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RESEARCH ARTICLE

Immunological and histopathological evaluation of *Eimeria tenella* oocysts Egyptian local isolate vaccine and its comparative efficacy with a commercial live vaccine

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ABSTRACT

Coccidiosis is the most important protozoan disease in broilers all over the world. Controlling of broilers coccidiosis via vaccination rather than chemicals is a new trend with promising results. Thus, the present work describes an evaluation of *Eimeria tenella* Lab-made vaccine of local Egyptian strain and its comparative efficacy with a commercial live vaccine "Fortegra®". Eighty broiler chickens one day old were used; they were divided in to 4 equal groups; 20 chicks each. Group 1 (G1) kept as control negative, G2 administrated orally with lab-made sporulated oocysts vaccine at 5 days old, the birds of G3 vaccinated orally with Fortegra® at day 6 of age, and G4 served as control positive. All birds were challenge by 50,000 sporulated oocysts of *E. tenella* at day 21. For testing the efficacy and comparison; OPG (oocyst per gram), serum Interleukin4 (IL4) levels, Immunoglobulin A (IgA) levels in both serum and ceca, cecal lesion score, as well as histopathological changes in ceca of tested groups were evaluated. The results demonstrated significantly elevated IL4 level in serum and IgA level in serum and cecum of G2 than G3. IgA in cecum significantly elevated in G2 than G3. OPG significantly decreased in both vaccinated groups (G2 and G3), and have lower lesion score than non-immunized group. Cecal tissues of vaccinated groups had mild pathological changes. Conclusively, good immunization by the currently tested vaccine, against experimental *E. tenella* infection was observed.

Keywords: Coccidiosis; *Eimeria tenella*; vaccination; histopathology; immunity.

INTRODUCTION

Chicken coccidiosis is one of the most pathogenic internal parasites of poultry which cause severe economic losses due to mortality, malabsorption, in-efficient feed utilization, impaired growth rate of broilers and reduced egg production in layers (Lillihoj & Dalloul, 2004). Moreover, avian coccidiosis causes economic global losses of more than 3 billion dollars per year in the poultry industry (Zhang *et al.,* 2012; El-Shazly *et al*., 2020). Control of poultry coccidiosis is based mainly on the use of prophylactic anticoccidial drugs. Traditionally, the disease is controlled by chemical feed additives that can inhibit the life cycle stages of *Eimeria* (Calnek *et al.,* 1997). Several disadvantages related to this strategy including withdrawal periods, development of drug resistance and drug residues in product's for human consumption (Dalloul & Lillehoj, 2005). Vaccines have been used in poultry industry for more than 50 years, primarily in broiler breeder and replacement layer flocks (Chapman *et al.,* 2002). Different types of vaccines have been made to immunize chicken against coccidiosis throughout the world by using low doses of sporulated oocysts (Shafiya *et al.,* 2017), irradiated sporulated oocysts (Raymond *et al.,* 2014), sporozoites (Garg *et al.,* 1999), merozoites, recombinant merozoite antigen (Jenkins, 1998), recombinant refractile body antigen (Kopko *et al.,* 2000) and sonicated oocyst (Akhtar *et al.,* 2001; Kadhim, 2014). Generally, vaccination against *Eimeria* spp. thought to stimulate host immune response (Allen & Fetterer, 2002; Awad *et al.,* 2013), that may help in protection against infection. In a vaccine production, the use of local strains may give a better results than using foreign strains of a pathogen. Fortegra® is a commercial live vaccine, distributed in Egypt but manufactured aboard, and it contains oocysts of several chicken *Eimeria* spp. So, we tried herein to make a vaccine from sporulated sonicated oocysts of a local field strain of *E. tenella* and to evaluate its efficacy as compared with a commercial live vaccine "Fortegra®".

MATERIALS AND METHODS

Ethical consideration: The experiment was carried out in Animal Health Research Institute, Tanta branch at the period from

October, 1st till November, 7th 2018. All procedures were carried out in accordance to national laws and regulations for the handling of animals to avoid harms and minimize their pain.

