EX-VIVO ANALYSIS OF B LYMPHOCYTE IN BURSA OF FABRICIOUS DURING PASSIVE IMMUNIZATION (A.GALLI) WITH THE SENSITIZED BURSAL CELLS IN WLH CHICKS

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ABSTRACT

Immunization has a great impact on the economics of poultry production than all other therapeutic and prophylactic treatment combined and that even with the significant advancement in modern medicine, vaccination may become a feasible control alternative (Emery and Wagland 1993). Vaccine holds the key for indication of any disease through immunization. The present study comprises immunization by conventional vaccines through transfer of sensitized antibody forming cells of bursa against GI nematode-*A galli*. B-lymphocytes (mature in bursa of fabricious) are precursor of plasma cells which synthesize and release immunoglobulins. The B-lymphocytes have easily detectable immunoglobulins on cell membrane. These cells act as specific antigen recognizing system and react accordingly against antigens of *A. galli*. Weight of the bursa is effective method to find out the status about the proliferation of B cell in BOF of the chick. After passive immunization it has been noticed that the weight of the bursa does not show any significant change while at the time of sensitization, the size of the bursa effectively increased. Immunopathological studies were also performed for getting the confirmed information according to experimental design. So passive immunization can be a effective tool or the preventive measure for suppression of the *A.galli* infection in chicks.

Key words: Ascaridia galli, Passive immunization, Bursa of fabricious, B lymphocyte,

Introduction

The development of vaccine against parasite has a long way to go despite a massive research efforts all over the world It is the time to move away from the tradition approach to the massive holistic view of the immune response in such a way that it favors protection and inhibits any pathophysiological side effects and the later functional may be more important than the former. Immunization may be active mediated by antigens or passive mediated by antibodies or transfer of antibody producing cells like bursa, bone marrow, thymus and spleen.(Gilmour et al.1970,Jankovic et al.1972).Passive immunization has great medical and veterinary importance. The present studies comprise immunization by conventional vaccines through

transfer of sensitized antibody forming cells of bursa against *A galli.* The vital role of bursa in the humoral immunity was discovered by Glick and Chang (1956).The parasitic infection tends to be long and chronic and is associated with inappropriate immune response and immunopathological damage. The development of antibodies aginst ascaridiasis is important in the protection of animals; however, chicks do not rapidly develop higher antibody litres, vaccination might provide a method to enhance immunological protection against infection in chicks. Understanding the immunization through lymphoid cells involved in an immune response will help to modulate the immune response, which may help in designing more effective vaccine.

MATERIAL METHOD AND EXPERIMENTAL DESIGN

-Experimental host - Male white leghorn chick

-Experimental parasite - *Ascaridia galli*

-Dose of infection - 2000 eggs/chick

The experimental host were obtained from local hatchery according to necessity and maintained in the well maintained animal house under the hygienic condition. *Ascaridia galli* were collected from intestine of fowl. Male and female worms were kept in petridish separately. Eggs were collected by squeezing the parasite in the petridish containing saline. These dishes were kept at 37°C for 2-3 weeks and 0.1% formaline had been added to each petridish to avoid any fungal infection. The embryonated eggs were counted by dilution method.

Experimental design-

During present investigation,chicks were divided into 4 group-control(C) having 8 chicks,infected group with the embryonated eggs of A.galli(C_1) having 8 chicks,immunized with sensitized bursal cells and challengd with 2000 embryonated eggs(RC_1),immunized with nonsensitized bursal cells and challenged with 2000 embryonated eggs (RC_2). Both group having 6 chicks.15 days old chicks were infected with 2000 embryonated eggs of *A. galli* .chicks from each group were sacrified at 15^{th} ,30^{th} ,45^{th} day of the infection .

Infection to experimental host-

15 days old chicks were infected with decided dose of embryonated eggs of *A.galli* orally(C_1).

