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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Immune Response of Sonicated Coccidial Oocyst in Chickens

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Abstract

Immune response of sonicated coccidial oocyst was observed in chickens. Indirect haemagglutination (INA) test was developed for detecting antibodies to coccidia and serum antibody levels were measured against soluble oocyst (sporulated) antigen. IHA antibody titre was significantly higher ($p < 0.05$), in chicks vaccinated with inactivated sonicated vaccines as compared to the chicks vaccinated with inactivated sporulated vaccine. The IHA antibody titre vaccinated with inactivated sonicated vaccine ranged from 1 : 64 to 1 : 1:512, among the 15 samples processed, 1:64 in two samples, 1:128 in four samples, 1: 256 in seven samples and 1: 512 in two samples. It gave 100 percent protection to the challenge chicks. Their faeces were normal and no clinical sign was recorded even 40 days post vaccination.

Key words: Immune response, sonicated oocyst, coccidia, chicks

Introduction

Coccidiosis is one of the major menace for poultry industry through out the world, In countries like Pakistan where the farming is substandard, the disease become more serious and causes heavy economic losses. Although the exact losses due to coccidiosis in Pakistan are not known due to the lack of statistical indices but these will be definite in billion of rupees.

The conventional methods to control the disease is by using certain coccidiostat as a prophylactic measure or coccidioidal for therapeutic purpose (Otto, 1979) in combination with certain growth promoters. But due to the development of resistance against commonly available coccidiostat/coccidioidal has out dated the conventional methods. The research on coccidiosis has been focused on the immunological control. It has been known that birds which recover from coccidiosis are resistant to further attack (Tyzzer *et al.*, 1932), accordingly there has always been the prospect of producing a vaccine. In this context, a live coccidiosis vaccine, containing small number of oocyst, was developed and marketed in USA but could not achieved the desired results and failed (Fitz-Coy and Edgar, 1992). Time to time different attempts have been made on the development of vaccine(s) against coccidiosis. There has been a recent interest in the use of hybridoma techniques to produce monoclonal antibodies and speculation about their application in the immunological control of coccidiosis (Davis *et al.*, 1979), The inoculation of antibodies per se does not seem to be viable procedure. The value of the monoclonal antibodies rests with their potential to identify the antigens responsible for the protective response. If this could be done by appropriate genetic engineering techniques, then large quantities of a suitable antigen could be produced. Having produced vast amounts of 'dead' antigen, the problem remains of how to deliver it to young chickens. Parental inoculation of dead antigens, although capable of stimulating circulating antibodies to the antigens, may not protect the local

mucosal site in the intestine. A method of delivering such antigens orally may be required but the secretory antibodies (IgA) are not produced in the intestine of chickens in response to dead antigens (Davis *et al.*, 1979). Recently, "Immunocox" has been launched in the field to control the disease. This is an imported live vaccines; may cause the disease due to vaccine failure in certain cases, and thus the results are not promising (Shaker, 1997). The present paper reports the immune response of sonicated coccidial oocysts.

Materials and Methods

Collection and examination of materials: Chicken guts suspected to be naturally infected with coccidia were collected from different poultry shops of Faisalabad city. Guts were opened and the contents thus collected were examined by direct microscopy (Soulsby, 1982). The positive contents were subjected to concentration of oocysts using salt floatation technique (Ryley *et al.*, 1976).

Extraction and sporulation of oocyst: Oocyst (mixed species of coccidia) recovered from the saturated salt solution were squirted into an excess volume of distilled water at least five volumes to bring the specific gravity below 1.03. The oocysts were recovered by centrifugation at 1500 rpm for 2 min and were transferred into 2.5 percent potassium dichromate solution in petridishes for sporulation at 30-32°C with 80 percent humidity (Hayat *et al.*, 1996). Sporulated oocysts were stored at 4°C until use. The oocyst per mL count was done by McMaster counting technique (Gordon and Whitlock, 1939).

Preparation of sonicated antigen: Sporulated oocyst collected in potassium dichromate solution were given 3-4 washing with physiological saline solution (pH 7.2) and a concentration of 4,000 mL⁻¹ was maintained. Washed sporulated oocysts were subjected to ultra-sonication (Ultra Turrax, Janke & Kunkel GmbH & Co, Germany) for 2x30 sec in a jacketed vessel with cool water.

Preparation of vaccine: Inactivated vaccine was prepared from the sonicated suspension by treating with 0.3 percent Formalin (33% formaldehyde) for 96 h at 37°C (Fu and Lee, 1976) and stored at 4°C until use.