Birds and management: One day-old, broiler chicks of the "Avian 48 strain" were purchased from a Fat Hens hatchery. Upon arrival, the chicks were, housed in clean, disinfected cages. Chicks raised according to routine management practice as outlined by the National Research Council requirements.

Fortegra® vaccine: It is a live oocysts of *E. acervulina,* precocious and classic strains of *E. maxima*, *E. mivati* and *E. tenella.* It was obtained from MSD Animal Health (Phils.), Inc. Company. Intervet Inc. Omaha, NE 68103, USA. U.S.Vet Lic. No.165A. Philippines: VBPR No:R-2127.

Lab-made vaccine preparation: Field strain isolate of *E. tenella* was collected from the ceca of dead broiler chickens during natural outbreak of cecal coccidiosis. Briefly, cecal contents were sieved, washed and centrifuged at 2000 rpm/5 min. Then, initial microscopic identification of the collected species was done. Collected oocysts were morphologically similar to *E. tenella.* Further identification was done as described before (Desouky *et al.,* 2015). A clear *E. tenella* oocysts pellet was concentrated by using saturated salt solution and centrifugation at 4000rpm/10 min. The upper third of solution was collected by rubber pipette. Then washed and kept in sufficient amount of 2.5 % potassium dichromate "$K_2Cr_2O_7$" for sporulation to avoid over growth of fungi and bacteria at 28°C (Davis, 1973). Then after that, potassium dichromate was removed by washing the pellets 4 times with distilled water followed by centrifugation at 4000 rpm/10 min. The harvested oocysts were counted using McMaster chamber and aliouqted in phosphate buffer saline (PBS) and stored at 4°C until use.

About 4000 *E. tenella* sporulated oocysts /ml stirred continuously on a magnetic stirrer for twelve hours at 4-8°C (Akhtar *et al.,* 2001), followed by ultra-sonication at 60 kHz for 5 shots of one minute each with an interval of 30 seconds in jacketed vessel at 4-8°C (Ultra Sonics Homogenizer 4710 series Cole Parmer Instrument Co. Chicago, Illinois 60648) (Akhtar *et al.,* 1998), Centrifugation at 10.000 x g for 30 min at 4°C. Supernatant of sonicated suspension was used as antigen, collected in 100 µl aliquots (Akhtar *et al.,* 2001).

Experimental design: A total of 80 broiler chicks one day old were used. Upon arrival, chicks were divided into 4 groups (20 chicks each):

G1: Non challenged, non-vaccinated and kept as control negative group.

G2: Immunized with lab-made *E. tenella* vaccine by oral route at day 6 of age (Akhtar *et al.,* 2001) and challenged by 50,000 sporulated *E. tenella* oocysts at day 21 of age.

G3: Vaccinated by Fortegra® vaccine at day 6, then challenged by 50,000 sporulated oocysts of *E. tenella* at day 21 of age.

G4: Infected by 50,000 sporulated oocysts of *E. tenella* at day 21 of age but non vaccinated and kept as control positive.

Evaluation parameters: The experiment was terminated at day 7 after challenge (28 days of age). During the whole period of the experiment all groups were observed daily and clinical signs were recorded. Blood was collected from wing vein at 16, 21 and 28 day of age. Humeral immunity was estimated by measuring Immunoglobulin A (IgA) level in serum and cecum (ELISA Kit Catalog No: MBS2507630 96T) (MyBiosource), cellular immunity was evaluated through estimation of InterLukin 4 (IL4) level in serum (ELISA Kit Catalog Number. MBS704068, MyBiosource).

Also, five birds from each group were sacrificed by cervical dislocation on day 7 post challenge and their ceca were collected for lesion scoring in accordance to Johnson and Reid (1970). Specimens from cecum were collected and processed for histopathological examination according to method described by Bancroft *et al*. (1994) at 4th week (end of experiment). Birds dropping (in each group separately) were collected at zero day of challenge and at days 6 and 7 post challenge for counting oocysts per gram (OPG) using McMaster chamber according to Lillehoj and Ruff (1987).

