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## Immunization of Broiler with Dead Sporozoites as Vaccine Against *Eimeria tenella* Parasite

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**Abstract:** Chickens were protected against coccidiosis induced by *Eimeria tenella* by using 125, 000 dead sporozoites that were injected in neck subcutaneously with two doses at 3rd and 16th days of age. Efficacy of this vaccine was estimated after challenge by determination some parameters like protection rate which was around 96.1-96%. Number of oocysts and cecal lesion score from the chickens in the immunized groups were decreased significantly ( $p < 0.05 < 0.01$ ). This vaccine was not effected change on the body weight gain. Also, the immunized groups were higher body weight gain as compare with control groups after challenge. The Anti coccidial index measurement revealed the effectiveness of vaccine considerably. The immunogenicity of vaccine was studied by using SDS-PAGE and Western blot. 7 polypeptides had been estimated more immunogenic after probed with chickens serum at 39 days of age, their molecular weight are (12.3, 13.68, 18.7, 39, 59.5 and 77.3) KD. The results were obtained the feasibility of immunizing Broiler against *E. tenella* infection by using dead sporozoites as a vaccine.

**Key words:** Vaccine, sporozoite, *Eimeria tenella*, western blot

### INTRODUCTION

Coccidiosis is caused by intracellular protozoan parasites belong to several different species of *Eimeria*. There are seven species of *Eimeria* that parasitize chicken (*E. maxim*, *E. brunetti*, *E. acervuline*, *E. necatrix*, *E. mevati*, *E. praecox* and *E. tenella*) (Long, 1990). The Eimeriid coccidia exhibit a complex life cycle comprising stages both inside and outside of the host. In general, oocysts shed in feces undergo sporogony (ameiotic process) in the external environment and this process requiring oxygen. The sporulated oocysts contain four sporocysts, each containing two sporozoites. Upon ingestion by a suitable host, sporozoites excyst within the intestinal lumen. This process is aided by trypsin, bile and CO<sub>2</sub>. The released sporozoites penetrate the villous epithelial cells. Sporozoites either develop within cells at site of penetration or transported to other sites according to the species of *Eimeria*. Where they undergo development, within the host cells, sporozoites undergo of asexual reproduction (schizogony or merogony), resulting merozoites that break free and penetrate other host cells. Sexual reproduction or gametogony follows the last merogonic cycle. Merozoites enter host cells and develop into either male (microgamonts) or female (macrogamonts) forms. The microgamonts give rise to many micro gametes that exit, seek and fertilize the macrogamonts, then develop into oocysts (Allen and Fetterer, 2002).

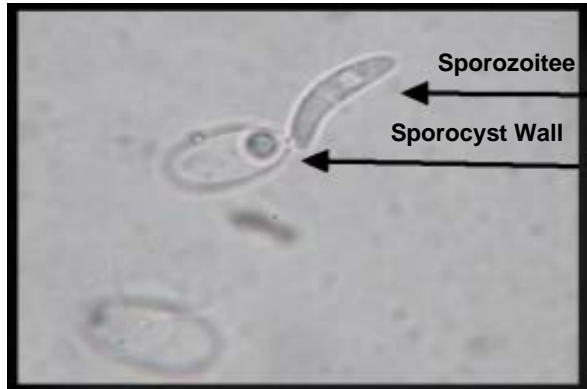
*E. tenella* is the most important *Eimeria* which infect the cecum of chickens and cause losses from the morbidity and mortality (Williams, 1999). Pathological changes

may occur by this obligate intra-cellular pathogens, these changes differ from destruction of local mucosal barrier and underling tissues to systematic effects such as blood loss, shock syndrome and ever death (Vermeulen *et al.*, 2001). It accounts for annual loss of billions of dollars for the poultry industry (Sun *et al.*, 2009). Treatment and prevention of coccidiosis was mostly based on chemotherapy but the drug resistant strain has recently become a major problem (Chapman *et al.*, 2010). Also, residues of the drug in the poultry raise serious health and safety concerns (Sharman *et al.*, 2010).