Collection and calculation of bursal cells-

After the 15^{th} day of the infection(C_1),Donar chicks were sacrificed on 15^{th} days of infection to obtain sensitized bursal cells. On the same day noninfected donar chicks(C) were also sacrified to form nonsensitized bursal cell suspension. Bursa were carefully removed from both type of chicks and kept separately in Ringer's solution (0. 9 gm NaCI. 0.042 gm KCI. 0025 gm $CaCl_2$ in 100 ml of distilled water) maintained at 4°C and suspension of sensitized and nonsensitized bursal cells had been prepared separately**.** Cell number and viability **was** assessed in a haemocytometer and a phase contrast microscope after eosin staining.

Number of viable cells/ml = average number of viable cells in large square x 10^4 x 2

After counting the cells, from that suspension the total number of $5xl0^5$ cells for each recipient was adjusted with in 0.2 ml of suspension.

Transfer of immunity through sensitized and nonsensitized bursal cells

The sensitized and nonsensitized bursal cells were injected subcutaneously with $5xl0^5$ into respective day old synenic recipient groups .

Counting of B lymphocyte

The blood of different groups of chicks was collected from the heart by the cardiac puncture with the sterilized dry glass syringe for separation of B lymphocyte.B cell were separated by ficoll hypaque and identified according to size and density(Deys et. al 1996).For counting of B lymphocyte 10 ml aliquots was kept on the counting slide. A cover slip was kept over the counting slide very carefully. The counting slide was kept under light microscope and B cells were counted. Counting of B cell were repeated three times and mean was taken.

Y = Mean of number of B cells and Tcells in 10µl

Y×100 = X

X = Number of B cell per ml blood

$$\text{Percentage of B cells} = \frac{\text{Number of B cell} \times 100}{\text{Total number of T cells and B cells}}$$

Tissue Processing for Morphohistological Study

The chicks were sacrified to take out the bursa of Fabricius according to the experimental design. In Fig. 1 Bursa of Fabricius (arrow) in its normal position. Bursa of Fabricius were carefully removed and their diameters were measured using a bursameter and their average and relative weights are recorded. The Bursa obtained from chickens were fixed in Bouin's fluid for 24 hours and were dehydrated in the series of ascending grade of alcohol followed by clearing in two changes in xylene, and the tissues then infiltrated with different grades of melted paraffin in the oven. The tissues were then embedded in paraffin and finally the sections were cut at 5µ thickness using sliding microtome (R. .Darboux et.al 1994).After cutting, the sections were floated on Luke-warm water in a 3 floatation bath at 38° C for stretching and then the sections were mounted on clean slides using egg albumin and dried on a slide warmer at 38° C(l.luna et. al 1963). The sections were stained using Mayer's Hematoxylin and Eosin (H & E). The histological structures of the bursa of Fabricius were observed using light microscope under low (×10) and high (×40) magnification.

Table-1 Percentage of B lymphocyte during passive immunization with sensitized bursal cells at 15th ,30th and 45th day of the infection

Days	CONTROL (C)	INFECTED GROUP (C1)	IMMUNIZED WITH SENSITIZED BURSAL CELLS(RC1)	IMMUNIZED WITH NONSENSITIZ BURSAL CELLS(RC2)
15th	30.5	34.4	37.4	35.4
30th	32.6	36.6	38	37.6
45th	33.4	38	40	38.2

