



Resistance to cecal coccidiosis following sonicated oocysts immunization of *Eimeri tenella* in broilers

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Abstract

Seventy five broiler chicks were used to determine the immunization of *in ovo* inoculation or intramuscular (I/M) injection of sonicated oocysts (SO) antigen against *E. tenella* manifestation. The chicks were divided in to 3 equal groups comprising of 25 chicks each. The 1st group inoculated *in ovo* with SO of *E. tenella* and repeated by I/M injection at 21 days old. The 2nd group injected I/M with SO at day old and repeated at 21 days old, The birds of the 3rd group remains as control. All birds were challenge by 50,000 sporulated oocysts of *E. tenella* at day 28. No significant ($P > 0.05$) group difference was detected between the immunized and non-immunized groups for mean, WBC count, H/L ratio, lesion score, mortality and oocysts shedding until *E. tenella* challenge at day 28. After challenge test the *in ovo* and I/M immunized groups showed a great protective immunity against *E. tenella* infection documented by significantly ($P < 0.05$) reduced mortality, lesion score and decreased fecal oocyst shedding, compared with non-immunized group. It is concluded that *in ovo* and I/M immunization of sonicated oocyst stimulates a remarkable protection in broilers following *E. tenella* infection comparing with non-immunized groups. There was no significance difference between the two procedures of immunization.

Key words: *E. tenella*, immunity, sonicated oocysts, broilers

المقاومة ضد الإصابة الاعورية لطفيلي الايميريا تينيليا بعد التمنيع بالبيوض المكسرة في دجاج اللحم

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الخلاصة:

أجريت هذه الدراسة على خمسة وسبعين فرخاً من افراخ اللحم للتمنيع ضد الإصابة بطفيلي الايميريا تينيليا باستخدام مستضد أكياس البيض المكسرة لطفيلي الايميريا تينيليا عن طريق الحقن بالبيض والعضلة. قسمت هذه الأفراخ إلى 3 مجاميع متساوية تحتوي كل مجموعة على 25 فرخاً، حقنت المجموعة الأولى بالبيضة بعمر 18 يوماً من عمر الجنين وأعيد حقنها بعمر 21 يوماً بعد الفقس بأكياس البيض المكسرة لطفيلي الايميريا تينيليا. المجموعة الثانية حقنت بالعضلة بعمر يوم واحد، وأعيد حقنها بعمر 21 يوماً بمستضد أكياس البيض المكسرة

لطفيلي الايميريا تينيليا. المجموعة الثالثة تركت كمجموعة سيطرة. تم إجراء اختبار التحدي لكل الطيور بإعطائها 50,000 كيس بيض متبوغ لطفيلي الايميريا تينيليا بعمر 28 يوماً. لم تلاحظ أي فروقات معنوية ($P > 0.05$) بين المجاميع الممنعة والغير ممنعة من ناحية معدل المعايير الدموية المنتخبة, والآفات العيانية, ومعدل الهلاك ومعدل طرح أكياس البيض في البراز حتى إجراء اختبار التحدي بعمر 28 يوماً. بعد إجراء اختبار التحدي أظهرت المجاميع الممنعة عن طريق الحقن بالبيضة والحقن بالعضلة مستويات حماية عالية ضد ألخمج بطفيلي الايميريا تينيليا وسجلت اختلافاً معنوياً عند ($P > 0.05$) من ناحية انخفاض عدد الهلاكات و الآفات العيانية, وانخفاض معدل أكياس البيض المطروحة مقارنة مع المجموعة غير الممنعة. من هذا يمكن الاستنتاج بأن التمنيع بواسطة الحقن بالبيض والحقن بالعضلة بأكياس البيض المكسرة حفزت المناعة ووفرت حماية يمكن ملاحظتها ضد الخمج بالاييميريا تينيليا مقارنة مع المجاميع غير الممنعة. ولم يكن هناك أي فرق معنوي بين طريقتي التمنيع بواسطة الحقن بالبيض أو الحقن بالعضلة.

Introduction

Coccidiosis is an economically important disease that seriously impairs the feed utilization and growth of chickens due to intestinal infection with the protozoa *Eimeria* (1). Seven species of *Eimeria* are generally accepted to be causative agents of avian coccidiosis, *E. tenella* found to be the most prevalent and pathogenic species throughout the world (2).

At present, prophylactic chemotherapy is still the main strategy of controlling coccidiosis, but their extensive use over the past 40 years has resulted in the development of drug resistance by these parasites (3). Due to food safety concerns and the cost of new drug development, recent emphasis has centered on elicitation of protective immune response to parasite infection by development and effective use of live or inactivated parasite vaccines (4).