The sporozoite antigens are present on the surface and some of these molecules may be involved in recognition and penetration of host cells (Long, 1990). Sporozoites represent a likely target for protective immune response since in immune chickens they undergo a very restricted development or even fail to penetrate cells in the intestinal tract (Murray and Galuska, 1986). With public health commitment rising, alternative methods for controlling the parasite are much needed, low molecular weight antigens of *E. tenella* elicit strong humoral responses in birds (Wisher, 1986; Tennyso and Barta, 2000). Current study use the sporozoite as vaccine to *E. tenella*, in Broiler and also study further investigation of this antigens *in vivo* by western blots.

### MATERIALS AND METHODS

**Parasite propagation:** Local isolate of *Eimeria tenella* were obtained from (Dr. Katranji M.M., Parasit Lab./College of Veterinary medicine/Hama/Syria) and



Pic. 1: Excystation of *E. tenella* sporozoite 100X

propagated throughout 3 weeks old chickens (Broiler, Ross, 308).

Oocysts were collected from the ceca of infected chickens at 7th day post infection. After sporulation with potassium dichromate at 28°C for 6-7 days, oocysts were purified by standard salt flotation technique and sterilized by sodium hypochlorite treatment as described previously (Schamatz *et al.*, 1984). Sporulated oocysts were stored in phosphate buffer saline (PBS PH = 7.6) at 4°C until further use.

**Sporozoites isolation and purification:** About 2 ml ( $4 \times 10^7$ ) of sterile sporulated oocysts 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 were separated from the glass beads by washing with PBS and suspension has been contained sporocysts, oocyst walls and a few intact oocysts. The suspension was centrifuged at 2500 rpm for 3 min. The pelleted sporocysts were suspended in excystation fluid 0.25% trypsin, 5% chicken bile (v/v) in PBS pH = 7.6 and placed in shaking water bath at 41°C for 3 hours. When the majority of the sporozoites had exited Picture (1), the excystation halted by 3-fold dilution with PBS at 2500 rpm for 3 min. The pellet containing sporozoites, sporocyst walls, unexcysted sporocysts and some intact oocysts was then suspended in PBS. Purification of sporozoites were carried out by use either percoll gradient as described previously (Dulski and Turner, 1987), or by use ion exchange chromatography (Schamatz *et al.*, 1984; Riggs and Perryman, 1987). Purified sporozoites were counted by use haemocytometer, freeze defreeze 3 time and stored in 1 ml PBS at -75°C until use.

**Chickens field experiment:** 120 chicks of Broiler (Ross 308) at age of one day-old, coccidiosis free, were obtained from (Hama, Syria) hatcheries. The source of drinking water was from main water supply and feeding

Table 1: Types of groups used in design of the experimental

Groups	Type of groups
G1	Vaccinated with Sporozoite+Adjuvant, challenged group (20 Birds)
G2	Vaccinated with Sporozoite, challenged group (20 Birds)
G3	Vaccinated with Adjuvant, challenged group (20 birds)
G4	Unvaccinated, challenged group(30 Birds)
G5	Unvaccinated, Unchallenged group (30 Birds)

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 five separated floor pens and on litter composed of wood shaving to a depth 5 cm. Temperature in the floor pens was maintained 20-30°C. Extreme management was taken to avoid accidental exposure of chicks to coccidia during immunization period. Also 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 in Table 1.

**Immunization:** A total number of 120 broiler chicks (Ross, 308) one-day old were divided as (Table 1) into 5 groups G1, G2 are immunized subcutaneously (S/C) in the neck with two doses: first dose at 3rd day of age with 125,000 sporozoites emulsified in Freund' s Complete Adjuvant (FCA-Sigma, St Louis USA) or without Adjuvant and booster dose was given at 16th day of age with 125,000 sporozoites emulsified in Freund' s Incomplete Adjuvant (FICA-Sigma, St Louis USA) or without Adjuvant. Chicks in group G3 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  $10^4$  of virulent *Eimeria tenella* sporulated oocysts for all groups except G5 which kept as unimmunized unchallenged control. Chicks in group G4 challenged only but didn't immunize.

