

IMMUNO-MODULATORY EFFECT OF INACTIVATED *EIMERIA TENELLA* VACCINE AND LIVE IMPORTED COCCIDIAL VACCINE ON NEWCASTLE DISEASE VIRUS VACCINATED BROILER CHICKS

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ABSTRACT

A total of 160 one-day-old broiler chicks were used to evaluate the immunomodulatory effects of an inactivated *Eimeria tenella* vaccine and a live polyvalent imported anticoccidial vaccine (Coccivac). This study indicated that both of these vaccines did not adversely affect the development of serum antibody against Newcastle disease virus (NDV) and the chicks vaccinated with either of the anticoccidial vaccines resisted the virulent NDV challenge. A study of the lymphoid organs such as bursa of fabricius, thymus and spleen from the experimental chicks indicated that those organs were comparable with those from the chicks not vaccinated with these coccidial vaccines. The overall findings of this study indicate that anticoccidial vaccines do not have any effects on the immune functions of the vaccinates. In fact these vaccines prevented the occurrence of clinical coccidiosis in the vaccinates.

Key words: Immuno-Modulatory, *Eimeria tenella*, Newcastle Disease Virus

INTRODUCTION

Various *Eimeria* species infect and cause coccidiosis in the broiler chickens in Pakistan. Infection with these organisms may cause high mortality, morbidity and adverse effects on the growth of infected birds (Muneer and Sameera, 1998). Coccidiosis has been controlled by administration of anticoccidial medicines (coccidiocidals and coccidiostats) and through vaccination. Coccidial vaccines may contain one or more species of *Eimeria*. Currently vaccination against coccidiosis is carried using the imported vaccines, which cost a lot of foreign exchange to the country. Keeping in view the economical importance of coccidiosis for the broiler industry of Pakistan use of foreign exchange on the import of anticoccidial vaccines and to minimize the expenses on vaccination against coccidiosis a vaccine against *Eimeria tenella* (local isolate) was prepared and its efficacy was tested in the broilers. Since effects of many immunomodulating agents (drugs, chemicals, viruses, antibiotics etc.) have already been reported (Koller, 1982; Muneer *et al.*, 1988), the purpose of this investigation was to evaluate the immunomodulatory effects of a locally prepared *E. tenella* vaccine and imported vaccine (coccivac) containing 5 attenuated *Eimeria* species using the broiler chicks. Parameters of this study included (a) development of antibody titers against Newcastle Disease virus in the chicks inoculated with either of the coccidial vaccines (b) potential of the NDV and *Eimeria* resist virulent NDV challenge and (c) to

observe the morphometric changes are lymphoid organs following *Eimeria*

MATERIALS AND METHODS

A 160, one-day-old broiler obtained from M/S Hi-Tech poultry Breeding Company, Lahore, were identified by wing-banding and divided into 4 groups (A, B, C, and D) each consisting of 40 chicks. Chicks in group A were kept as unvaccinated controls. Those in groups B, C and D were vaccinated subcutaneously with an oil-based NDV vaccine on day 12 of their age. Chicks in group C were also vaccinated on days 3 and 10 with the 1000 *E. tenella* formalin treated oocysts (locally prepared vaccine) per chick and those in group D were vaccinated on days 3 and 10 with an attenuated polyvalent imported vaccine (Coccivac; Vetec Laboratones, Canada). All the chicks in groups B, C, and D were administered NDV LaSota strain vaccine through drinking water on day 28 (16 days post killed NDV vaccination).

Haemagglutination and Haemagglutination Inhibition Tests

The haemagglutination (HA) and haemagglutination-inhibition (HI) tests were conducted in the "U" bottom wells of microtitration plates (GIBCO Laboratories, Grand, Island, NY, USA) using the chicken embryo propagated LaSota strain of NDV according to the procedure described by Alexander and Chettle (1977). Eight HA units of the NDV were used

in the HI test. The HI titre was reciprocal to the highest dilution of the serum that inhibited haemagglutination. All the chicks of experimental groups of A, B, C and D were bled at days 1, 8, and 12 (prevaccination) and at weekly intervals thereafter (post NDV vaccination) and sera were separated for the determination of HI antibody titre against NDV.

