DEVELOPMENT OF IMMUNITY TO COCCIDIOSIS IN CHICKEN ADMINISTRATED SONICATED COCCIDIAL VACCINE

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ABSTRACT

Humoral immune response of Vaccine-I (supermatant from sonicated sporulated oocyst), Vaccine-II (sediment from sonicated sporulated oocyst) and Vaccine-III(un-sonicated sporulated oocyst) against coccidiosis in chickens was determined by indirect haemagglutination (IHA) test. IHA antibody titre was significantly higher($P<0.05$) in chicks vaccinated with Vaccine-I as compare to Vaccine-II and Vaccine-III. The IHA antibody titre of chicks vaccinated with Vaccine-I ranged from 1:8 to 1:8192, 1:16 1:512 for Vaccine II and 1:2 to 1:64 for Vaccine III. Vaccine I gave 100 per cent protection and oocysts appeared in the faeces (100-200 per gram) on day 10 post challenge which gradually increased (600-900 per gram) on day 16 and 200-400 on day 20 post challenge. Vaccine II gave 60 per cent protection and oocyst appeared in the faeces (3,50,000-4,50,000 per gram) on day 8 post challenge which gradually increased (700,000-900,000 per gram) on day 16 and 100, 000-200,000 on day 20 post challenge. Vaccine III gave 30 per cent protection and oocyst appeared in the faeces (8,50,000-9,00,000 per gram) on day 7 post challenge which gradually increased (11,400,000-12,00,000 per gram) on day 16 and 8,40,000-9,00,000 on day 20 post challenge. In control group, characteristic bloody diarrhoea was observed in all the chicks and oocysts appeared in the faeces (10,00,000-12,50,000 per gram) on day 5 post challenge of faeces which gradually increased (15,00,000-17,50,000 per gram) on day 16 post challenge. Results of the humoral and challenge response indicated that the Vaccine I induced a strong protection as immune chicks contained high level of antibodies that resisted heavy dose of challenge and gave 100 per cent protection.

Key words: Immunity, Coccidiosis, Sonicated Vaccine

INTRODUCTION

Immunity to coccidia is of a considerable academic interest because of the complicated life cycle of the organism and its obligate intracellular habitat, principally in the intestine of the host (Rose, 1976). Development of chemo-resistant strains had lead the investigators to search for the development of vaccine. Several attempt had been made in different parts of the world to immunize the chicks against coccidiosis by using attenuated vaccine (McDonald *et al.*, 1982), irradiated attenuated *Eimeria (E.) maxima* sporozoites vaccine (Jenkins *et al.*,1993), avian gut associated vaccine (Lillehoj, 1993), killed vaccine (Long, 1994), "Paracox" attenuated anticoccidial vaccine (William, 1994), live attenuated vaccine (Shirley *et al.*,1995), *E.maxima* gametocyte antigens vaccine (Wallach *et al.*, 1995), live coccidial vaccine (Bushell *et al.*,1995), tissue culture vaccine (Brake *et al.*, 1997) and sonicated coccidial oocyst (Akhtar *et al.*, 1998). To date no single vaccine is available which gave promising results.

Different characteristics are involved in inducing the protective immunity in chicks against coccidia including cell mediated immunity, antibody mediated response (Suigusaar and Parre, 1976; Davis *et al.*, 1985), T-cell activation and lymphocyte blastogenesis (Rose, 1985).

Recently, Immunocox (Vetch Labs., Canada) has been launched in the field to control the disease. This is an imported live vaccine that does not provide complete protection and disease occur in spite of vaccination as reported by Shaker (1997). The present paper reports the preparation of inactivated sonicated vaccine(s) from local strains of coccidia and their evaluation on the basis of humoral and challenge responses in chicken.

MATERIALS AND METHODS

Collection and Sporulation of oocyst:

Oocyst (mixed species of coccidia) recovered from the naturally infected chickens with coccidia were sporulated (Akhtar *et al.*,1998). The oocyst per mL count was done by McMaster counting technique (Hayat and Akhtar, 2000).

Preparation of sonicated antigen and vaccines:

Sporulated oocysts were given 3-4 washings with phosphate buffered saline (PBS:pH 7.2) and a

concentration of 4,000 per mL was maintained with PBS. These were stirred continuously on a magnetic stirrer for twelve hours and then subjected to ultra-sonication (Akhtar *et al.*, 1998). Supernatant and sediment as antigen(s) were collected for vaccine(s) preparation.

Following vaccines were prepared from the unsonicated sporulated oocyst, supernatant and sediment by inactivating with formalin (Ayaz, 1999).

Vaccine-I contain supernatant from sonicated sporulated oocyst

Vaccine-II contain sediment from sonicated sporulated oocyst

Vaccine-III contain un-sonicated sporulated oocyst

All the vaccines were kept in refrigerator for future use.

Experimental design

One hundred day old broiler chicks were purchased from local market of Faisalabad city. Chicks were reared under the standard managemental conditions in the Department of Veterinary Parasitology, University of Agriculture, Faisalabad. The birds were fed commerical ration (National Feed, Pvt). Fresh and clean water was given through out the experiment. Chicks on day 6 were divided into four groups viz. I, II, III and IV, having 25 chicks in each group. The detail for each group is as under.