Statistical analysis: Data were represented as mean±SE (standard error). One way analysis of variance (ANOVA)-Tukey test was used to compare the mean values of the various groups at significance level of $P \leq 0.05$. Statistical analysis was performed using the method cited in Petrie and Watson (1999) and computerized using SPSS 20 (2011).

RESULTS

Regarding the parameters evaluated in the current work; the IL4 level in serum of chicken during the experimental period is shown in Table 1. There was no significance difference between all experimental groups at day 16 and 21, but at day 28 of age (seven days after challenge) there was significant difference between control group (G4 22.60±2.09523) and vaccinated groups (G2 and G3); G2 (17.6333±1.2147) showed higher IL4 level than G3 (12.6333±3.1599).

There was no significance difference between experimental groups at days 16 and 21. At day seven after challenge (i.e. day 28), the level of IgA of G2 (5.3900±0.1809) was higher than other groups, but there was no significance difference between G3 (2.9283±1.3042) and G4 (1.2673±0.758). The levels of IgA in sera of chicks during the experimental period are clarified in Table 2.

There was no significance difference between all groups at day 16 and 21 of age, but there was significant increase. Whereas, at day 28 IgA level in G2 (4.4657±1.75943) was higher than that of G3 (2.7790±0.45525). While IgA level in cecum of chicks during experiment is shown in Table 3.

At day 21 of age there was no significant difference in OPG between all groups, whereas at day 27 (day 6 post challenge) vaccinated groups (G2 and G3) showed significant decrease in OPG as compared to G4. At day 28 of age, G2 showed no significant difference in OPG with G3. OPG in all groups of chicks are shown in Table 4.

Lesion score of ceca of chicken at day 7 post challenge showed that there was no significance difference between vaccinated groups (G2 and G3) whereas, control positive group (G4) showed higher lesion score. Lesion score in ceca of all groups is shown in Table 5.

Histopathological examination of ceca of all groups is shown in Fig. 1. Briefly, intestines of chicken in G1 showed normal histopathological features as normal intestinal glands with its normal small basophilic nuclei. While, intestines of chicken in G2 showed normal intestinal glands with very few numbers of *E. tenella* oocysts, proliferated activating intestinal gland cells present with presence of some inflammatory cells. Intestine of chicken in G3 showed moderate infection by schizonts stages only of *E. tenella* with infiltration of inflammatory cells. Lastly, G4 chicken intestines showed infection by different developmental

Table 1. Interleukin 4 (IL4) levels in serum of chickens of all groups detected during the experiment

Age by day	Experimental groups			
	G1	G2	G3	G4
16	4.9000 ± 0.32146[a]	5.5000 ± 0.47258[a]	5.3333 ± 1.6448[a]	4.9000 ± 0.32146[a]
21	6.0333 ± 0.40552[a]	7.8000 ± 2.12838[a]	9.9667 ± 2.72906[a]	6.0333 ± 0.40552[a]
28	6.1667 ± 0.61734[c]	17.6333 ± 1.2147[ab]	12.6333 ± 3.1599[bc]	22.60 ± 2.09523[a]

[a,b] Values bearing similar superscript between rows do not differ at ($P \leq 0.05$).

Table 2. Immunoglobulin A (IgA) level in serum of chickens of all groups detected during the experiment

Age by day	Experimental groups			
	G1	G2	G3	G4
16	0.5603 ± 0.030[a]	0.5930 ± 0.1422[a]	0.5203 ± 0.1909[a]	0.5603 ± 0.0309[a]
21	0.5993 ± 0.039[a]	1.0213 ± 0.4244[a]	1.1417 ± 0.4351[a]	0.5993 ± 0.0393[a]
28	0.5683 ± 0.052[c]	5.3900 ± 0.1809[a]	2.9283 ± 1.3042[b]	1.2673 ± 0.758[bc]

[a,b] Values bearing similar superscript between rows do not differ at ($P \leq 0.05$).

