EFFICACY OF EGG YOLK IMMUNOGLOBULINS (IGY) AGAINST ENTERIC PATHOGENS IN POULTRY*

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ABSTRACT

Day old chicks, under treatment groups were administered orally the egg yolk with specific antibodies ((IgY) against E.coli, Salmonella pullorum, Clostridium perfringens *and* Campylobacter jejuni *organisms whereas the control groups were left as such. When challenged with respective pathogens, treatment groups did not show pathognomonic symptoms, and specific lesions, whereas the control groups exhibited typical symptoms and specific lesions. The specific antibacterial egg yolk immunoglobulins could be used orally for offering protection against enteric bacterial pathogens.*

Key words: *Egg yolk antibodies (IgY), Prophylaxis, Enteric Pathogens and Efficacy,*

INTRODUCTION

Prevention of bacterial infections especially those due to *Salmonella*, *Campylobacter* and *E.coli* in meat animals and poultry has gained importance in the last few years due to highly published food borne illness in human beings. Due to concerns about use of antibiotics in animal production and the increase in the evolvement of enteric antibiotic resistant pathogens, an alternative to antibiotics need to be developed.

Chicken egg yolk was recognized as an inexpensive alternate antibody source (IgY) as its immunotherapeutic application has proved successful for treatment of a variety of gastro-intestinal infections. Though the systemic administration of specific antibodies would be degraded in the stomach and intestine, a fraction of immunoglobulins retain their neutralization activity, atleast locally in various segments of the gastro-intestinal tract. This concept led to oral dosage forms of antibodies that would have the advantages of reduced costs, simplicity of administration with a potential for treating localized conditions in the gastro-intestinal tract. They do not cause any hypersensitivity reaction.

The paper deals with the production and purification of egg yolk immunoglobulins against enteric pathogens in poultry, *In vivo* assessment of bacteriostatic potency of egg yolk immunoglobulins

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thereby assessing the effectiveness and safety of egg yolk immunoglobulins in the prevention of enteric infections.

MATERIALS AND METHODS

Isolation of *E. coli, S. pullorum, C. jejuni* and *C. perfringens*

Escherichia coli isolate (O:2 strain) was obtained from Nagpur Veterinary College, Maharastra. *Salmonella pullorum* and C*lostridium perfringens* isolates were obtained from Department of Veterinary Microbiology, Veterinary College and Research Institute, Namakkal. *Campylobacter jejuni* was isolated from local poultry farms in and around Namakkal and it was confirmed by PCR technique with DNA amplification at 402 bp as per method described by King *et al.* (1997). The above Isolates were revived and subcultured for production of specific immunoglobulins and challenging immunized chicks.

Production and purification of IgY

Sixteen layer chickens of 20 weeks age and 80 day-old chicks were purchased from a commercial poultry farm and hatchery in Namakkal. One milliliter of Salmonella antigen was homogenized with 1 ml (1:1) of Freund's complete adjuvant and 1 ml of this emulsion was given intramuscularly to chickens. Two booster doses of 0.5ml, one with Freund's incomplete adjuvant, one without adjuvant was given on 14th day and 21st day respectively by the same route. Antibodies were detected by slide agglutination method on 14th day after second booster and the eggs were collected daily and stored at 4°C until further use. Similar method was employed to produce the IgY against *E.coli*, *Clostridium* perfringens and *Campylobacter jejuni*. The above immuniglobulins were purified by using IgY purification kit (Genei, Bangalore) and compared with standard chicken IgY (Genei, Bangalore) by SDS-PAGE analysis. The purity of the IgY was checked by slide agglutination method using corresponding antigens.

