



Short Communication

Immune response of chicken to an experimental sonicated coccidia oocyst vaccine

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ABSTRACT

A total of 120 one-day old broiler chicks (Ross breed) were used to evaluate the humoral immune response of vaccine-I (supernatant from sonicated sporulated oocyst), vaccine-II (sediment from sonicated sporulated oocysts) and vaccine-III (un-sonicated sporulated oocyst) against coccidiosis in chicks 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 1:7 to 1:7012, 1:16 to 1:410 for vaccine II and 1:2 to 1:64 for vaccine III. Vaccine I gave 100 percent protection and oocysts appeared in the faeces (100-200 oocysts per grams) on day 11 post challenge which gradually number of oocysts increased, 650 to 900 oocysts per grams on day 17 and 200-400 on day 21 post challenge. Vaccine-II gave 62 percent protection and oocyst appeared in the faeces (300,000-400,000 oocysts) on day9 post challenge which gradually increased (800,000-900,000 oocysts) on day 17 and 100,000-200,000 on day 21 post challenge. Vaccine III gave 33 percent protection and oocyst appeared in the faeces (800,000-900,000) on day 8 post challenge which gradually increased (100,000-1,250,000 per grams) on day 17 and 840,000 - 900,000 on day 21 post challenge. In control group, characteristic bloody diarrhoea was observed in all the chicks and oocysts appeared in the faeces (1,000,000-1,250,000 per grams) on day 6 post challenge of faeces which gradually increased (1,400,000-1,750,000 per grams) on day 17 post challenge. The overall findings of this study indicate that the humoral and challenge response of the Vaccine I indicated a strong protection as immune chicks contained high level of antibodies that resisted heavy dose of challenge and gave 100 percent protection.

Keywords: Immunity, Coccidiosis, Sonicated vaccine

INTRODUCTION

Over the years, there has been tremendous research interest in the immune response to

coccidiosis in poultry. A number of significant reviews of this subject are available (Rose *et al* 1985). 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

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(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 *Eimeria (E) maxima* sporozoites vaccine (Jenkins *et al* 1993), avian gut associated vaccine (Lillehoj 1993), killed vaccine (long 1984), "Paracox" attenuated anticoccidial vaccine (William 1994), live attenuated vaccine (Shireley *et al* 1995), 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). Today, there is not available a single vaccine which gave promising results. *E. maxima* Different characteristics are involved inducing the protective immunity in chicks coccidia including cell mediated immunity, antibody mediated response (Suigusaar and Parre 1976, Davis *et al* 1985), T-cell activation and lymphocyte blastogenesis (Rose *et al* 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 coccidia of oocysts. Oocysts (mixed species of coccidia) recovered from the naturally infected chicks with coccidia were sporulated (Akhtar *et al* 1998). The oocysts counts were done by McMaster counting technique (Hayat & Akhtar 2000).

Preparation of sonicated antigen and vaccines. Sporulated oocysts which are *E. maxima*, *E. tenella*, *E. necatrix* and *E. accervulina* as vaccines for use in

pullets should contain three species at minimum, were given 3-4 washings with phosphate buffered saline (PBS, pH 7.2) and a concentration of 4,000 sporulated oocysts per ml and maintained in PBS. These were stirred continuously on a magnetic stirrer for twelve hours and then subjected to ultrasonication (Akhtar *et al* 1998). Supernatant and sediment as antigen(s) were collected for vaccine(s) preparation. The oocyst counts were done by McMaster counting technique (Hayat & Akhtar 2000).

Sporulated oocyst, anticoccidial vaccine which provided a recombinant peptide with a novel epitopes. The recombinant peptide has an amino-terminal amino acid sequence that is unique among known *Eimeria* antigens. (European publication No. 0349071 of AKZO N.V. 1991). Discloses polypeptide of *Eimeria* which can be used to immunize poultry. Molecular clones were isolated from both *E. acervulina* and *E. tenella*.

With a view to producing a coccidiosis vaccine (European patent publication 1991), prepared extract from sporozoites or sporulated oocysts of *Eimeria tenella*, which contain at least 15 polypeptides, many of which were associated with the surface of the sporozoites injection of these extract into chickens reduced cecal lesion following oral inoculation with virulent *E. tenella* sporulated oocysts. The coccidial sporozoites or sporulated oocysts by killing it with chemical, gets deactivate they cannot reproduce at all, yet the presence of the dead in the body still generates response by B-cells, producing antibodies and memory records.

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

Vaccine I contains supernatant from sonicated sporulated oocysts. Vaccine II contains sediment from sonicated sporulated oocyst. Vaccine III contains un-sonicated sporulated oocysts. All the Vaccines were kept in refrigerator between 4-16.85 °C. Freezing will kill the parasites and render the vaccines ineffective (Mike optiz 2005).

Experimental design. 120 one-day old broiler chicks (Ross breed) were purchased from market, chicks were reared on alitter of wood shavings. To help avoid contamination with extraneous coccidia or bacteria in the clinic of veterinary science departement Ilam university, the bird accomadation was fumigated consecutively with formaldehyde and ammonia gas, feed has been prepared under standard formula for broiler and treated by 25 mg/kg with amprolium to develop the resistant against coccidiosis and all food were sterlized by irradiation, table 1 shows the formulation of diet. Fresh and clean water was given through out the experiment as it is source of energy to small chicks , the water was tested and shown to be *Costredia*-free, and the wood-shaving litter was sterlizedby autoclaving. A specimen bag of feed was checked for bacterial contamination. (William *et al* 2003) Chicks on day 7 were divided into four groups viz. I, II, III and IV, having 30 chicks in each group. On day 16 post vaccination, 25 birds from each group were slaughtered to collect the blood. Sera were collected, labeled and stored at 4 °C in the refrigerator for further use.