Table 3. Immunoglobuiln A (IgA) level in ceca of chickens of all groups detected during the experiment

Age by day	Experimental groups			
	G1	G2	G3	G4
16	1.105 ± 0.088[a]	1.1433 ± 0.20042[a]	1.0480 ± 0.02715[a]	1.1053 ± 0.08872[a]
21	1.322 ± 0.108[b]	2.8057 ± 1.32602[ab]	3.0123 ± 0.96684[ab]	1.3223 ± 0.10890[b]
28	1.51 ± 0.189[b]	4.4657 ± 1.75943[a]	2.7790 ± 0.45525[ab]	2.5323 ± 0.68290[ab]

[a,b] Values bearing similar superscript between rows do not differ at ($P \leq 0.05$).

Table 4. Oocyst per gram (OPG as x 10^4) for chickens in all groups detected during the experiment

Age by day	Experimental groups			
	G1	G2	G3	G4
21	0.0350 ± 0.00764[a]	0.2233 ± 0.05859[a]	0.0767 ± 0.01453[a]	0.0633 ± 0.14970[a]
27	0.9590 ± 0.0870[c]	1.2333 ± 0.14530[c]	2.3667 ± 0.08819[b]	19.0333 ± 0.57831[a]
28	0.1500 ± 0.2783[d]	2.6533 ± 0.08667[b]	2.1900 ± 0.43715[b]	21.0833 ± 0.58333[a]

[a,b] Values bearing similar superscript between rows do not differ at ($P \leq 0.05$).

Table 5. Cecal lesion score (LS) for chickens in all groups detected during the experiment

Age by day	Experimental groups			
	G1	G2	G3	G4
LS	0[c]	2[b]	2[b]	4[a]

[a,b] Values bearing similar superscript between rows do not differ at ($P \leq 0.05$).

stages of *Eimeria* i.e. schizonts, macrogametes and microgametes. Also, intestines of infected chickens showed different stages of parasite, villous atrophy, severe inflammation, mononuclear inflammatory cell infiltration, sever hemorrhagic areas.

DISCUSSION

In Egypt, chicken coccidiosis is a serious problem in poultry production sector. *E. tenella* is a major pathogenic species, with wide prevalence and high detection rates (Abu-Akkada