**Evaluation of immune protection:** The protective efficacy of the sporozoite antigen used in immunized groups were measured according (Table 2, 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 caecum of each bird was examined and 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 which were collected separately between 7th and 9th days post challenge and the numbers of oocysts per gram feces were calculated using McMaster technique as previously described (Long *et al.*, 1976). Oocyst score of each group was determined by the formula. The criteria as following: O.P.G. of the vaccinated group in relation to the control group = (O.P.G. of the vaccinated challenged group) ÷ (O.P.G. of the unvaccinated challenged group)

Table 2: Some parameters of the protective efficacy for the tested vaccine in immunized chickens

	Types of vaccine				
	Negative G5	Positive G4	Adjuvant G3	S+adj. G1	S G2
Controls					
Oocyst score	0	40	40	10	10
Cecal lesion score	0	3	2.4	0.86	0.76
Percentage of protection%	100	0	45.5	96.1	96
ACI index	200	100.2	108.9	174.8	176.2

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: 1.1 to 25%+10: 26 to 50%+20: 51 to 75%+40: 67 to 100% (Kodama *et al.*, 2006).

Cecal lesion scores were determined as average 7, 8, 9 days after challenged with *E. tenella* according Johnson and Reid (1970).

Percentage protection = (the number of oocysts from unvaccinated challenge group - the number of oocysts from vaccinated challenge group) ÷ the number of oocysts from unvaccinated challenge group × 100 (Li *et al.*, 2012).

ACI index = (Relative weight gain rate+survival rate) - (Lesion score × 10+oocyst value). Criteria: 180 or higher: very effective; 160 to 179: considerably effective; 120 to 161 slightly effective; less than 120: not effective (Geriletu *et al.*, 2011; Yang *et al.*, 2012).

\*p = 0.01, \*0.05 significantly different from the control groups (G3, G4).

Table 3: Some parameters of the tested vaccine 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)

	Types of vaccine				
	Negative G5	Positive G4	Adjuvant G3	S+adj. G1	S G2
Controls					
Average weight gain (g) <sup>A</sup>	839	581	612	784	787
Relative weight gain (%) <sup>B</sup>	100	70.2	72.9	93.4	93.8
Average feed intake(g) <sup>C</sup>	2212	1940.6	2100	2096.2	2236
Percentage feed conversion (%) <sup>D</sup>	37.9	29.9	29.1	37.4	35.1
Survival rate(%)	100	100	100	100	100
Average OPG x(10) <sup>7, 8, 9th day</sup>	-	7.0966	3.8675	0.2716	0.28

<sup>A</sup>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.

<sup>B</sup>Relative weight gain (%) = {weight gain of the vaccinated group} ÷ {weight gain of unvaccinated unchallenged group} × 100.

<sup>C</sup>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}.

<sup>D</sup>Percentage feed conversion (%) = {Average weight gain of each group at period} ÷ {average feed intake of the same group at this period} × 100.

100: Criteria: 0 to 1%, +5: 1.1 to 25%, +10: 26 to 50%, +20: 51 to 75%, +40: 67 to 100% (Kodama *et al.*, 2006). 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 (Geriletu *et al.*, 2011). Percentage of protection also determined as described (Li *et al.*, 2012). Survival rate also determined and the Anti-Coccidial Index (ACI) is a Comprehensive indicator of medicine or vaccine as described by Geriletu *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.

**Blood collection:** Blood was collected from a wing vein from all chickens post first dose of at 7th days old chicken and after second dose of sporozoites antigen at 28 days old chicken and also collected at the end of experiment at 39th day of age chicken post challenge. Sera were stored at -20°C until use.

**Antigens characterization:** *E. tenella* sporozoite antigens are identified by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). *E. tenella* sporozoites are extracted with (0.5% Nonidet P40, Tris-HCl 10 µM, Aprotinen 0.1 U/ml, 1% Triton X-100) by taking 100 µl that added to the pelleted of purified sporozites for 24h at 4°C with vortex, then centrifugation at 10000 rpm for 10 min and the supernatant were taken as source of protein to electrophoresis. Concentration of proteins were determined by the method of Bradford assay (Bradford, 1976).

