

Trials for immunization against coccidiosis in broiler chickens as a control measure

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Abstract

Coccidiosis is an important diseases facing intensive poultry rearing. Prevention of coccidiosis is mainly based on the use of anticoccid drugs, The use of vaccines against coccidiosis in chickens is used many countries. This attract our attention to use various dose of oocysts inoculated by different methods at different time as a method immunization of chickens against coccidiosis. In the present study 10 chicks were used in 5equal groups, two groups were oral inoculate two groups were injected by S/C and I/P route , one group left as control +ve group. First dose were given at 4 day old .Second dose were given after one week from the first dose to each group except the control +ve group and then after one week birds were challenge. The parameters used for match up to different groups are monitoring of oocysts production, detection of serum proteins (albumin and globulins) and Antibody titer in serum by Indirect Haemagglutination test. Results revealed that inoculation of chickens orally by low dose of oocysts (5,000 and 10,000 sporulated oocysts / bird) reduced fecal oocysts count, while S/C and I/P injection lack to affect on fecal oocysts count, and slight increase of the total protein and A/G ratio compared with other investigated groups. The challenge infection showed significant decrease of the total protein, significant decrease of albumins and decrease of A/G ratio 0.47%. The IHAT revealed 1/512 ,1/512, 1/256, 1/128, 1/512 for the experimental groups .

Introduction:

Avian coccidiosis is a cosmopolitan disease that is universally found wherever chickens are raised, caused by the genus *Eimeria* of the phylum Apicomplexa, class protozoa (Long et al., 1979). Coccidiosis is a self limiting infectious disease of the digestive tract (small intestine and ceca), a considered as one of the major problems that cause high economic losses a decrease in egg production in the intensive chicken industry and in meat chickens, so continuous prophylactic medication system must be carried parallel with hygienic measures. (yun et al. 2000 and vermeulen et al. 2004) Most of the anticoccidial drugs can not be given to birds in the egg production period yet most adult birds live in an infected environment, so that they must have develop immunity to coccidiosis before reaching the egg production stage (long et al., 1979).

The medication with the available anticoccidial drugs is effective on preventing serious outbreaks among birds reared for broiler market, however the life of most of these drugs is limited due to the emergence of resistant strains therefore a pressing need for an alternative method of control, so that the scientists are also now directing their attention towards the immunization of chickens against coccidiosis through vaccination (Crouch et al. 2003 and Wulet et al. 2004).

The present study is aimed to make trials for immunization against coccidiosis in broiler chickens as a control measure.

Materials and methods

Materials:

- chicks: One – hundred Hubbard chicks, one – day old, from a local commercial broiler hatchery were kept on the floor "using shaving wood as litter." under normal breeding temperature in six separate groups each of 25 chicks. To avoid the risk of exposure to environmental contamination, chickens were kept under strict hygienic conditions and controlled temperature according to Harrison & Harrison (1986), the experimental room and stands used for separation of the room were fumigated with Formalin 1L + 500 g potassium permanganate / 1m³ of the room volume. The chicks were fed on hand made rations (not less than 21% crude protein, not less than 2.8% crude fat and not more than 3.1% crude fiber). The rations were free from any anti-coccidial agents or any drug additives. The rations and water were provided ad-libitum.

- coccidia strain: Field strain of sporulated *Eimeria* oocysts.

Methods:

1- Experimental design: chicks are divided randomly into six groups at 1st day old according to the doses of a mixture of oocysts given to each chick and the method of inoculation.

At the age of 4 days first dose of infection given to the chicks groups orally by means of a syringe to whose tip a rubber tubing was fixed then it was introduced intra esophagus

Group(1) was infected with 5,000 sporulated oocysts/bird according to Group(2) was infected by 10,000 SP. oocysts / bird oral inoculation. Group(3) was infected with 20,000 sporulated oocysts / bird (C+ve group)

Where, Group (4) was injected with 0.2 mg sporozoite antigen / chick sub. Cut. Group (5) was injected with 0.2 mg sporozoite antigen / chick I / P. Group (6) left as non - infected group (C - ve group).