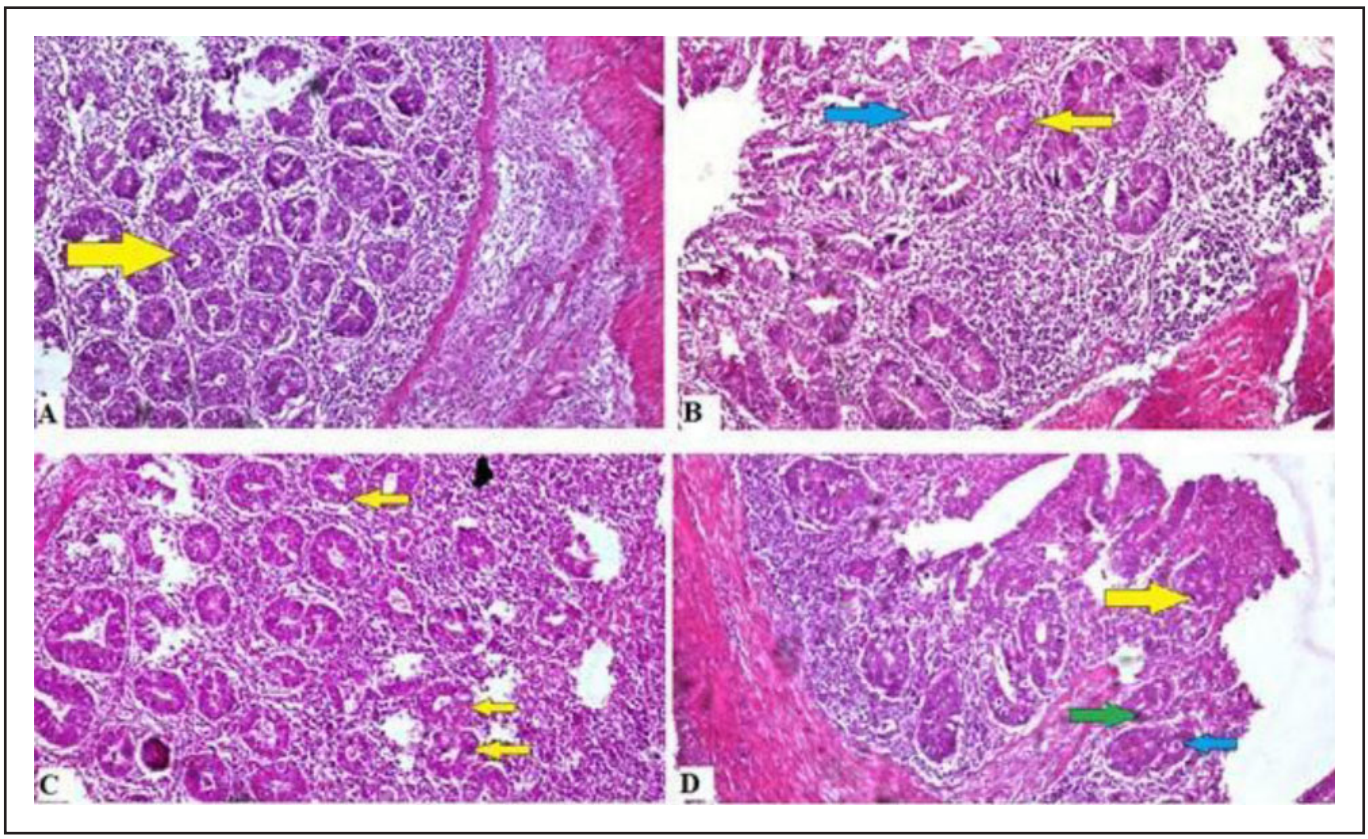

Figure 1. Histopathological pictures of ceca of chickens of all groups detected at day 28. Stain Haematoxylin and Eosin X100. **(A)** Cecum of chickens in G1 showing normal intestinal glands with its normal small basophilic nuclei. **(B)** Cecum of chickens in G2 showing normal intestinal glands with very few numbers of coccidial cysts, proliferated activating intestinal gland cells present (blue arrow) with presence of some inflammatory cells. **(C)** Cecum of chickens in G3 showing moderate infection by schizonts stages only of the coccidial life cycle stages (yellow arrow) with infiltration of inflammatory cells. **(D)** Cecum of chickens in G4 showing active infection by *E. tenella* and the presence of different *Eimeria* stages including schizonts, macrogametes and microgametes.

& Awad, 2012). There are many past and ongoing on researches dealing with vaccination and immunization against avian coccidiosis with different theories and application in order to achieve the best results (Shivaramaiah *et al.,* 2014; Ahmad *et al.,* 2016). But, it is well known that coccidial antigen can vary geographically according to strain (Allen & Fetterer, 2002; Awad *et al.,* 2013). So, the current work comprised the use an antigenic material made from local Egyptian strain to immunize broilers against the pathogenic strain of *E. tenella,* and to compare the efficacy of the lab-made vaccine with a commercial one.

IL4 is produced mainly by CD4+ TH2, CD8+ T cells, NKT cells, and granulocyte, basophils, eosinophils, and mast cells; elevated levels of IL-4 are typically associated with tissue injury and TH2 diseases caused by infection with parasites or extracellular pathogens (Martinez, 2008). The results represented herein agreed with the findings of Chapman *et al.* (2005) who reported that the primary infection with *E. tenella* oocyst induced complete protection against homologous challenges, and agreed with Davou *et al.* (2018) who reported that the prominent cytokines detected in the infected broilers were IFN-γ, IL2, IL4, IL6, TNF and TGF. However, Cacho *et al.* (2012) reported that the numbers of cells secreting the Th2 cytokines IL4 and IL10 were diminished in immunized and infected chickens compared with the non-immunized/ non infected one. The results were disagreed with Hong *et al.* (2006) who reported that IL4 decreased after primary or secondary infection of *E. acervulina* and *E. tenella.* This difference in the results may be due to difference in chicken age during vaccination, the strain of *Eimeria* used in vaccine preparation and other environmental factors.