Experimental design: Forty five day old broiler chicks were procured from the local market. Chicks were reared under the standard managemental conditions. Chicks at the days 5 were divided into three groups viz A, B and C having 15 birds in each group. Group A and B were given inactivated sporulated and inactivated sonicated vaccines orally (0.25 mL per bird), respectively. Birds in group C were kept as control and were given physiological saline (0.25 mL per bird).

Blood samples were collected from each group at 15 days post vaccination and serum was separated and stored at 4°C for further use.

Five birds in each group were challenged with 50,000 sporulated oocyst (mixed species) at days 15, 22 and 29 post vaccination. Faecal examination were conducted daily and number of oocysts per gram of droppings were determined upto 40 days. Clinical symptoms were also observed daily in each group.

Immunological response: Indirect haemagglutination (IHA) test was performed to assess the antibody titres. For IHA, sheep erythrocytes were sensitized with glutaraldehyde and sonicated antigen followed the method of Tokuda and Warrington (1970) with modifications. The sensitized erythrocytes were finally resuspended in phosphate buffered saline (PBS) to make 1.5 percent suspension.

A two fold serial dilution of the serum samples was made with PBS. Equal volume (0.05 mL) of sensitized erythrocytes (1.5%) was added in each well of the microtitration plates. The plates were tapped to ensure even mixing of erythrocytes and kept at 37°C for 90 min. The degree of haemagglutination in each well was recorded in comparison with the control.

Results and Discussion

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). Various attempts have been made to immunize the chicks against coccidiosis by using live (Shirley *et al.*, 1995), attenuated (McDonald *et al.*, 1982), killed (Long, 1984) and irradiated (Augustine and Danforth, 1990) vaccines with variable success. Present studies report the immunizing effect of inactivated sporulated oocyst and inactivated sonicated vaccines against avian coccidiosis. Indirect haemagglutination (IHA) test was developed for detecting antibodies to coccidia. Serum antibody levels in chickens were measured against soluble oocyst (sporulated) antigen. IHA antibody titre was significantly higher ($p < 0.05$), in chicks vaccinated with inactivated sonicated vaccines as compared to the chicks vaccinated with inactivated sporulated vaccine. The IHA antibody titre vaccinated with inactivated sonicated vaccine ranged from 1: 64 to 1: 1:512, among the 15 samples processed, 1:64 in two samples, 1: 1:28 in four samples, 1: 256 in seven samples and 1: 512 in two samples. The IHA antibody titre in chicks vaccinated with inactivated

sporulated oocyst vaccine ranged from 1: 4 to 1: 128, among 15 samples processed, 1:4 in three samples. 1:8 in six samples, 1: 16 in four samples and 1: 128 in two samples.

Results of the challenge experiments revealed that the inactivated sonicated vaccine gave 100 percent protection to the challenge chicks. Their faeces were normal and no clinical sign was recorded even 40 days post vaccination. The oocyst appeared in their faeces on day 7 post challenge showing 600 oocyst per gram of faeces, which gradually increased to 1100 on day 13 post challenge (PC) and again decline gradually to 900 by the end of the experiment.

The chicks vaccinated with inactivated sporulated (unsonicated) vaccine also gave 100 percent protection to the challenge chicks. But the oocyst count was comparatively higher than the sonicated vaccine. Tere oocyst appeared in their faeces on day 7 of post challenge showing 1000 oocyst per gram of faeces, which gradually increased to 2000 on day 13 PC and then decline gradually to 1400 by the end of the experiment.

In the unvaccinated group the oocyst count per gram was 21300, 18000 at day 5 and 13 PC, respectively and 14100 by the end of the experiment. The oocyst count per gram of faeces was significantly high ($p < 0.05$) in control group as compared with the vaccinated groups. As the vaccine was given orally, the antibodies produced are principally of IgA, secretory antibodies (Rose, 1976) which protect the immune chicks from challenge infection. The means by which the host immune response actually kill, inhibit development, or prevent the establishment of the parasite is not clearly known. The parasite is at its most vulnerable stage when extracellular and the host immune responses are most effective against the invasive stages as they move from one cell to another, although sometimes not manifest until after penetration. It is also difficult to investigate that how serum antibodies, in particular, makes contact with the parasite. However, the immunized gut become hypersensitive to challenge inoculi of oocyst. It increased the permeability of the gut lumen that result in a leakage of serum protein into the tissue and the gut lumen. These serum proteins contain the secretory immunoglobulin (IgA) in gut lumen that may limit the coccidia replication and thus the immune chicken may prevent from infection (Lebacqz-Verheyden *et al.*, 1972). Further studies are under way to follow the effect of sonication on the oocyst and sporozoites.

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