Efficacy of *Eimeria tenella* (Oocyst and Sporozoite) Proteins as a Vaccine in Broilers Against Coccidiosis

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**Abstract:** The protective efficacy against homologous challenge in chickens evaluated by using two proteins of a (Sporozoite) and (Oocyst) from *Eimeria tenella*. Immunization was applied on 3rd and 18th day of age subcutaneously with this two types of protein in separate groups at dose (25µg per chicken). Vaccinated birds were challenged at 30th day of age, demonstrated that sporozoite protein could provide chickens with protection rate around 99.2-99.5%, while oocyst protein gives 67-69% protection, number of oocysts and cecal lesion from chickens in the immunized groups with sporozoite protein decrease significantly and this protein was more effective from another groups. The body weight gain not affected (higher) in sporozoite immunized groups when compared with oocyst protein immunized groups and control positive groups, also we estimated ACI which was demonstrated that sporozoite protein was very effective while the oocyst protein was slightly effective.

**Key words:** Vaccine, sporozoite, oocyst, *Eimeria tenella*

**INTRODUCTION**

*Eimeria tenella* is an apicomplexan parasite which causes coccidiosis in the chickens, represents a severe problem for the poultry industry throughout the world due to the losses from mortality and morbidity (Williams, 1999). *Eimeria* infects the epithelial cells of intestinal lining. Pathological changes may occur by this obligate intracellular pathogen, these change differ from destruction of local mucosal barrier and underlying tissues to systematic effects such as blood loss, shock syndrome and ever death (Vermeulen et al., 2001). The disease is controlling today by preventive medication using polyether ionophores or chemical agent as anticoccidial drugs. Long usage promotes the development of drug resistance and cause great losses in the poultry industry, it also cause large concerns for public about the chemical residues in food (Vermeulen, 1998; Allen and Fetterer, 2002; Williams, 2002). Coccidiosis is highly immunogenic, primary infections can stimulate solid immunity to homologous challenges. Therefore, it would seem obvious that vaccines could offer excellent alternatives to drugs (Allen and Fetterer, 2002). So, there are many procedure to control coccidiosis involving immunological, biotechnological and genetically methods of these, immunological approach is considering more important, live vaccines containing virulent or attenuated strains of *Eimeria* are available but their use is limited in poultry industry due to its high cost. Additionally these vaccines consist of several *Eimeria* species, makes them labour as well as intensive cost to produce (Vermeulen, 1998). Also, these types of vaccine may reverting back to a pathogenic form (Sharman et al., 2010). Therefore, our research efforts have been invested in the development of anticoccidial protein vaccines composed of antigens as an alternative to live vaccines. Since the sporozoite was believed to be the target for protective immunity (Brothers et al., 1988, Danforth and Andew, 1987). This was taken in our consideration, the present study use the oocyst and sporozoite extract as a vaccine to protect broilers from *Eimeria tenella* parasite.

**MATERIALS AND METHODS**

**Parasite propagation and oocyst, sporozoite protein preparation:** Local isolation of *Eimeria tenella* were obtained from (Dr. Katranji M.M., Parasit Lab /Collage of Veterinary medicine/Hama/Syria) and propagated throughout 3 weeks old chickens (Broiler, Ross. 308). Oocysts were collected from the ceca of infected chickens at 7th days post infection. After sporulation with potassium dichromate at 28°C for 6-7 days, oocysts were purified by standard salt flotation techniques and sterilized by sodium hypochlorite treatment as described previously (Schramatz et al., 1984). Sporulated oocysts Pic. (1) were stored in phosphate buffer salin (PBS PH = 7.6) at 4°C until further use.

**Preparation of sporozoite protein:** Sterile sporulated oocysts about 2 ml (4 × 10⁶) were used for excystation of sporozoites. Sporocysts were released from their oocysts by vortex with 3.3 gm of glass beads contained in glass vial for about 2-3 min. The released sporocysts

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Pic. 1: Sporulated Oocysts (40x)

Pic. 2: Sporocysts after cracked oocysts (40x)

Pic. 3: Sporozoites after excystation (100x)

100) which was added to the pelleted of purified sporozoites for 24 h at 4°C with vortex. Then centrifugation at 10000 rpm for 10 min and the supernatant were taken as source of protein (vaccine). Concentration of protein were determined by the method of Bradford assay (Wallach et al., 1994).