Group-I Given Vaccine I @ 0.25ml per bird, orally

Group-II Given Vaccine II @ 0.25ml per bird, orally,

Group-III Given Vaccine III @ 0.25ml per bird, orally.

Group-IV Given PBS @ 0.25ml per bird, orally.

On day 15 post vaccination, 20 birds from each group were slaughtered to collect the blood. Serum was separated, labeled and stored at 4°C in the refrigerator for further use.

Challenge experiment

All the remaining chicks in group I, II, III and IV (5 chicks in each group) were given oral doze of 60,000 sporulated oocyst of mixed species of coccidia on day 15 post-vaccination. Their faecal sample were collected daily up to day 35 post-vaccination. Number of oocyst per gm of dropping were calculated from each group by using McMaster counting technique.

Chicks of the experimental and control groups were examined daily to record their general body appearance, droppings appearance, abnormal sign and symptoms if any and feed and water intake etc.Mortality occuring in all the experimental groups during experimental period and autopsical findings were recorded. The intestines and caeca of birds died during the experiments and those of slaughtered at the end were examined to asses severity of the disease.

Immunological response

Indirect haemagglutinatin (IHA) test was applied (Ayaz, 1999) to assess the humoral immune response of immune chicks vaccinated against coccidiosis.

RESULTS AND DISCUSSION

IHA antibody titre (geomean titre) was significantly higher ($P<0.05$) in chicks vaccinated with Vaccine-I as compare to Vaccine-II and Vaccine-III.

The IHA antibody titre of chicks vaccinated with Vaccine-I ranged from 1:8 to 1:8192 with geomean titre 955.4. Among the 20 samples processed, 1:8 antibody titre for two samples, 1:512 for five samples, 1:1024 for six samples 1:4096 for four samples and 1:8192 for three samples.

The antibody titre of chicks vaccinated with Vaccine-II ranged from 1:16 to 1:512 with geomean titre 48.5. Among the 20 samples processed, 1:16 antibody titre for three samples, 1:32 for eight samples, 1:64 for six samples, 1:256 for two samples and 1:512 for one sample.

The antibody titre of chicks vaccinated with Vaccine-III ranged from 1:2 to 1:64. Among the 20 samples processed, 1:2 antibody titre for five samples, 1:4 for six samples, 1:16 for four samples, 1:32 for two samples and 1:64 for three samples.

Results of the challenge experiments revealed that the Vaccine-I gave maximum protection (100%) followed by Vaccine II (60%) and Vaccine III (30 per cent) to the challenge.

Birds in group I were found absolutely normal. They were alert, active and healthy. They were jumping in normal ways. Their water and feed intake was normal. Their faeces were normal. No mortality was recorded in this group after challenge. Oocyst appeared in the faeces on day 10 post challenge (25 days age) showing 100-200 oocyst per gram of faeces which gradually increased to 600-900 EPG on day 16(31 days age) post challenge. Oocyst number per gram of faeces decreased to 200-400 on day 20(35 days age) post challenge.

Birds in group II were partially normal. Some of the birds were lazy, huddling together in the corner, tired, exhausted, dropping of wings. They consume less feed and water as compared to the control group. Eight birds from this group died after challenge. Oocyst appeared in the faeces on day 8 post challenge (23 days age) showing 3,50,000-4,50,000 oocyst per gram of

faeces which gradually increased to 700,000-900,000 EPG on day 16 (31 days age) post challnege. Oocyst number per gram of faeces decreased to 100,000-200,000 on day 20 (35 days age) post challenge.

Birds in group III were not normal. Majority of the birds were diseased and showing tiredness, laziness, unthriftiness and were exhausted. They were dull, depress, huddle together in corners with the bloody diarrhoea. Oocyst appeared in the faeces on day 7 post challenge (23 days age) showing 8,50,000-9,00,000 oocyst per gram of faeces which gradually increased to 11,400,000-12,00,000 EPG on day 16(31 days age) post challenge. Oocyst number per gram of faeces decreased to 840,000-900,000 on day 20 (35 days age) post challenge. It gave only 30% protection.

On day 5 post challenge, change in the behavior of the birds of group IV was observed. The birds were found uninterested in feeding but were drinking water normally. They were dull, depressed and were looking tired. They huddle together in the corners with dropping of wings and looking very much exhausted. Characteristic bloody diarrhoea was observed in all the birds of this group. All the birds died due to coccidiosis at different intervals. Oocyst appeared in the faeces on day 5 post challenge (20 days age) showing 10,00,000-12,50,000 oocyst per gram of faeces which gradually increased to 15,00,000-17,50,000 EPG on day 16(31 days age) post challenge. Oocyst number per gram of faeces decreased to 10,00,000-13,75,000 on day 20(35 days age) post challenge.

Results of the humoral and challenge responses indicated that the Vaccine-I induced a strong protection as immune chicks contained high level of antibodies that resisted heavy dose of challenge and gave 100 per cent protection. Similar findings were also observed by Lillehoj and Trout (1996) and Lillehoj *et al.*(1986) who reported that antibodies mediated responses play role in protectin against coccidiosis and immune chicks can resist the infection to coccidia. Although humoral and cell mediated immune responses both are essential for complete protection against coccidiosis (Jenkins *et al.*,1991).

Further studies are underway on the *in vitro* propagation of oocyst on large scale for the production of vaccine on commercial scale.

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