Effects of Coccidial Vaccines on the Lymphoid Organs

Five chicks from each treatment group were killed on days 5 and 12 (2 days after vaccination) and their thymus, bursa of Fabricius, and spleen were aseptically removed, cleared of tissue debris and weighed in order to observe whether there were any differences in their weight or those organs had any abnormal lesions.

Fifty per cent of the chicks from each experimental group were challenged with lethal dose of virulent Newcastle disease virus having 4.36×10^3 /ml LD₅₀. Observations were recorded daily upto day 56 of age, when all the surviving chicks were sacrificed, autopsied. Postmortem lesions were recorded, and blood from each experimental bird was collected for sero-analysis against NDV.

RESULTS

Pre-vaccination geometric mean (GM) HI antibody titers in chicks of groups A, B, C, and D on day one were 36, 38, 35 and 36, respectively and on day 8 those titers in groups A, B, C, and D dropped to 24.5, 22.3, 21.8 and 21.6, respectively. On day 12, the GM HI antibody NDV titers in chicks of groups A, B, C, and D were 15, 18, 14.99, and 16, respectively. The HI antibody titre in the sera on day 21 were 11.49, 15.99, 34.88 and 42.98 for groups A, B, C, and d, respectively (Table 1).

This study indicated that birds in all the treatment groups had almost similar GM HI titer against NDV at the day 1. The GM NDV HI titer recorded at day 1 were 36, 38, 35 and 36 in chickens of groups A, B, C, and D, respectively. The maternal antibody titres in unvaccinated chickens waned progressively through day 56. These maternal levels on days 1, 8, 12, 21, 28, 35, 42, 49 and 56 were 36, 24.5, 15, 11.49, 13.5, 10.37, 11.8, 11.31 and 7.66, respectively. It was also observed that the HI titers against NDV titer indicated a downward trend at day 56, in birds of groups B and C. It was also observed that the HI titers against NDV in the oil-based NDV inoculated chickens started rising on day 28 (16 days post vaccination). The GM titers in chicks of group B, on days 18, 12, 21, 28, 35, 42, 49, and 56 were 38, 22.3, 18, 15.9, 21.88, 32.62, 41.48, 112.86, and 96.12, respectively. The GM HI NDV titers

in the locally prepared *E. tenella* vaccinated birds on days 21, 28, 35, 42, 49 and 56 were 18.88, 29.16, 40.34, 82.98, 128.72, and 112.22, respectively. On days 21, 28, 35, 42 and 49 the GM titers of chicks in group C were higher than those in group D (Table 1).

Table 1: GMT HI Antibody Titre of Experimental Chicks in Groups A to D on Various time intervals.

Time in Days	Observations	Group			
		A	B	C	D
1	16	36	38	35	36
8	16	24.5	22.3	21.8	21.6
12	16	11.5	18	14.99	16
21	16	11.49	15.99	18.88	15.98
28	16	13.5	21.88	29.16	23.94
35	16	10.37	32.62	40.34	36.62
42	16	11.8	41.48	82.98	72.54
49	16	11.31	112.86	128.72	108.78
56	16	7.66	96.12	112.22	116.02

A= Non vaccinated control.

B= NDV oil based vaccination at day 12.

C= Vaccinated with oil based NDV vaccine at day 12 and locally prepared *E. Tenella* vaccine at days 3 and 10.

D= Vaccinated with oil based NDV vaccine at day 12 and coccivac vaccine at days 3 and 10.