The extraction of *E. tenella* sporozoites protein (50 µg) per lane were lysed by boiling in reducing loading buffer {25 %glycerol, 5% β-Mercaptoethanol, 10% Sodium Dodecyl Sulfate (SDS), 0.01% Bromophenol blue in Tris/HCl (PH = 6.8) 16mM}. Then separation was done by using (SDS-PAGE) on a 5-15 gradient gel. Polypeptide in the gel were transferred electrophoretically to nitrocellulose paper (BA85; 0.4 µM; Schleicher and Schull, Inc.) In a Transblot transfer cell (Bio-Rad

Laboratories). Electrophoresis was done with transfer buffer at 4°C for 1.15 h at constant 250 A. After transferring, the nitrocellulose paper containing the polypeptid was washed two times, for 5 min each time, with distilled water. Excess binding sites on the nitrocellulose paper were blocked by washing the paper with Tris buffer saline-tween (TBS-T) PH = 7.5 {10mM Tris-Hcl, 154mM NaCl and 0.1% Tween -20} plus to 3% povine serum albumin, for 24 h in 4°C.

The nitrocellulose paper was further washed 3 times, for 5 min each time with (TBS-T) buffer on rotater shaken. The membrane was then cut into strips which were separated into individual container and exposed to a 1:200 dilution of the experimental chicken sera in (TBS-T) buffer for 1h. Following washing, the nitrocellulose membrane was incubated for 1h in a 1:1000 dilution of {Rabbit anti-chicken IgG (H and L) conjugated to hoers radish peroxidase (Invitrogen Company/California/USA)}, washed (3 x 5 min) in TBS-T buffer, finally incubated in OPD (Ortho Phenil diamin) substrate solution for 15-20 min with rotate until band had been appeared. The reaction was stopped by washing nitrocellulose stripes 2 times with D.W.

Data (Cecal lesion scores and Oocysts output) were analyzed statistically by using ANOVA (Analysis of Variance) test.

**RESULTS**

The efficacy of *E. tenella* frozen sporozoites (summarized in Table 2 and 3) were described as.

**Oocysts output:** There was significant decrease in oocysts output between immunized groups G1 =  $0.2716 \times 10^4$ , G2 =  $0.28 \times 10^4$  per gram feces (OPG) as compared with unimmunized control G4 =  $7.0966 \times 10^4$ , G3 =  $3.8675 \times 10^4$  (P<0.01) Fig. 1.

**Oocyst score:** The oocyst score of each group was determined and showed decrease in immunized groups G1 = 10, G2 = 10 but in unimmunized control groups oocyst score were G4 = 40 and G3 = 40.

**Lesion score:** The immunized groups had means of lesion score G1 = 0.86, G2 = 0.76 which different significantly from unimmunized control groups G4 = 3 (p<0.01) and G3 = 2.4 (p<0.05) Fig. 2.

**Percent protection:** The observed percent protection for immunized groups of sporozoite vaccine G1, G2, was 96%.

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

**Anti-coccidial Index (ACI):** ACI for immunized groups of sporozoite vaccine was considerably effective G1 = 174.8, G2 = 176.2, while in control groups were not effective G4 = 100.2, G3 = 108.9 Table 1.