The results of IgA level in serum of chicks agreed with Ayaz *et al*. (2008). Also, agreed with results of other studies Anwar *et al.* (2008); Bahram and Bahrami (2006); and Akhtar *et al.* (1998) that indicated IgA level was elevated in vaccinated birds with different types of *E. tenella* vaccines e.g., local gametocytes, sonicated sporulated sporocysts and inactivated sonicated vaccines.

Meanwhile, our results about IgA in ceca agreed with Davis *et al.* (1978) who showed that cecal contents from immune birds contained high levels of IgA. Girard *et al.* (1997) observed an elevation in secretory IgA in mucosal tissue from the duodenum and cecum 14 days post-infection. Parker *et al.* (2007) found that chicken vaccinated with a commercial coccidia vaccine showed *E. tenella* infection, even if manifested by high lesion score, does not have to decrease the cecal production of IgA. Akhtar *et al.* (1998) used sonicated coccidial oocyst vaccine orally, and found that the antibodies produced in cecum were principally IgA.

Results of OPG in the current work agreed with Anwar *et al.* (2008), they detected significant higher oocysts count in LivaCox®-vaccinated group as compared to local gametocyte-vaccinated chickens. Akhtar *et al.* (2001) found that the supernatant of sonicated sporulated oocysts vaccine gives the lowest OPG post challenge as compared with the sediment of the same vaccine. Bahram and Bahrami (2006)

found that sonicated sporulated oocysts gives the lowest no. of OPG appeared in feces from day 5 to 7 post challenge. According to Suprihati and Yunus (2018) the oocysts firstly appeared on the day 6 pi, then reached peak on the day 9 pi before numbers declined rapidly and the fewest oocysts were detected on day 12 pi. The same pattern of daily oocysts output was seen in both *E. tenella* primary and challenge infection, but oocysts output per day of the *E. tenella* challenge infected chicken were significantly lower than *E. tenella* primary infected chicken.

Lesion score results agree with those of Anwar *et al.* (2008) who detected that local gametocyte and LivaCox® immunized chickens developed lesions (1.0–2.0) respectively. Ritzi *et al*. (2016) reported that birds received Immucox® vaccine through gel droplet administration at the hatchery, had significantly lower lesion scores than control birds. At post-mortem examination of naturally infected chicken with *Eimeria* sp. Ayaz *et al.* (2008) observed minimum lesion scoring in group of birds immunized with sonicated gametocytes followed by group received gametocytes inactivated with formalin and group received intact gametocytes. Madison (2015) stated that Fortegra® vaccine resulted in lower lesion scores compared to the conventional and control groups at 14, 17 and 21 days post-vaccination.

The present histopathological findings were similar to those reported by Akhtar *et al.* (2001). These results were similar to those reported by Madison (2015) and Rafiqi *et al.* (2017). Suprihati and Yunus (2018) detected that *E. tenella* showed considerable numbers of oocysts in lamina propria of cecum, severe hemorrhage, complete desquamation of epithelium and edema of lamina muscularis. G4 revealed infection by different coccidial schizonts, macrogametes and microgametes. These results agree with Zulpo *et al.* (2007) in the presence of different stages of the parasite life cycle, villous atrophy, and severe inflammation in group infected with 2 x 10^4 sporulated oocysts of *E. tenella.*

CONCLUSION

Oral administration of Fortegra® was compared with lab-made vaccine of *E. tenella* sonicated sporulated oocysts local Egyptian strain in this study. Better results were obtained regarding to IL4 using the commercial vaccine, but the lab-made vaccine showed higher IgA level in both serum and cecum. There were no significant differences between two groups concerning the other parameters. So, a vaccine based on sonicated sporulated oocysts of *E. tenella* local strain when given orally, showed potentiation of immune response against challenge infection. This may be of interest in further studies on vaccination and immunization against cecal coccidiosis in broilers.

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Conflict of interest

The authors declare that they have no conflict of interest. This research is a part of N.I. Ammar's study to acquire the Ph.D. in Parasitology.

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