Result and discussion

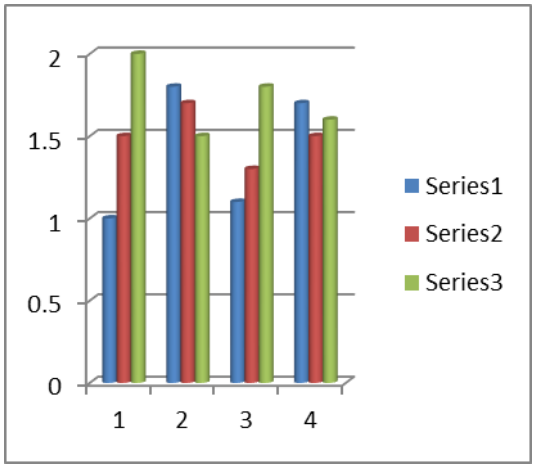
During present investigation it has been observed that the percentage of lymphocyte were increasing in all group in comparison to control group (table-1) but it has been noticed that the weight of bursa was increased in infected group at the 15th and 30th day of the infection while it decresed at 45th day of the infection while it remain constant in a group immunized with sensitized bursal cells. Immunized group with nonsensitized bursal cells have similar values like infected group. These finding showed that against infection bursal cells produce more lymphocyte at the site of infection so the weight and diameter (E. Ciriaco. et.al 2003) decreased with the more day of the infection. (histogram-1 &2).Histologically the capsular wall was found to be more or less thickened. The follicles were found to be atrophied(Fig 2). The irregular inflammatory and non-inflammatory edema and depletion of lymphocytes were observed in present study. Lymphoid follicle atrophy was probably the result of the migration of the' lymphocytes to the site of infection. It may be attributed to the leakage of endotoxins into the bursa, released during antigen-antibody interaction. It may be due to severity of infection. Immunopathological lesions in infected chicken are formed resulting into hypoplasia and atrophied bursa of fabricius(fig-3) (Bagust et al. 1979; Taniguchi et al., 1977). Immunopathologically the Bof revealed severe reterogressive changes of lymphocytes in most of the lymphoid follicle (fig-4) and many of the lymphocytes were replaced by reticuloendothelial cells, Interfollicular edema, fibroplasia associated with infilteration of lymphocytes, monocytes and plasma cells (Singh and Rao, 1987) Passively immune chicks do not induce protection against bursal atrophy (Lucio and Hitchner, 1979) whereas hypoplasia of bursa was observed by Bangust et al. (1984). The follicular hyperplasia was due to increase in the number and size of secondary follicles but Degenerative changes and depletion of lymphoid cells were observed in bursa of fabricius of vaccinated broiler chicks (Jeurissen et al. 1998; Stoev et al., 2000). The immunized chicks which received sensitized and non-sensitized bursal cells showed severe reterogressive changes in lymphocytes and size of follicles significantly smaller. There were marked interfollicular fibrosis. The sections of lymphoid organs reveal a disproportionate decrease in lymphocyte content.

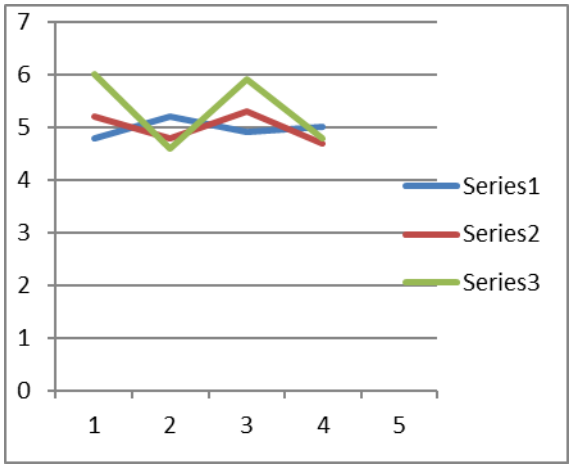
Recipient chicks which received non-sensitized bursal cells showed significant changes while chicks immunized with sensitized bursal acells appeared as normal structure with some exceptions.

Conclusion

To prevent host tissues from harmful effect of antibiotics which may be immunosuppressive also, it is better to inject prepared antibodies to treat infections. Immune response in such a way that it favors protection and

inhibits any pathophysiological side effects and the later functional may be more important than the former. Antibodies are documented to suppress the humoral and cellular immune response.