Vaccination is an alternative option for coccidiosis control (5). Compared with virulent or attenuated live vaccine, recombinant protein vaccine can induce good antibody response and has more efficiency to protect birds against challenge of *Eimeria* oocysts (5). Parental inoculation of dead antigen is although capable of stimulating circulating antibodies against coccidiosis antigen (6). In spite of these limitations, vaccination remains

the most efficient means of preventing disease and reducing economic losses (7). The purpose of the present study was to protect chicken against coccidiosis by immunized it with whole sonicated oocyst trial in alleviating *E. tenella* infection.

Materials and Methods

Experiment was done in the poultry house of Pathology and Poultry Diseases Department, College of Veterinary Medicine, Baghdad University.

Birds and management

After cleaning and disinfection, one-day-old, broiler chicks of the "Cobb strain" were purchased from a local hatchery. Upon arrival, the chicks were raised according to routine management practice as outlined by the National Research Council requirements (8). All nutrients including water were supplied *ad libitum* to meet the NRC (8).

Sonicated antigen and vaccine preparation

Field isolate of a highly virulent strain of *E. tenella* was obtained from a broiler farm with an outbreak of bloody diarrhea with pathognomonic cecal lesion and high mortality was used in this study for oocysts collection. After oocysts collection in potassium dichromate solution (% 2.5) and sporulation, the oocysts were

washed 3 times by physiological saline solution (pH 7.2) at 3000 rpm for 10 minutes each and concentrated to 5000-6000 per ml using the hemocytometer (9). The washed sporulated oocysts were subjected to ultra-sonication by Soniprep150 (SONY Company) for 2 by 30 seconds in jacketed vessel with cool water. The inactivated vaccine was prepared from sonicated suspension by treating with 0.3 percent formalin (33% formaldehyde) for 96 hours at 37°C (10; 6) and stored at 4°C until use.

Experimental Design

A total of seventy five newly hatched commercial broiler chicks "Cobb strain" was purchased from a local hatchery. Upon arrival, the chicks were divided randomly into three equal groups of 25 chicks each, 1st group chicks were inoculated *in ovo* with 0.2 ml of sonicated oocyst at day 18 embryonated eggs and repeated by I/M injection at 21 days old. The 2nd group chicks were receives 0.2 ml of sonicated oocyst by I/M injection at day one and repeated by same route at 21 days old. The 3rd group chicks remain as control. All groups were feed a basal diet without anticoccidial drugs and challenged by 50,000 viable sporulated oocysts of *E. tenella* at day 28 (11).

Performance parameters

Morbidity and mortality rate

The morbidity and mortality monitored by recording the clinical signs and dead chicks from 5th day to 8th day after challenge infection with *E. tenella* (12).

Effects on selected haemograms

Blood (3 ml) was collected from the birds from the jugular vein into

(EDTA), WBC were counted using the haemocytometer (13), and heterophil to lymphocyte ratio were determined by counting encountered elements on blood smears under the microscope according to Hawk (14).

Lesion scores

Means of the lesion scores of the different treatment groups were determined. On days 28, 33, 34 and 35 five birds per group were randomly selected and sacrificed by slaughtering for scoring of lesions from cecal coccidia. Lesions were scored in the ceca on a scale from 1 to 4 with 4 being the most severe (15).

Determination of oocysts shedding

The oocysts output was assessed using a haemocytometer chamber method which was documented by (16; 17). Litter was collected from several representative areas of the chicken house for oocyst counts at 10 days post infection. The samples are mixed together and 5 g of material are weighed, and purification, flotation and a haemocytometer counting was done. Total oocyst output= total oocyst count per gram x weight of feces.

Results

The mean total WBCs count of broilers during the experimental period is summarized in Table 1. No significant differences were shown between all experimental groups at day 28. Then after, statistically significant differences were indicated in the immunized groups ($P < 0.05$) when compared with non-immunized group at days 33, 34 and 35. At that time the vaccinated ones had the low values.

Table: 1 the WBCs counts ($10^3/\text{mm}^3$) of broilers during the experimental period (mean \pm SD)

Groups	Before challenge	After challenge		
	d 28	d 33	d 34	d 35
Inovo	20.48 \pm 0.509 a	20.79 \pm 0.750 b	20.95 \pm 0.840 b	21.42 \pm 0.916 a
I/M	21.48 \pm 1.377 a	20.88 \pm 0.993 b	20.99 \pm 0.809 b	21.81 \pm 0.777 a
Control	20.39 \pm 1.042 a	22.95 \pm 1.375 a	22.74 \pm 0.890 a	22.58 \pm 0.891 a

a, b Values bearing similar superscript between column do not differ at ($P < 0.05$).