Preparation of oocyst protein: About 2 ml (4x 10^7) of purified sterilized oocysts as mention previously were vigorously mixed with glass beads for 10 min. on vortex, then glass beads washed with minimal amount of PBS. The suspension of oocysts, sporocysts, sporozoites and walls was Frozen –186°C in liquid nitrogen and defreeze in water bath at 45°C for 3 times. Lysate buffer were added to the suspension (200 µl/1.5 ml) and incubated for 24 h at 4°C with vortex. Centrifugation was done for the suspension at 2000 rpm for 10 min and the supernatant was taken as a source of protein (vaccine). Concentration of protein were determined by the method of Bradford assay (Wallach et al., 1994).

Chickens field experiment: 160 chicks of Broiler (Ross 308) at age of one day-old, coccidiosis free, were obtained from (Hama, Syria) hatcheries. The source of drinking water from main supply and feeding on non medicated broiler diet (according to animal nutritional requirement of local feed tables) (Kussibati et al., 2003) as mash ad libitum. Throughout the study birds were maintained in 7 separated floor pens, and housed on litter composed of wood shaving of 5 cm depth, temperature in the floor pens was maintained 20-30°C. Extreme care was taken to avoid accidental exposure chicks to coccidia during immunization period and feces were examined periodically by the flotation technique for the absence of coccidial oocysts. The birds were grouped (20-30 chicken per group) at first day of hatch as Table 1.
Table 1: Types of groups used in the experimental design

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type of groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1SA</td>
<td>Vaccinated with Sporozoite protein+Adjuvant, challenged group (20 Birds)</td>
</tr>
<tr>
<td>G2S</td>
<td>Vaccinated with Sporozoite protein, challenged group (20 Birds)</td>
</tr>
<tr>
<td>G3OA</td>
<td>Vaccinated with Oocyst protein+Adjuvant, challenged group (20 Birds)</td>
</tr>
<tr>
<td>G4O</td>
<td>Vaccinated with Oocyst protein, challenged group (20 Birds)</td>
</tr>
<tr>
<td>G5</td>
<td>Vaccinated with Adjuvant, challenged group (20 Birds)</td>
</tr>
<tr>
<td>G6</td>
<td>Unvaccinated, challenged group (30 Birds)</td>
</tr>
<tr>
<td>G7</td>
<td>Unvaccinated, Unchallenged group (30 Birds)</td>
</tr>
</tbody>
</table>

Table 2: The protective efficacy to some parameters of the used proteins in immunized chickens

<table>
<thead>
<tr>
<th>Control</th>
<th>Type of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G7</td>
</tr>
<tr>
<td>Oocyst scorea</td>
<td>0</td>
</tr>
<tr>
<td>Cecal lesion scoreb</td>
<td>0</td>
</tr>
<tr>
<td>Percentage of protection (%)c</td>
<td>100</td>
</tr>
<tr>
<td>ACI indexd</td>
<td>200</td>
</tr>
</tbody>
</table>

a Oocyst score of each group was determined by the calculation and the criteria as described: O.P.G. of the vaccinated group in relation to the control group = (O.P.G. of the vaccinated challenge group) / (O.P.G. of the unvaccinated challenge group) * 100. Criteria: 0 to 1%: 5; to 25%: 10; to 50%: 20; to 75%: 40; to 100%: 67 to 100% (Kodama et al., 2006).
b Cecal lesion scores were determined as average 7, 8, 9 days after challenged with E. tenella according Johnson and Reid (1979).
c Percentage protection = (the number of oocysts from unvaccinated challenged group - the number of oocysts from vaccinated challenge group) / the number of oocysts from unvaccinated challenged group * 100 (Li et al., 2012).
d ACI index = (Relative weight gain rate + survival rate) - (Lesion score x 10 + oocyst value). Criteria: 180 or higher: very effective; 150 to 179: considerably effective; 120 to 161 slightly effective; less than 120: not effective (Gerl et al., 2011; Yang et al., 2012).