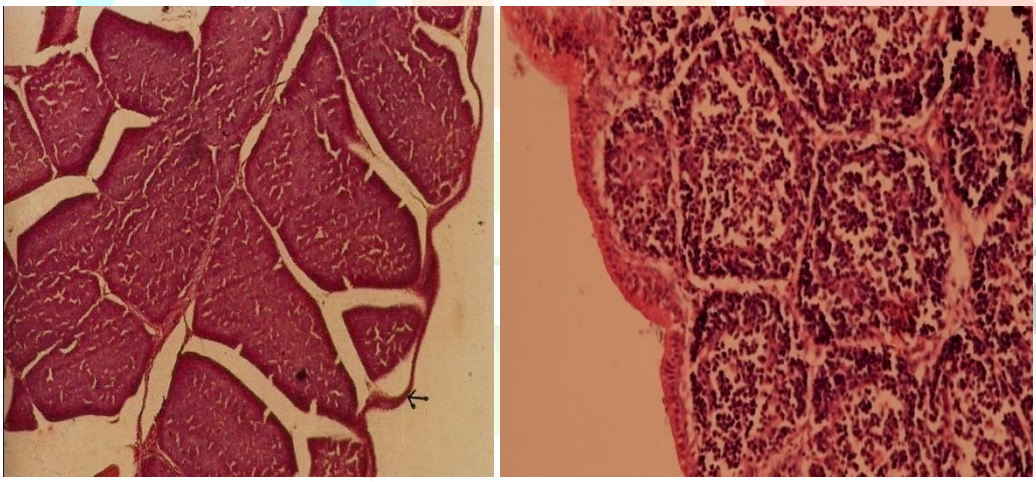
Histogram 1:Weight of bursa in different groups at 15th day, 30th day, 45th day of the infection

Histogram 2:Diameter of bursa in different groups at 15th day, 30th day, 45th day of the infection

Y axis showing weight in gram in histogram-1and diameter of bursa in mm in histogram-2

X showing 4 different group(1-C),(2-C_1),(3-RC_1),(4-RC_2)

Series showing 15th day, 30th day, 45th day



(a) (b)

Fig: 1 T.S passing through the bursa of control group, showing

(a) several plicae(→)(10x) (b) a plicae having polyhedral follicles(↑)(40x).

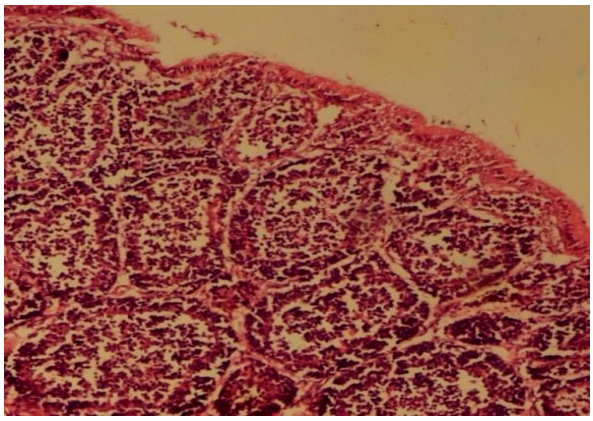


Fig: 2

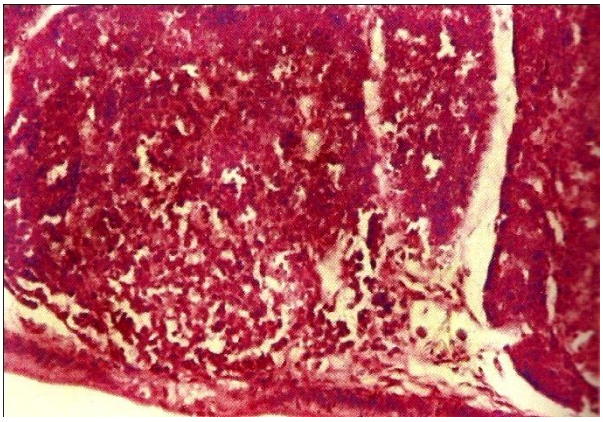


Fig:3

Fig:2 T.S passing through the bursa of infected with dose of eggs at 15th day, showing ruptured surface epithelium at several places(←), moderate enlargement of follicles size(→), and increased lymphocytes(↑) in the bursa of the sensitized chicks (40x)

Fig:3 T.S passing through the bursa of infected chicks at 45th day, revealing depletion of lymphocytes(↓) in the cortex region, interfollicular spaces filled with oedematous fluid(←)(40x).

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