Postchallenge Mortality

At day 50 of their age, fifty per cent of the chickens from each treatment group (A, B, C, and D) were challenged with virulent ND virus (local isolate). It was observed that the NDV isolate used as challenge virus in the present study was highly virulent as it caused over 83 per cent mortality in the unvaccinated control chickens (Table 2). However, the NDV vaccinated chickens in treatment group B, C, and D resisted the effects of virulent ND virus. The protection percentages in chicks of group B (NDV-vaccinated), group C (NDV- and locally prepared *E. tenella* vaccine inoculated) and group D (NDV-vaccine and coccivac inoculated) were 83.33, 83.33 and 75, respectively, indicated that (a) the NDV vaccine used in the present experiment was very effective and lead to sero-conversion in the inoculated birds; and (b) the vaccination of experimental chicks with the locally prepared *E. tenella* vaccine or with the imported coccivac vaccine did not interfere with the development of immunity against ND virus, as the birds vaccinated with those anticoccidial vaccines resisted the virulent NDV challenge. However, the protection percentage after vaccination with the locally prepared *E. tenella* vaccine was higher (83.33 percent) than the immunity conferred by the imported coccivac vaccine (75%).

Postchallenge Clinical and Postmortem Findings

The feed and water intake of NDV- vaccinated virulent virus challenged chicks in various treatment groups was normal upto 10 day postchallenge. The initial sings of Newcastle disease in the unvaccinated

Table 2: Post-Challenge Mortality in Various Treatment Groups.

Group	Number ^a challenged	Number dead	Number survived	Survival percentage
A = Unvaccinated control	12	10	02	16.66
B = NDV-oil based vaccinated	12	02	10	83.33
C = NDV-oil based and Locally prepared <i>E. Tenella</i> vaccine	12	02	10	83.33
D = NDV-oil based and imported coccivac vaccine	12	03	09	75.00

A challenge with local NDV isolate.

NDV-challenged chickens were noted on day 3 postchallenge (PC). The major clinical signs in the NDV unvaccinated challenged chicks were anorexia, dullness, depression, ruffled feathers, and reluctance to move. The infected birds gasped for air, some birds having salivary and ocular discharges. Most of the infected chicks voided greenish watery fecal droppings, and were dehydrated, with cyanosis of the comb and wattles. Involvement of the CNS (involuntary movements of head and neck; paralysis of wings and legs) were quite evident. A few birds exhibited torticollis at the terminal stage of illness. The sick birds became comatose at the final stage of illness and most of them died within 18-48 hours after the onset of clinical signs. The most frequent lesions observed in the dead birds were muscular and pulmonary congestion, haemorrhages in the proventriculus, intestine and caecal tonsils. Congestion of lungs and mucoid exudates in trachea were noted.

Effects of Coccidial Vaccines on Various Immune Organs

The mean thymus weights of chicks in treatment groups A, B, C, and D sacrificed on day 5 (2 days post-coccidial vaccination) were 45, 47, 46, and 45 grams, respectively. The difference in the mean body weights of chicks in various treatment groups were non significant (Table 3).

The mean thymus weights of chicks in various groups on day 5 were 0.0495, 0.0498, 0.0493 and 0.496 grams for the groups A, B, C, and D, respectively (Table 3). The thymus-body weight ratios for groups A, B, C, and D were 1.1785, 1.7986, 1.1755, and 1.1762 respectively. There were non significant differences in thymus weights of chickens in various treatment groups.

The bursal weights of chicks in treatment groups A, B, C, and D on day 5 were 0.02714, 0.02737, and 0.02623, 0.2635 gms, respectively (Table 3). The bursal body weight ratios of chicks in group A, B, C, and D at day 5 were 0.6461, 0.6345, 0.6516 and 0.6373, respectively. The differences in bursal weights in chicks of various treatment groups at day 5 were not significant.

Table 3: Morphometric Analysis of Lymphoid Organs on Days 5 and 12 Post Anticoccidial Vaccination.