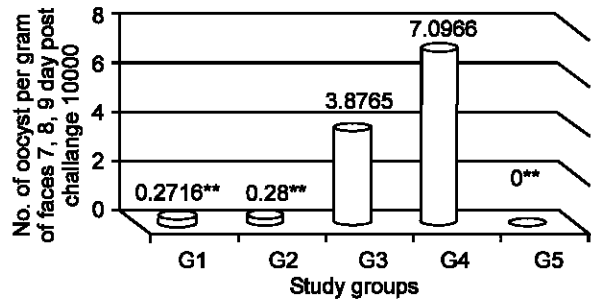


Fig. 1: Oocyst output

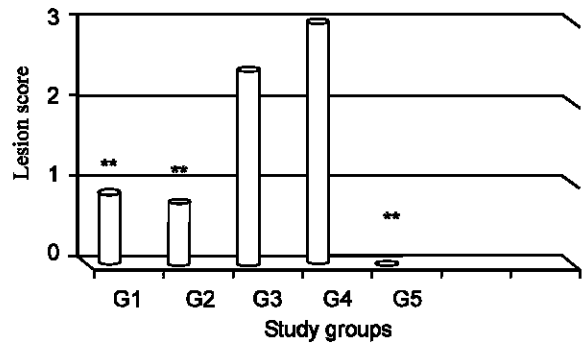


Fig. 2: Average lesion score

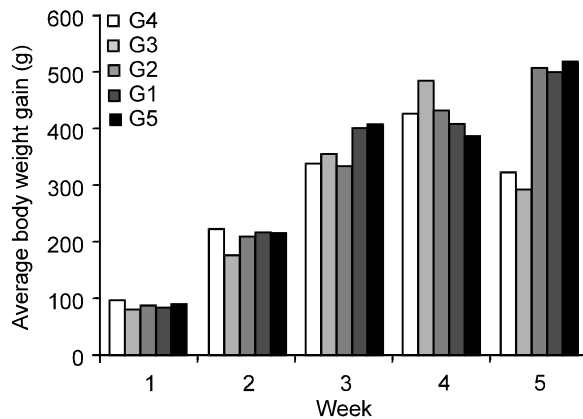


Fig. 3: Average body weight gain (g) weekly

**Body weight gain:** BWG in each group was determined weekly (5 week) as Fig. 3. There was no differences in BWG after immunization with first and second dose of vaccine in all groups at four weeks. BWG after challenge the birds with *E. tenella*, revealed reduction in BWG in unimmunized control groups G4, G3. Figure 4 described the BWG estimated at the period before challenge (28th day of age) and at end of the experimental (39 day age of chicken). Reduction in the BWG of unimmunized challenge groups was observed G4 = 581g, G3 = 612g, while unvaccinated unchallenged control G5 = 839g, the BWG of sporozoite vaccine

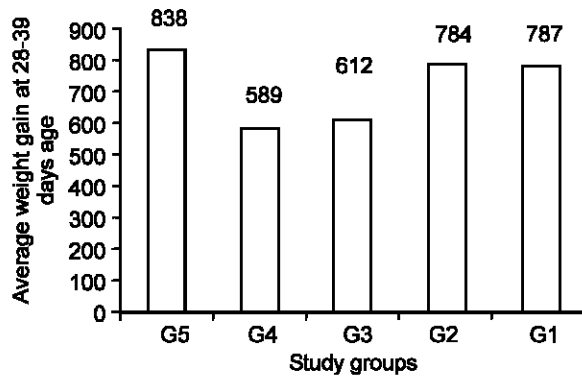


Fig. 4: Average body weight gain (g) for 28-39 days age of chickens

groups had been reported closely to the control group G1 = 784g, G2 = 787g.

**Percentage of relative weight gain:** The percentage of relative weight gain in immunized groups was appeared better for both G1 and G2 (93.4, 93.8%), respectively, as compare with unimmunized control groups G4 = 70.2 and G3 = 72.9.

**Average feed intake:** Average feed intake was decrease in unimmunized control groups G4 = 1940.6g, G3 = 2100g as compare with immunized groups were G1 = 2069.2g, G2 = 2236g and control groups G5 = 2212.

**Percentage feed conversion (%):** Had been estimated for G5 = 37.9 and for immunized groups was G1 = 37.4, G2 = 35.1, while for unimmunized control groups appeared less G4 = 29.9 and G3 = 29.1.

**Antigens analysis:** Polypeptides are obtained for analysis by the separation of sporozoite antigens by SDS-PAGE. There are 11 polypeptides were prominent stained strongly with coomassie blue. Their molecular weight were 10.9, 11.6, 13.9, 25.9, 37.5, 51.1, 77, 100, 107, 113 and 155 KD. Polypeptides also aggregated upon 155 KD which had molecular weight greater than our used marker protein Picture 2.