The previous treatments were repeated after one week, then after two weeks a challenged dose given to each group (20,000 sp. oocysts / bird oral inoculation)

2- Sampling and Examination of the Birds:-Fecal samples were collected from the freshly evacuated feces at least 2gm of samples taken in clean labeled plastic packages from 5th dpi(1) till 10th dpi(1), from 13th dpi(1) till 18th dpi(1) and from 20th dpi(1) till 22nd dpi(1) and examined for oocyst count by using

Mc Master technique according to Gordon and whitlock(1939) as the follow formula

(Number of oocyst out put/gm=No.of oocyst in two chamber/2×100).

Results

(oocysts count/gm) results in Graph (1, 2) showed that :

1-G1(5,000 sporulated oocysts /bird orally), after 1st dose oocysts count gradually increased until reach the maximum level at 7th dpi (2200) gradually regression in oocysts count till 9th dpi (1500), after given 2nd dose oocysts count was gradually increased until reach the maximum level at 7th dpi (3500) then gradually regression in oocysts count till 9th dpi (2000). After challenge infection The oocysts count increased gradually as Follow:- at 5th dpi (3500) then increased at 6th dpi reach (3600) then gradually regression in oocysts count till 9th dpi (2000)

2-G2(10,000 sporulated oocysts /bird orally), oocysts count was gradually increased after 1st dose from 5th dpi (3000) until reach the maximum level at 7th dpi (6500) then gradually regression in oocysts count till 9th dpi (3000). The oocysts count began to increase again after given 2nd dose from 5th dpi (4500) until reach the maximum level at 7th dpi (6800) then gradually regression in oocysts count till 9th dpi (3900). After challenge infection The oocysts count increased gradually as Follow:- at 5th dpi (5000) then increased at 6th dpi reach (6000) then gradually regression in oocysts count till 9th dpi (4000)

3- G3 (0.2 mg sp. Ag / bird S/C), after challenge infection The oocysts count increased gradually as Follow :- at 5th dpi (9000) until reach the maximum level at 6th dpi (10000) then gradually regression in oocysts count till 9th dpi (5000) .

4- G4 (0.2 mg sp. Ag / bird I/P), after challenge infection The oocysts count increased gradually as Follow :- at 5th dpi (7000) until reach the maximum level at 6th dpi (9500) then gradually regression in oocysts count till 9th dpi (5000) .

5-G5 (20,000 sporulated oocysts /bird orally) control +ve group , after 1st dose oocysts count was gradually increased until reach the maximum level at 7th dpi (30000) then gradually regression in oocysts count till 9th dpi (15000), after given 2nd dose oocysts count was gradually increased until reach the maximum level at 7th dpi (350000) then gradually regression in oocysts count till 9th dpi (15000). After challenge infection The oocysts count increased gradually as Follow:- at 5th dpi (36000) then gradually increase reach the maximum dose at 7th dpi (45000) then gradually regression in oocysts count till 9th dpi (27000) .

Total serum protein , albumins and globulins were evaluated in all experimental groups of chickens after each dose of infection . Results shown in table (1) showed that. At the 7th dpi (after challenge infection) revealed non significantly changes in all experimental groups when compared with the control +ve group, but G1 (5,000 sporulated oocysts / bird orally) showed slight increase of the total protein and A/G ratio in compared with other investigated groups. G5 (challenge infection): showed significant

decrease of the total protein (2.13 ± 0.14) gm / dl , significant decrease albumins (0.68 ± 0.08) gm / dl and decrease of A/G ratio 0.47% .

antibody titer in broiler chickens that infected with different doses of Eimer spp. 1, 2, and 3 weeks after infection with sporulated oocysts by using indirect Haemagglutination test. The results are showed in table (2):-, showed positive reactions with titers for the experimental groups after one week w 1/32, 1/64 , 1/32, 1/512, and 1/64 respectively. After 2nd dose of infection chickens of the experimental groups gave titer of 1/128, 1/256, 1/64, 1/ and 1/256 respectively.

After challenge infection the titer of antibody for the experimental groups were 1/512 , 1/512, 1/256, 1/128, 1/16 , 1/512 respectively.

Discussion:

Immunization of chickens by different dose and different routes with sporulated oocysts and or oocyst extraction, it gave different levels of immunity. Immunization of chicken with low doses of sporulated oocyst induce high protection against challenged infection . chicken immunized with oocyst extraction by intraperitoneal injection revealed low protection rate after challenge . Simovart et al., (1993) stated that birds can acquire a strong immunity without showing any evidence of clinical diseases after immunization with low number of living sporulated oocyst mixture.