Table 3: Some parameters of the used proteins in immunized chickens which estimated to the period before challenge (28th day of chicken age) to the end of experiment (39th day of chicken age)

<table>
<thead>
<tr>
<th>Control</th>
<th>Type of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G7</td>
</tr>
<tr>
<td>Average weight gain (g)d</td>
<td>839</td>
</tr>
<tr>
<td>Relative weight gain (%)</td>
<td>100</td>
</tr>
<tr>
<td>Average feed intake (g)</td>
<td>2212</td>
</tr>
<tr>
<td>Percentage feed conversion (%)</td>
<td>37.0</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>100</td>
</tr>
<tr>
<td>Average OPG x (10t7, 8, 9th day)</td>
<td>-7.0996</td>
</tr>
</tbody>
</table>

(A) Average weight gain = Weight of each group at 39 day age of chickens subtracting weight of the same group at 28 day age of chicken.

(B) Relative weight gain (%) = (weight gain of the vaccinated group) / (weight gain of unvaccinated unchallenged group) * 100.

(C) Average feed intake (g) = {Amount of feed consumption in each group at period} / {mean of the chickens number in the same group at this period}.

(D) Percentage feed conversion (%) = {Average weight gain of each group at period} / {Average feed intake of the same group at this period} * 100.

Immunization: A total number of 160 chicks of broiler (Ross, 308) at age one-day old were divided as Table 1 into 7 groups. Groups (G1SA, G2S, G3OA, G4O) were immunized subcutaneously (S/C) on the neck with two doses: first dose at 3rd day of age with 25 μg antigen (sporozoite protein or oocyst protein) emulsified in Freund’s Complete Adjuvant (FCA-Sigma, St Louis USA) (or without Adjuvant) and booster dose was given at 18th day of age with 25 μg antigen (sporozoite protein or oocyst protein) emulsified in Freund’s Incomplete Adjuvant (FICA-Sigma, St Louis USA) (or without Adjuvant). Chicks in group (G5) were inoculated (S/C) with FCA emulsified in PBS as first dose and booster with FICA emulsified in PBS. After two weeks of last immunization an oral inoculation with 10⁴ of virulent Eimeria tenella sporulated oocysts for all groups except (G7) which kept as unimmunized unchallenged control. Chicks in group (G6) challenged only but didn’t immunized.

Evaluation of immune protection: The protective efficacy of the proteins used in immunized groups were measured according Table 2 and 3 to the: Cecal lesion scores which were determined at 7th, 8th, 9th days after chickens being challenged with sporulated oocysts of E. tenella and three chicks from each group were chosen randomly then euthanized. The caecal of each bird was examined, the gravity of lesions were scored between 0
and 4 according to the method of Johnson and Reid (1970). Oocysts output, also measured, by taking feces from each group separately between 7 and 9th days post challenge and the numbers of oocysts per gram feces were calculated using Mc Master technique as previously described by (Long et al., 1976). Oocyst score of each group was determined by the calculation and the criteria as following: O.P.G. of the vaccinated group in relation to the control group = (O.P.G. of the vaccinated challenge group) ÷ (O.P.G. of the unvaccinated challenge group) 100: Criteria: 0 to 1%, +5: 1.1 to 25%, +10: 26 to 50%, +20: 51 to 75%, +40: > 75% to 100% (Kodama et al., 2008). The Body Weight Gain (BWG) of the chickens in each group was determined weekly and the body weight also determined at end of the experiment subtracting the body weight at the time of challenge (Gerile et al., 2011). Percentage of protection also determined as described by (Li et al., 2012). Survival rate also determined and the Anticoccidial Index (ACI) is a Comprehensive indicator of medicine or vaccine as described by Gerile et al. (2011) and Yang et al. (2012) were determined. The parameters of: Percentage of relative weight gain, percentage of feed conversion and average feed intake (g) also determined.

