsils, and cecal glands (vertical arrow) (H&E, x100).

stages of *Eimeria* (Vanparijs et al., 1989). A previous study by the authors (Gadelhaq et al., 2017) found that the exposure of *Eimeria* oocysts to diclazuril (0.2%) for 72 h caused destruction of the oocyst wall. Additionally, diclazuril has been reported to suppress the invasion activity the merozoites in an *in vivo* study (Zhou et al., 2010).

Therefore, the treatment of oocysts with a high dose of diclazuril (0.2%) for 48 h (a long time) affects the invasion capacity of sporozoites, which is confirmed by observation of very low numbers of parasitic stages in the epithelial cells and proliferation of the cecal lymphoreticular tissue in the submucosa in the immunized chicks.

The oocyst counts in faeces at day 7 post-challenge in all the immunized groups were significantly lower than the infected unimmunized control group. Decreased fecal oocyst shedding was the result of significant production of interleukins (IL-8, IFN-γ, IL-15, TGF-4, and IL-1), which increased host protection against coccidiosis and reduced intracellular development of *Eimeria* spp. in chicken (Lillehoj et al., 2004; Zhang et al., 2012). Moreover, IFN-γ secretion slows down sporozoite replication in chicken (Dimier et al., 1998), and decreases fecal oocyst shedding (Yun et al., 2000).

The weight gain was significantly ($P < 0.05$) higher in all the immunized groups compared to the infected unimmunized control group. This finding may be a result of a minimal intestinal tissue damage, which led to increased absorption and food conversion. Zhang et al. (2012) reported that an increase in IFN-γ and lymphotactin levels post-imunization (Zhang et al., 2012). However, body weight gain decreased in the infected unimmunized control group after challenge with *Eimeria* species oocysts because infected chicks must divert energy from growth to fight the infection, thus disrupting weight gain (Klasing et al., 1987; Lin et al., 2015).

Consistent with the previous results, the mRNA expression of IL-2, IL-12, and IFN-γ showed a significant increase in the immunized groups (3, 4, and 5) compared to the uninfected challenged control group (G2). The adverse reactions of coccidiosis were caused by the invasion and development of intracellular stages of *Eimeria* spp. in the gut, which are associated with the induction of local inflammatory response.

In addition, increases in several proinflammatory reactions were

observed in the chicken gut following infections with *E. tenella*, *E. acervulina*, and *E. maxima*. Moreover, soluble inflammatory mediators are responsible, in part, for intestinal damages during coccidiosis (Hong et al., 2006a,b; c). Consequently, the immunized groups showed a mild degree of coccidiosis adverse effects. In contrast, the infected unimmunized control group showed severe clinical signs and high oocyst counts. Therefore, we think that less severe coccidiosis in the immunized groups may be a result of successful immunization. The later may have elicited an adequate immune response that enabled birds to resist the challenge infection with wild parasites (Chapman et al., 2005). Additionally, the primary immunization with *E. tenella* increased leukocytes infiltration in the lamina propria, including macrophages and T cells (Vervelde et al., 1996). In addition, the challenge infection resulted in increased $CD8^+$ lymphocytes (Allen and Fetterer, 2002). Consequently, production of IFN-γ at days 6 and 7 during primary infection increased (Heriveau et al., 2000; Lowenthal et al., 1997), which inhibited the penetration of sporozoites of *E. tenella* in host epithelial cells *in vitro* as well as *in vivo* (Lillehoj and Choi, 1998). During *Eimeria* spp. infection *in vivo,* an increase in IFN-γ up-regulates production of the pro-inflammatory cytokines IL-1β and CC chemokine K203 (Laurent et al., 2001).

Interestingly, Chickens, which were medicated with diclazuril, were protected for several days after withdrawal of medication (McDougald and Seibert, 1998). Furthermore, Mortier et al. (2005) found that depletion of diclazuril residues in chicken breast and thigh muscle was 9 days after withdrawal of medicated feed. However, our study included the treatment of oocysts with diclazuril, and the immunization of chicks with the diclazuril-treated oocysts was subsequently carried out, which precludes diclazuril residues in poultry meat. A more detailed study is required to explore the effect of residual diclazuril in treated animals using supernatant from the treated oocysts and untreated oocysts and the content of diclazuril in the treated oocysts. Additionally, among the important issues that should be addressed in future investigations are the cecal immune response in the context of diclazuril-treated oocysts against coccidiosis in layer chicks.

In conclusion, chicks, which were immunized with the diclazuril-treated sporulated oocysts achieved better results than the other groups, and the treatment of sporulated oocysts with 0.2% diclazuril had an attenuation effect on eimerian oocysts. Diclazuril is effective in attenuating *Eimeria* oocysts and thus provides an alternative approach for using the diclazuril-treated sporulated oocysts to protect chicks against *Eimeria* challenge. In addition, the current study could provide the most cost-effective vaccination strategy, particularly for developing countries.

Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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Appendix A. Supplementary data

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