In western blot patterns reaction of parasite-specific IgG (H and L) antibodies with sporozoite antigens differed when each of 3 immune sera were used as a probe in the immunoblotting procedure. These differences were most obvious between molecular weight 12.3 and 77.3KD Picture 3. Immunized serum of 39 day age of chicken consistently identified and reacted more intensely with more numbers of antigens in sporozoite preparations than immunized serum at 28-7 day age of chicken. These antigens with molecular weight 77.3, 59.5, 39, 18.7, 13.68 and 12.3 KD were identified with immune serum post of challenge dose, with *E. tenella* parasite.

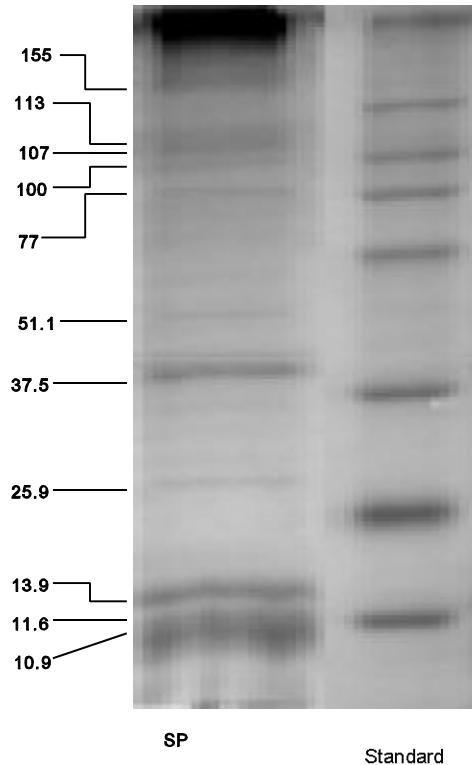


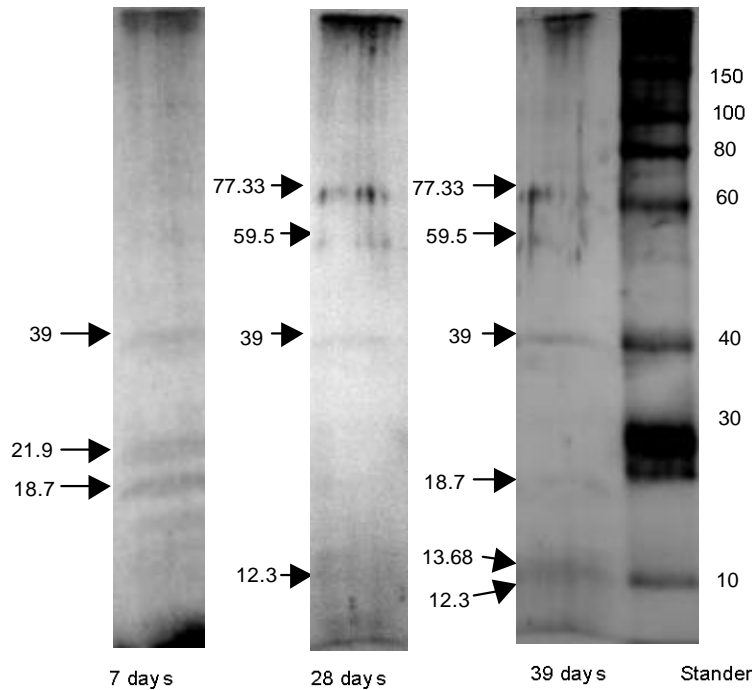
Fig. 2: SDS-PAGE(5-15%) stained with Coomassie blue after electrophoretic separation of sporozoites proteins (SP)

The immunized serum at 7 days age was reacted slightly when used as a probe. Three antigens had been reported with molecular weight 39, 21.9 and 18.7 KD. One of these antigen 39 KD and further other antigens with molecular weight 77.3, 59.5 and 12.3 were identified with immunized serum at 28 day age.

All three immunized serum were reacted with sporozoite antigen of molecular weight 39 KD. Immune serum of 28 and 39 days age reacted with four common antigens (77.3, 59.5, 39 and 12.3KD).