Data (Cecal lesion scores, Oocysts output) were analyzed statistically using ANOVA (Analysis Of Variance) test.

**RESULTS**

The efficacy of *E. tenella* vaccine of oocyst or sporozoite (summarized in Table 2 and 3) were described as

**Oocyst output:** Significant decrease in oocysts output between immunized groups G1SA = 0.0566 x 10^4, G2S = 0.03 x 10^4, G3OA = 2.2963 x 10^4, G4O = 2.16 x 10^4 per gram feces (OPG) as compared with unimmunized control G6 = 7.2966 x 10^4 (P = 0.01) and G5 = 3.8675 x 10^4 (P = 0.05) (Fig. 1).

**Oocyst score:** The oocyst score of each group was determined and showed decrease in immunized groups G1SA = 5, G2S = 5, G3OA = 20, G4O = 20 but in unimmunized control groups oocyst score were G6 = 40 and G5 = 40.

**Lesion score:** The immunized groups had mean lesion score G1SA = 0.73, G2S = 0.64, G3OA = 0.98, G4O = 1.4 which different significantly from unimmunized control groups (p = 0.01) G6 = 3 and G5 = 2.4 (Fig. 2).

**Percent protection:** We could see percent protection for immunized groups of sporozoite vaccine G1SA, G2S, more effective than in oocyst vaccine G3OA, G4O. (99.2, 99.5, 67, 89%), respectively.

**Mortality:** There was no mortality in all studied groups.

**Anti-coccidial index (ACI):** The anti-coccidial index (ACI) for immunized groups of sporozoite vaccine was G1SA = 183.2, G2S = 184.3 very effective but with oocyst vaccine was G3OA = 159, G4O = 154.1 slightly effective, while in control groups G6 = 100.2, G5 = 108.9 not effective (Table 2).

**Body weight gain:** The body weight gain BWG in each group were determined weekly (5 week) as Fig. 3. There was no differences in BWG after immunization with first and second dose of vaccine in groups at four weeks. BWG after challenge the birds with *E. tenella*, we saw reduction in BWG in unimmunized control groups G6, G5. As in Fig. 4 which described the body BWG estimated at the period before challenge (28th day age of chicken) and at end of the experimental (39 day age of chicken), we saw reduction in the BWG in unimmunized challenge groups G6 = 581g, G5 = 812g, while unvaccinated unchallenged control G7 = 839g, the BWG of sporozoite vaccine groups well and close the control group BWG which was G1SA = 802g, G2S = 803g. The BWG of oocyst vaccine groups was G3OA = 714g, G4O = 723g less than control group G7.
strains can vary geographically (Allen and Fetterer, 2002) and certain *Eimeria* species may exhibit immunological variation infra specifically (Chapman et al., 2005). Live vaccines had many problem related reverting back to a pathogenic form (Sharan et al., 2010), high cost preparation and limited period shelf-life. Therefore we use local Syrian isolation to prepare protein vaccines from most important stages in the life cycle of *E. tenella* parasite (sporozoite and oocyst).

No oocysts were detected in the faeces of group G7 chickens throughout this study, demonstrating the success of the procedure adopted to prevent contamination by extraneous coccidia. FCA was found not successful in potentiating immunogenicity of sporozoite and oocyst protein. This result agree with study when use FCA with cell line as vaccine against avian coccidia (Miller et al., 1998) but disagree with another study when use FCA with sporozoite protein which was given higher effective (Badawy and Aggour, 2006). The ineffective of FCA in recent study may relative with higher immunogenicity of our used vaccines so there are no differences between groups with adjuvant and without it.