Immunization and Challenging of chicks

Eighty, day old chicks were divided into 8 groups (Group 1-8) of10 birds each. First four groups were immunized with 3 ml of IgY against *E. coli, S. pullorum, C. jejuni* and *C. perfringens* and remaining four groups were kept as controls. After three days of immunization, Group 1 and 5, Group 2 and 6, Group 3 and 7 and Group 4 and 8 were challenged orally with 10^9 cfu of *E.coli* (O:2 strain) *Salmonella pullorum*, Clostridium *perfringens* and *Campylobacter jejuni* organisms respectively for consecutive three days. Group three and seven were orally administered with oocysts of *Eimeria necatrix* and *Eimeria tenella* along with *Clostridium perfringens* to induce necrotic enteritis.

Detailed necropsy was conducted on dead birds in the control groups as well as slaughtered birds in the treatment groups and the gross lesions were recorded. Histopathological examinations using haematoxylin and eosin (H&E) stain were conducted in the representative pieces of tissues from liver, intestine and heart collected during necropsy. Bacteriological examinations from dead birds were carried out to identify the above pathogens.

RESULTS AND DISCUSSION

Antibacterial IgY against *E. coli*, *Salmonella pullorum*, C*lostridium perfringens* and *Campylobacter jejuni* were tested and identified by slide agglutination method. Terzolo *et al*. (1998) also utilized slide agglutination method for testing and

identifying the IgY. Immunoglobulin Y purification kit yielded 60-70mg of pure immunoglobulin per egg and ammonium sulphate precipitation method could yield approximately the same quantity of IgY as reported by Garcia *et al.* (2005). Similar type of bands was observed for all egg yolk immunoglobulins by SDS-PAGE analysis and is suggestive of purification as observed by Imberechts *et al.* (1998).

In the *E.coli* challenge study, treatment group survived the challenge, showed better body weight gain and did not manifest diarrhoea. Similar observations were made by Yokoyama *et al.* (1992). Control group birds exhibited reduced appetite, loss of body condition and body weight. Four of them had diarrhoea. Necropsy lesions in the internal organs and histopathology were suggestive of *E. coli* infection, which are in accordance with the findings of Gross (1966).

In the Salmonella challenge study, treatment group did not show morbidity and mortality, as observed by Fulton *et al*. (2002). General weakness, depressed appetite, poor growth and adherence of chalky white material to the vent were observed in chicks of control groups. Mortality was also observed six days after challenging. Gross lesions and histopathology in control group were suggestive of Salmonella infection. Similar findings were reported by Wray (1996) and Shivaprasad (2003).

In the *C. perfringens* challenge study, treatment group did not show morbidity and mortality, which is in agreement with the findings of John and Williams (1998). In control group, general depression and decreased appetite were observed and the chicks were reluctant to move. Mortality was observed ten days after experimental infection, which concurs with the findings of Wages and Opengart (2003). Coagulative necrosis of epithelial cells of intestinal villi was observed. Similar reports were made by Olkowski *et al*. (2005). *Clostridium perfringens* could be isolated on EYA from the necropsy specimens and identified by biochemical tests, as done by Wages and Opengart (2003).

In the *C. jejuni* challenge study, treatment group was normal with no morbidity and mortality, as observed by Tsubokura *et al*. (1997). The control group showed diarrhoea, degeneration and necrosis of villi epithelium, which are in accordance with the findings of Welkos (1984). Accumulation of watery fluid and distention of the intestinal loop were also observed in dead chicks which was similar to the findings of Sanyal *et al*. (1984). C*ampylobacter jejuni* was isolated from the clinically affected chicks from cloacal swabs, as described by Stern *et al.1988.* The antibodies that escaped digestion in the stomach are still functional in the small intestine (Ikemori *et al*., 1997).

The passive immunotherapy with specific egg yolk antibodies resulted in significant protection in chickens against challenge with *E.coli (*O:2 strain), *Salmonella pullorum, Clostridium perfringens* and *C. jejuni* infections and the survival rate against these enteric infections (*E. coli, S.pullorum, C. perfringens and C.jejuni*) was 100 per cent as chicks treated with specific antibody did not show clinical symptoms, mortality and postmortem lesions. Hence, antibacterial IgY could be used prophylactically against enteric infections in poultry.

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