Groups I, II, III and IV of chicks were adminstered orally 0. 25 ml of per bird, Vaccine I, II, III and PBS respectively.

Challenge experiment. All the remaining chicks in groups I, II, III and IV (5 chicks in each group) were given oral doze of 60,000 sporulated oocysts of mixed three species of coccidia which are of *E. tenela*, *E. necatrix*, *E. accervulina* and *E. maxima* on day 15 post-vaccination, their faecal samples were collected daily up to day 35 post-vaccination. Number of oocysts 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, dropping appearance, abnormal sign and symptoms if any, feed and water intake. Mortality occuring in all the experimental groups

during experimental period and autopsical findings were recorded. The intestines and caeca of birds died during the experiment and those of slaughtered at the end were examined to assess severity of the disease.

Table1. Formulation of diets

Components	w/w %
Fishmeal 65% protein	3. 00
Rapeseed solvent extract	10. 00
Soft wheat	56. 62
Soy oil	4. 44
DL_methionine	0. 14
Lime stone	1. 04
Dicalcium phosphate	0. 94
Soybean meal 48%protein	27. 02
Sodium chloride	0. 30
Vitamins/minerals premix	0. 50

Immunological response. Indirect heamagglutinin (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 1:7 to 1:7012 with geomean titre of 966. 4. Among the 25 processed samples, the results were obtained as follows: a ratio of 1:7 antibody titre for three samples, 1:410 for six samples, 1:1924 for seven samples, 1:4096 for five samples and 1:7012 for four samples.

The antibody titres of chicks vaccinated with vaccine-II ranged from 1:16 to 1:512 with geomean titre 46.5. Among the 25 samples processed, 1:16 antibody titre for four samples, 1:32 for nine

samples, 1:64 for seven samples, 1:146 for three samples and 1:512 for two sample. The antibody titre of chicks vaccinated with Vaccine III ranged from 1:2 to 1:64. Among the 25 samples processed, 1:2 antibody titre for six samples, 1:4 for seven samples, 1:16 for five samples, 1:32 for three samples and 1:64 for four samples.

Results of the challenge experiment revealed that the Vaccine I gave maximum oritECTION (100%) followed by Vaccine II (62%) and Vaccine III (33 %) to the challenge.

Vaccinated birds showed either no significant difference in live weight compared to medicated birds or some cases, finished significantly heavier. this trial was performed in Italy. Also, the trials were performed in France, Spain and UK in which comparable results were produced It was reported that vaccination to be as good or better than in-feed anticoccidiosis in terms of efficacy and bird performance (Long 1984).

Birds in group I were found absolutely normal. They were alert, active and healthy. They were jumping ion normal ways. Their water and feed intake was recorded in this group after challenge. Oocysts appeared in the faeces on day 10 post challenge (25 days age) showing 100-200 oocysts per grams of faeces which gradually increased to 650-900 oocysts on day 16 (31 days age) post challenge to 200-400 oocysts 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 consumed less feed and water as compared to the control group, two birds from this group died after challenge. Oocysts appeared in the faeces on day 8 post challenge (23 days age) showing 300,000 oocyst per gram of faeces which gradually increased to 800,000-900,000 oocysts on day 16 (31 days age) post challenge. Number of oocysts 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 tiredness, laziness, unthriftiness and were exhausted. They were dull, depress, huddle together in corners with the bloody diarrhea and excreted a lot amount of mucous. Oocysts appeared an the faeces on day 7 post challenge (23 days age) showing 800,000-900,000 oocysts per gram of faeces which gradually increased to 10,400,000 - 1,200,000 oocysts on day 16(31 days age) post challenge. Number of oocysts 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 behaviour 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 diarrhea was observed in all the birds of this group. All the the birds died to coccidiosis at different intervals, Oocyst appeared in the faeces on day 5 post challenge (20 days age) showing 1000,000-1250,000 oocysts per gram of faeces which gradually increased to 1,400,000 – 1,750,000 oocysts on day 16 (31 days age) post challenge. Number of oocysts per gram of faeces decreased to 1,000,000-1,375,000 on day 20 (35 days age) post challenge.

The most easily recognized clinical sign of severe cecal coccidiosis is the present of bloody droppings. mortality is highest in the fourth and six days *E. tenella* is one of the most pathogenic (disease producing) coccidiosis in ceccum of infected chicken while *Enecatrix* develops in small intestine in early stage and later in ceccum (sexual stage) this both speceis develop in deep tissues of small intestine and is are major pathogen of poultry. *E. accervulina* and *E. maxima* both develop in epithial cell primarily in the upper part of the small intestine (Kennedy & Hanson 2003).

Results of this study shows that the humoral and challenge responses indicated that Vaccine-I induced

a strong protection as immune chicks contained high level of antibodies that resisted heavy dose of challenge and gave 100 percent protection. Similar finding were also observed by akhtar *et al* 2001, Lillehoj & Trout (1996) and Lillehoj *et al* (1986) who reported that antibodies mediated responses play role in protection against coccidiosis and immune chicks can resist the inflection to coccidian. Although humoral and cell mediated immune reponses both are essential for complete protection against coccidiosis (Jenkins *et al* 1991). A successful vaccination will provide long -lasting immunity and no need for anti-coccidial feed medication.

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