The efficacy of sporozoite vaccine was determined primarily on comparative oocysts output which decrease as compare with control groups or other vaccinated groups with oocyst vaccine (p = 0.05). We noticed lower mean of lesion score for sporozoite vaccine which differs significantly as compared with control groups but not appeared significantly with oocyst vaccine groups. Studies were used sporozoite protein as vaccines reached same results but use different procedure for preparation vaccines (Murray and Galuska, 1986; Karkhanis et al., 1991; Badawy and Aggour, 2006). Percent protection for sporozoite vaccines were (99.2, 99.5%) represent substantial degree from control groups and oocyst vaccinated groups of protection. Sporozoite that used as protein vaccine gives 66.7% percent protection (Badawy and Aggour, 2006), while in another studies by Subramanian et al. (2008) and Geriletu et al. (2011) gives (60, 77.3%), respectively percent protection when use recombinant *E. tenella* sporozoite antigen. In this study the sporozoite vaccine gave more protection and it might be returned to the procedure use (lysis buffer) for preparation and also we use total protein of sporozoite not subunit of sporozoite protein. ACI for sporozoite vaccine had been shown very effective while oocyst vaccine less effective, another study which use sporulated oocysts protein of *E. tenella* gives partially protected chickens (Karkhanis et al., 1991) and Geriletu et al. (2011) was recorded ACI of DNA vaccine on chickens against *E. tenella* very effective for two types of these vaccine and low lesion scores (1.1, 1.18) but percent protection was (60.4, 65.99%).

Average weight gain in vaccinated groups was higher as compared with non vaccinated control groups in the

**Percentage of relative weight gain:** The percentage of relative weight gain in immunized groups was better (G1SA = 95.5, G2S = 95.7, G3OA = 88.9 and G4O = 86.1%), respectively, as compare with unimmunized control groups G6 = 70.2 and G5 = 72.9.

**Average feed intake:** Average feed intake in grams decrease in unimmunized control groups G6 = 1940.8g, G5 = 2100g as compare with immunized groups were G1SA = 2218.7g, G2S = 2235.9g, G3OA = 2148.8g, G4O = 2150.4g and control groups G7 = 2212.

**Percentage feed conversion (%):** As compare between control G7 was 37.9 with immunized groups was G1SA = 36.9, G2S = 35.9, G3OA = 33.2, G4O = 33.6 and these exceptable, while unimmunized control groups less in percentage feed conversion which was G6 = 29.9 and G5 = 29.1.

**DISCUSSION**

There were very few studies on vaccines prepared against *E. tenella* parasite in Syria and only one study, on live attenuated oocysts vaccine by rays was reported (Al-attar and Al-Qshtiny, 1996). Antigencity of coccidial
period of challenge infections and there are no effective in average weight gain among the groups at the immunization period (4 week) when use first and second dose of vaccines. That means our vaccines were improved body weight gain as compare with control positive groups, this is might be due to specific antibody responses as well as cell-mediated responses which were detected in vaccinated groups (Badawy and Aggour, 2006). Eimeria, parasite invades the cells of the intestine that producing enteritis and diarrhea, resulting in disability to absorb dietary nutrients through the disruption of the integrity of the intestinal mucosa (Mansori and Modirsanei, 2012) and this lead to loss in weight of infected unvaccinated groups of chickens. Our results agree with another studies were use recombinant vaccine (Geriletu et al., 2011; Li et al., 2012).

Relative weight gain of immunization groups with sporozoite vaccines were (95.5, 95.7%) higher than oocyst vaccines groups (88.6, 86.1%) and control groups (70.2, 72.9%).

Average feed intake after challenge with E. tenella in control groups G5, G6 less than that of control group G7 and vaccinated groups, because of severe infection occur in unvaccinated groups were effected by lesion scores in cecum caused reduction in feed intake. Percentage feed conversion in chickens of sporozoite vaccinated groups was appeared close to the control group G7 but higher to control groups G6, G5 and oocyst vaccinated groups. Our results were showed similarity with study which was use antibody obtained from an egg of immunized chicken with an antigenic outer membrane proteins for sporozoite and merozoit of E. acervulina, E. tenella and E. maxima (Kodama et al., 2006). While in another study, there was no correlation between oocyst output, severity of lesions and bird weight gains has been discussed (Williams and Cotchpole, 2000).

Conclusion: Our results demonstrated clearly sufficient protection against coccidia by use the E. tenella sporozoite protein as vaccine in broiler after challenge. Further studies are recommended for determination and isolation of immune protective antigens.

REFERENCES