Parameter	A	B	C	D
Mean body weight (gm)				
1	45	47	46	45
2	107	109	106	109
Mean thymus weight (gm)				
1	0.0495	0.0498	0.0493	0.0496
2	0.193	0.201	0.199	0.197
Thymus-body ratio				
1	1.1785	1.7986	1.1755	1.1762
2	1.803	1.844	1.877	1.807
Bursal weight (gm)				
1	0.02714	0.02623	0.02737	0.02635
2	0.099	0.099	0.098	0.092
Bursal body ratio				
1	0.6461	0.6345	0.6516	0.6373
2	0.925	0.802	0.924	0.844
Mean splenic weight (gm)				
1	0.0225	0.0232	0.0227	0.0233
2	0.147	0.151	0.149	0.147
Splenic body wt. ratio				
1	0.500	0.493	0.493	0.517
2	1.373	1.385	1.405	1.348

Group A: Unvaccinated control chicks.

Group B: NDV-oil based vaccinated chicks.

Group C: NDV and *E. Tenella* vaccinated chicks.

Group D: NDV and coccivac inoculated chicks.

1= on day 5 2= on day 12

The mean splenic weight of chicks in treatment A, B, C, and D at day 5 (2 days post coccidial vaccination), were 0.0225, 0.0232, 0.0227, and 0.0233 gms, respectively (Table 3). These differences were also non significant.

The lymphoid organs were also weight on day 12 (2 days post boosting of chicks with the coccidial vaccine). The average weight of chicks in treatment groups A, B, C, and D at day 12, were 107, 109, 106, and 109 gms, respectively.

The mean thymus weights of chickens in treatment groups A, B, C, and D on day 12 were 0.193, 0.201, 0.199, and 0.197 gms, respectively (Table 3). The differences in thymus weights of various treatment groups were non significant.

The mean bursal weights of chickens in treatment groups A, B, C, and D at day 12 were 0.925, 0.802, 0.924, and 0.844, respectively (Non-significant difference).

The mean splenic weights of chickens in groups A, B, C, and D, at day 12 were 0.147, 0.151, 0.149, and 0.147 grams respectively (Table 3). The splenic body weight ratio in chicks of groups A, B, C, and D were 1.373, 1.385, 1.405, 1.348, respectively, and the differences in mean splenic weight of various treatment groups were non-significant.

The overall analysis of various lymphoid organs indicated that there were nonsignificant differences in weight of various organs in chicks belonging to various treatment groups. No abnormal lesion of any kind on any lymphoid organ was observed in the postcoccidial vaccination period upto 56 days indicating that the anticoccidial vaccines used had no adverse effects on the immune organs (bursa of fabricius, thymus, or spleen). Vaccination of chicks against coccidiosis did not interfere in the humoral HI antibody development, and the coccidial-and-NDV vaccinated chickens resisted the virulent NDV challenge.

DISCUSSION

Coccidiosis of chickens is an infectious, readily transmissible and economically important disease causing very high morbidity and mortality (Muneer and Sameera, 1998).

In a separate experiment it had been established that both the locally developed *E. tenella* vaccine and an imported coccivac vaccine by Dohms and Saif (1984), who reported that morphometric changes in the central or peripheral lymphoid organs, concentration or ratio of different classes of serum immunoglobulins, serum complement levels, functional activity of immune response and demonstration of the suspected agents interference with the results of vaccination and or increase in the course of disease produced by other infectious organisms be used to evaluated immunosuppression. Kwalli and Cho (1983) also used the same criteria for evaluating the immunomodulatory effects of levamisole in broiler chicken.

The GM HI antibody titers of chicks in various treatment groups were recorded (Table 1) as per Allan and Gough (1974). This study indicates that the sera of chicks in groups A, B, C, and D, collected on day 21 (9-days post administration of an oil-based NDV vaccine) exhibited GM HI titers of 11.49, 15.99, 18.88 and 15.98 in chicks of groups A, B, C, and D respectively. The chicks in group C had the highest GM titre on day 21. However, both the vaccines used against coccidiosis were able to elevate the GMT levels of chicks in group C and D as 18.88 and 15.98, respectively.