## DISCUSSION

The protective immune response in animals against Coccidial infection is directed against sporozoite stage but it was generally accepted that the asexual stages produce the strongest stimulus for development of immunity (Long, 1990). Antigenicity of coccidial strains can vary geographically (Allen and Fetterer, 2002). Also, certain strain of *Eimeria* exhibit immunological variation (Lee, 1993). For a better understanding of strain variation is needed for any vaccine to give promising results against local field strains of *Eimeria* (Anwar *et al.*, 2008). However, the host cell-parasite relationship at the invasive stage has remained unclear and it is considered that surface antigens of sporozoite are important for adhesion of sporozoite to epithelial cells



Pic. 3: Immunoblot of proteins transferred from a 5-15% SDS-PAGE. Lanes are immunoblots of sporozoite extract preparations probed with immune serum (7 days); after first dose of vaccine (28 days); after second dose of vaccine and (39 days) after challenge with *E. tenella* oocysts

before invasion (Martin *et al.*, 1997). So, the present study has been tested asexual stage of whole dead sporozoites of local strain of *E. tenella* as vaccine. Besides that the criteria for judgment of the immunizing efficacy of this antigen, humoral response for sporozoite antigen in chicken which challenged by *E. tenella* were studied.

There was no contamination by extraneous coccidian throughout this study, that demonstrated the success of the field experiment. In this study, we use 125,000 whole dead sporozoite as vaccine which represented (15, 625 oocysts) that were injected in the neck of chickens at 3rd day of age. This should not be given orally to chicks in this earlier age, because it might be the cause and of severe pathological effect.

FCA was failed in potentiating immunogenicity of dead sporozoite. Our results agreed with previous study when FCA used with cell line as vaccine against avian coccidia (Miller *et al.*, 1998). But these results disagreed with another study when FCA used with sporozoite protein which was given higher effectiveness (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 or without it.

This antigen was induced significant lower oocyst output and lesion scores than the challenge control groups G4, G3. The result of ACI were G1 = 174.8, G2 = 176.2

considerably effective, while ACI was not appeared effective in control groups G4, G3.

The body weight gain of the G1, G2 did not differ at the days of first and second immunization dose and challenge as compare with control negative group G5, while there was a reduction in body weight gain in control positive groups G3 G4 after challenge. This indicated that whole dead sporozoite antigen could be an effective candidate to develop vaccine against *E. tenella* infection. Tennyso and Barta (2000) studied the localization and immunogenicity of a low molecular weight antigen (125, 000 dead sporozoites) of *E. tenella* that showed highly immunogenic and conserved among all life stages of this parasite, although the protective nature of immune response was not investigated in the last study. The low molecular weight antigen elicited a strong and persistent serum IgG response during infection or when injected intramuscularly as a part of whole, dead sporozoite.

Our results agree with other studies that used sporozoite protein as vaccine against *E. tenella* infection (Murray and Galuska, 1986; Karkhanis *et al.*, 1991; Badawy and Aggour, 2006) which lower lesion score and oocyst output. In another study the immunization of Broiler chicks by *in ovo* injection of *E. tenella* sporozoites, sporocysts, or oocysts with different doses, were resulted in reduction of oocysts output and lesion score but weight gain was not affected (Weber and

Evants, 2003). In recent study sporozoite antigen was given 96% percent protection while Badawy and Aggour (2006) were recorded 66.7% percent protection by using protein of sporozoite to *E. tenella* vaccine in Broiler.

Relative weight gain in vaccinated groups were G1 = 93.4 and G2 = 93.8 which appeared sufficient as compared with control positive groups G4G3. Average feed intake of immunization groups were reported closely to control negative group G5. Percentages of feed conversion of control positive groups G3G4 were less than control negative G5 and immunization groups G1 G2.