In the present study total serum protein , albumins and globulins were evaluated in all experimental groups of chickens after each dose of infection revealed non significantly changes in all experimental groups when compared with the control +ve group. antibody titer in broiler chickens that infected with different doses of Eimeria spp. 1, 2, and 3 weeks after infection with sporulated oocysts by using indirect Haemagglutination test. After challenge infection the titer of antibody for the experimental groups were elevated till the control negative group

This results agreed with Hegazi (1988) said that repeated small doses of oocysts given orally and through subcutaneous inoculation, the results by both routes, vaccinating orally or subcutaneously, when challenged survival rates were 100% and Prominent lesions were not observed among the challenged after 6 weeks, Hasbullash et al (1992) recorded that, in broilers inoculated with oocysts at age of 15 days the antibody titers increased rapidly after 19 day post inoculation and reached the maximum level on day 23 : 32 post infection. After challenge infection

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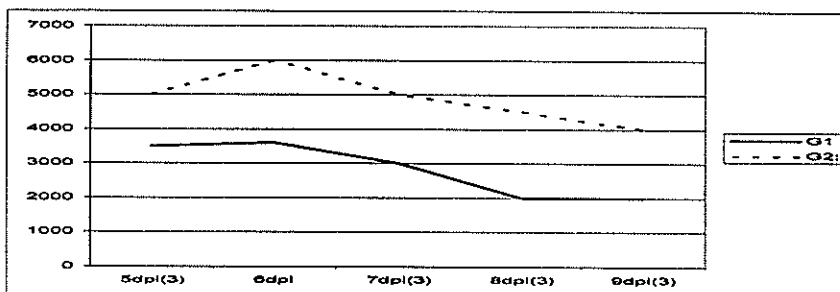
Table (1): Serum protein (Mean \pm S.E) in broilers after challenge infe

Groups	T. P. gm / dl	Albumin gm / dl	Glubulin gm / dl	A/G ratio
G(1)	2.97 \pm 0.17	1.09 \pm 0.03	1.88 \pm 0.02	0.58%
G(2)	2.67 \pm 0.12	0.85 \pm 0.02	1.82 \pm 0.1	0.47%
G(3)	2.43 \pm 0.01	0.90 \pm 0.03	1.53 \pm 0.01	0.53%
G(4)	2.33 \pm 0.02	0.78 \pm 0.05	1.55 \pm 0.01	0.50%
G(5)	2.85 \pm 0.13	0.98 \pm 0.08	1.87 \pm 0.05	0.52%

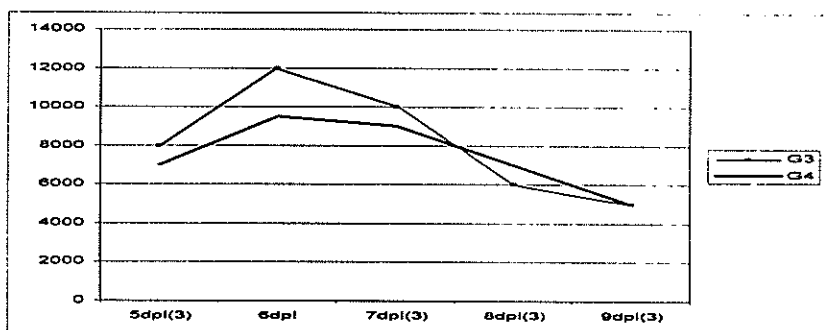
Table (2):- Diagnosis of coccidial infection by detection of antibody in broiler chickens.

Group	Titer of antibodies		
	After one week	After 2 weeks dose	After challen
G1	1/32	1/128	1/512
G2	1/64	1/256	1/512
G3	1/32	1/64	1/256
G4	1/32	1/64	1/128
G5	1/64	1/256	1/512

Graph (1) The oocysts count per gram of feces in broilers immunized *Eimeria* spp. by ingestion after challenge infection.



Graph (2) The oocysts count per gram of feces in broilers immunized *Eimeria* spp. by injection after challenge infection.



Graph (3) The oocysts count per gram of feces in non immunized broi with *Eimeria* spp. after challenge infection.