Sero-analysis on day 35, indicated the GM HI titres at 10.37, 32.62, 40.34 and 36.62 for the chicks in

groups A, B, C, and D, respectively. These findings clearly indicate that on day 35 the maternal titres of chicks in NDV unvaccinated group A had progressively waned to a low level as compared to chicks in other vaccinated groups (groups B, C, and D). However, chicks of group-C, which had been administered both the an oil based NDV and *E. tenella* vaccination had better NDV GMT HI titres as compared to NDV unvaccinated and other NDV vaccinated groups (groups A, B, and D). On day 49 it was quite clear that the chicks in group C continued to enjoy the best NDV GMT HI titer (titer=129) as compared to the chicks in groups A, B or D (Titers=11.31, 112.86, 108.78, respectively).

On day 56, subsequent to the challenge of virulent ND virus, administered through intramuscular and intranasal routes at day 50 of age, the seroprevalence of GMT HI antibody in chicks of groups A, B, C and D were 7.66, 96.12, 112.22, 11602 respectively indicated that the chicks in groups B, C, and D were quite immunocompetent to retain the seroconversion caused by NDV vaccination. Although the GMT HI antibody titers of chicks in group D were slightly higher than those of group C, the differences in those titers against NDV was non significant.

The overall evaluation of the NDV antibody titers of chicks in the post NDV and coccidial vaccination period indicates that the vaccinates remained immunocompetent throughout the experimental period and no untoward effects of coccidial vaccination on the immune response of the chicks were notable. Observations on post NDV challenge mortality (Table 2) also suggest that the vaccinated chicks (both the NDV and coccidial vaccine) were immunocompetent to resist the virulent NDV challenge upto 56th day of their age. Similarly, the study of organs like bursa, thymus and spleen of chicks in post coccidial vaccination period suggested that coccidial vaccines (local or imported) did not cause any lesions on these organs nor the weight of these organs significantly differed from that of the unvaccinated chicks. All those findings suggest that both the coccidial vaccines do not have any adverse effects on the vaccinates. In fact those vaccines were useful in maintaining the health of the birds against the ill effects of coccidiosis. Hence their use as a protective agent coccidiosis is advocated.

Morbidity and Mortality Record

None of the chicks in any in experimental group became sick or died upto day 49 of age, as they were not given any type of infection. On day 50, all the chicks were administered 10,000 LD₅₀ of virulent strain of ND virus per chick intraocularly and intramuscularly. Twelve hours following challenge

dose of NDV infection three birds belonging to group A (non-vaccinated control) indicated anorexia, dullness, depression, ruffled feathers and reluctance to move. The morbid chicks were seen gasping for air and some were exhibiting sticky, gelatinous discharge both from the mouth and nostrils. They suffered from high fever (Temperature 107-110 °F), dehydration and passed whitish-green-watery faeces during the acute illness. In more advanced cases, the affected chicks indicated CNS involvement. Sings observed in those chicks were involuntary movements, paralysis of wings and leg weakness. Torticollis was noted in 3 birds prior to their death. The affected birds became comatosed at the final stage of their illness and six birds of group A died within 24 hours post-challenge infection. By the day 53, from the remaining birds four died after showing the typical sings of the ND. A total 10 challenge birds in group A died within 72 hours post NDV challenge. Similar clinical picture was shown by some of the affected birds in the other groups. Ten birds of group B, 10 of C and 9 of group D were still alive on day 56, and those birds did not indicate any disease signs.

The postmortem examination of the carcasses indicated pinpoint haemorrhages of varying intensity in the proventriculus, muscular and pulmonary congestions, and haemorrhages in the intestines. Similar sings and lesions of ND infection have been reported by Cheville *et al.* (1972), Beard and Brugh (1975), Rehman (1976), Ahmad *et al.* (1981) and Chaudhry (1991).

Further investigations on the immunomodulatory effect of local coccidiosis vaccine on birds that are vaccinated with different viral vaccines are suggested.

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