Similarly, an increasing pattern of H/L ratio was seen in non-immunized group at days 33, 34 and 35 (Table 2). That of the immunized groups remained the lowest H/L ratio ($P < 0.05$) at days 33, 34 and 35.

Table: 2 the H/L ratio of broilers during the experimental period (mean \pm SD)

Groups	Before challenge	After challenge		
	d 28	d 33	d 34	d 35
Inovo	0.373 \pm 0.046 a	0.412 \pm 0.044 b	0.404 \pm 0.052 b	0.394 \pm 0.054 b
I/M	0.401 \pm 0.070 a	0.409 \pm 0.032 b	0.406 \pm 0.032 b	0.404 \pm 0.032 b
Control	0.402 \pm 0.059 a	0.554 \pm 0.049 a	0.502 \pm 0.048 a	0.509 \pm 0.048 a

a, b Values bearing similar superscript between column do not differ at ($P < 0.05$).

The lesions score of broilers during the experimental period (mean \pm SD) are illustrated in table 3. Lesion scores were zero for the all groups before challenge. After challenge, the immunized groups have the lowest mean lesion compared with non-immunized control group which was had the highest mean lesion scores with an average of 3.20.

Table: 3 the lesions score of broilers during the experimental period (mean \pm SD)

Groups	Before challenge	After challenge
	d 28	d 35
Inovo	0	1.00 \pm 0.707 ^b
I/M	0	0.80 \pm 0.447 ^b
Control	0	3.20 \pm 0.447 ^a

^{a, b} Values bearing similar superscript between column do not differ at ($P < 0.05$).

In the cross sectional study, noticeable signs of morbidity were observed as depression, ruffled feather, diarrhea and/or blood mixed droppings were recorded as clinical cases at day 5 post infection and peaked on days 6 and 7. The result of clinical infection was observed in non-immunized group (control). One bird died for each *in ovo* and I/M groups, then six birds was died from control group (table 4).

Table: 4 the mortality of broilers during the experimental period (mean \pm SD)

Groups	Number of challenge at d 28	After challenge till d 35	Percentage
Inovo	15	1	6.66
I/M	15	1	6.66
Control	15	6	40.00

The results of oocyst output of broilers during the experimental period are summarized in table 5. The immunized groups shed the lowest oocysts (0.051×10^6 , 0.055×10^6) of any of the infected groups. Nevertheless, the control group had the highest means with an estimate of 1.930×10^6 oocysts shedding.

Table: 5 the oocyst output ($\times 10^6$) of broilers during the experimental period (mean \pm SD)

Groups	d 28	d 38
Inovo	0	0.051 ± 0.016^b
I/M	0	0.055 ± 0.009^b
Control	0	1.930 ± 0.155^a

^{a, b} Values bearing similar superscript between column do not differ at ($P < 0.05$).

Discussions

The effectiveness of vaccination in the generation of immunity and protection against subsequent *Eimeria* infection has been well documented (4). The *in ovo* inoculation at day 18 embryonated eggs and I/M at 1 day old injection proved their ability to stimulating the immune system (18; 19). The main objectives of this research were to assess whether the sonicated oocysts antigen could reduce the negative effects to mean protection rate, cecal lesions and oocyst output and mortality by stimulating effective immune response associated with virulent *E. tenella* challenged in broiler chickens. Our results revealed that there were no statistically differences were detected in the immunized and non-immunized groups for mean, WBC count, H/L ratio, lesion score, mortality and oocysts shedding until *E. tenella* challenge at day 28, this proved that the dose of antigen not effected on the chicks.

White blood cells are the effectors cells of immune responses and have been assessed in many immunological studies of avian (20). The normal range limits of selected haemogram parameters values (21) in the immunized groups signified possible event of a usual situation compared with the deviation of WBC count and

H/L ratio from normal in the non-immunized groups, this increase corresponds to periods of severe tissue destruction to the heavy infestation of *E. tenella* (22).

In this study immunization with the *E. tenella* sonicated oocysts stimulated protective immunity against experimental challenge infection as indicated by reduced lesion scores among immunized groups compared with non-immunized groups (cecal lesions in the 2 to 3 range), vaccination against *E. tenella* reduced the cecal distraction (4). The mortality rate in non-immunized groups showed high levels comparing with the immunized groups which proved high protection against the *E. tenella*.

In general protective immunity has normally been measured through vaccination by reduction in lesion scores, cessation in total oocyst output (23). We can conclude that immunization of broilers chicks with a sonicated oocyst is an effective tool for generation of protective immunity to field strain of *E. tenella* following challenged of poultry with this antigen increased this protection and eliminate the disease by prevent the clinical signs, gross lesions and mortality rate.

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