In this study SDS-PAGE for sporozoites of *E. tenella* had been demonstrated many proteins (11 polypeptides) with Molecular Weight (MW) ranging from (10.9 to 155) KD, These polypeptides were (>10.9, 11.6, 13.9, 25.9, 37.5, 51.1, 77, 100, 107, 113 and 155KD). Murray and Galuska (1986) were detected (11 polypeptides) from sporozoite of *E. tenella* protein when used SDS-PAGE, their MW are (235, 175, 105, 94, 82, 60, 50, 45, 28 and 23KD). There are differences in molecular weight of polypeptides between two studies might be because of the differences in preparation of sporozoite proteins. Also, differences in condition of SDS-PAGE for each experiment.

In immunoblotting study there were differences in identification of antigens and staining intensities among sera that were collected from chickens at (7, 28 and 39) days of age which appeared after first dose of currently used vaccine (after four days from first vaccine dose). We recognized three polypeptides with molecular weight (93, 21 and 18.7KD). The number of proteins and intensities of reaction were increased after second dose of sporozoite antigen and after challenge with *E. tenella* parasite.

In immunoblotting study the protein of molecular weight 21.9 KD was appeared only after first dose of used vaccine. Another study had been shown 22 KD protein of sporozoite antigen when used western blot of sporozoite proteins (Karkhanis *et al.*, 1991). While Brothers *et al.* (1988) were observed (by using two neutralizing antibodies) 25 KD polypeptide on the surface of *E. tenella* sporozoites. This antigen consisted of two 17 and 8 KD polypeptides linked by a disulfide bond. The present study recognized 18.7 and 12.3 KD polypeptides after challenge but the 18.7 KD polypeptide has not appeared after second vaccine dose and this may be due to the lost of polypeptides during SDS-PAGE. Murray and Galuska (1986) had been reported 7 immunogenic surface sporozoite of *E. tenella* polypeptides (26, 45, 68, 82, 94, 105 and 235 KD). Another study was reported membrane proteins of 37 and 45 KD inhibited invasion of *E. tenella* but not *E. meleagridis* (Augustian and Danforth, 1985).

This study was observed 39 KD polypeptide in the serum that collected time of after first and second dose of vaccine and after challenge with *E. tenella* parasite.

But 59.5 and 77.3 KD polypeptides were observed after second dose of vaccine and after challenge.

Fetter *et al.* (2004) were demonstrated approximately 19 KD protein from extracts of *E. tenella* sporozoites, merozoites, sporulated oocysts and oocysts in various stage of sporulation when use rabbit antiserum to recombinant sporozoite (sz-1). While Numajiri *et al.* (2000) were found during their study by using Bioten-labeled proteins of *E. tenella* sporozoite more than 200 sporozoite proteins that were recognized with silver staining after 2D-PAGE, among them 7 biotin-labeled proteins were detected as surface molecules after western blotting. Their molecular masses ranged from 23 to 117 KD.

It is not clear whether these differences or similarity between the present results and previous ones depend on the labeling reagent and method or not. So, further studies are necessary.

Antibodies play a role in the protective immunity against *Eimeria* (Wallach, 2010) and *in vitro* studies have demonstrated that antibodies could have many effects, including agglutination, lysis, neutralization of infectivity and morphological changes on various developmental stages of *Eimeria* if they come in close contact with the parasites (Rose, 1987). Augustiane and Danforth (1985) had been suggested that antibody did not inhibit cell invasion by clumping sporozoites. Instead, it may operate by reacting with sporozoites secretory products which aid in the invasion process.

Badawy and Aggour (2006) have been shown during their study on sporozoite protein as vaccine when used Enzyme linked immune sorbent assay, antibodies were detected on day 16 and reached peak on day 40 post immunization. These observation agree with current study when 3 immune sera was used as a probe in the immunoblotting procedure. Immune serum on day 39 post immunization consistently identified and reacted more intensely with more antigens in sporozoite preparations than immune serum at 7 and 28 days age of chicken. While other studies were demonstrated that antibody responses played minor role in protection against coccidiosis (Speer *et al.*, 1985; Augustine and Danforth, 1986).

**Conclusion:** 125, 000 dead sporozoites were used as vaccine in chicks at 3 days age. This vaccine was given more effective protection in Broilers against *Eimeria tenella* parasite. The antibody was played an important role in the protection Broilers after challenge. Further studies are recommended for determination the role of cellular immunity.

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