IMMUNIZATION EXPERIMENTS TO STUDY MAST CELLS ON 15TH, 30TH AND 45TH DAYS OF POST INFECTION AND POST IMMUNIZATION AGAINST ASCARIDIASIS INDUCED BY SENSITIZED BURSAL CELLS

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ABSTRACT

Ascaridiasis is caused by Ascaridia galli, the largest internal parasitic nematode or roundworm causing helminthiasis in poultry. It infest the small intestine and can cause ill-thrift and intestinal compaction (enteritis). Economic losses and welfare issues that result from severe A. galli infections are an important problem in laying flocks, especially in hens kept in free-range and organic farming systems (Ruff, 1999; Martín-Pacho et al., 2005). Immunization has a great impact on the economics of poultry production than all other therapeutic treatment and that even with the significant advancement in modern medicine, vaccination may become a feasible control alternative (Emery, McClure and Wagland, 1993). Present work is to explore potentialities of both conventional and novel approach to the development of vaccines by bursal antigens and to take a more holistic view of the immune response with the object of manipulating the response in such a way that it favours protection in chicks from Ascaridiasis and inhibit any pathological side effects. Data from this study may assist in developing more efficient control measures for A. galli, for combating against economic losses in poultry industry. Studies have been taken with mast cells counting with respect to sensitised bursal cells with low (300 embryonated eggs of Ascaridia galli) and high dose (1000 embryonated eggs of Ascaridia galli) of infection.

Index Terms: Ascaridia Galli, Ascaridiasis, Sensitized Bursal cells

I. INTRODUCTION

During the present era, poultry farming has become an important small scale industry in India and other developing countries due to increased demand of egg and poultry meat. Indian Poultry Industry is booming and is emerging as the world's second largest market. The Poultry Industry is growing at a rapid growth rate of 12-15% per year on the back of the intelligent use of modern technology and all this while maintaining some of the world's lowest production costs.

Ascaridiasis is caused by *Ascaridia galli*, the largest internal parasitic nematode or roundworm causing helminthiasis in poultry. It infest the small intestine and can cause ill-thrift and intestinal compaction (enteritis). Droopiness, emaciation and diarrhoea are the common clinical symptoms. The most important clinical sign of *A. galli* infections is loss of body weight, which increases parallel to worm load (Reid and Carmon, 1958). Increased feed intake (Gauly et al., 2007), blood loss, reduced body weight, and increased mortality may also occur (Ikeme, 1971). Immunization has a great impact on economics of poultry production. So vaccination may

become feasible control alternative (Emery, McClure and Wagland, 1993). There is a paucity of information regarding development of immunity in chicks against *A. galli* infection. In view of this, experimental immunological studies were undertaken with the hope that some serodiagnostic technique may be helpful in the diagnosis of ascaridiasis.

The majority of chicks injected subcutaneously with extract of different lymphoid organ enriched in developed circulating antibodies and when challenged had a significant resistance to infection by *A. galli*.

Successful vaccination is hindered by lack of cold chain facilities, poor transport to the remote villages and physical control over the chickens and the vaccines. Records show that little effort has been done to control Newcastle disease using locally obtained strains. Lentogenic and mild virulent isolates have been used for production of live and inactivated vaccines in Europe and Asia (Palya, 1991, Mowat and Rweyemamu, 1997.

The present studies comprise immunization by conventional vaccines through transfer of sensitized antibody priming cells of bursa against *Ascaridia galli* a gastrointestinal nematode of chicks.

II. MATERIAL METHOD AND EXPERIMENTAL DESIGN

During present investigation Experimental host are white leghorn chicks. Experimental parasite is *Ascaridia galli*. Doses of infection are

Low dose - 300 embryonated eggs of *Ascaridia galli*.

High dose - 1000 embryonated eggs of *Ascaridia galli*.

Ascaridia galli were obtained from intestine of chicks. The eggs of *Ascaridia galli* were cultured and incubated for embryonation by Riedal method (1947). These pure embryonated eggs were used for experiments. Counting of eggs was done by dilution method.

Infections were given to the chicks with different doses of (300 and 1000) embryonated eggs of *A. galli*. Chicks were sacrificed at day 15^{th}, 30^{th} and 45^{th} of infection. Bursa was carefully removed and kept separately in Ringer's solution maintained at 4^0 C, separated from attached expaneous tissues. Bursa was washed three times in Ringer's solution and finally kept in diluted Ringer's solution.

The bursa was teased with forceps and gentle dispersion in a loose fitting glass homogenizer. The cells were separated and suspended into fresh ringer solution, then centrifuged at approximately 3000 rpm for 10 minutes to remove any adherent exogenous material. In addition, the dead cells were disrupted during centrifugation and so the percentage cell viability increases during the washing procedure. The supernatant was decanted off using a pasture pipette and fresh Ringer's solution added. This washing procedure was repeated three times. Cell number and viability was assessed in a haemocytometer and a phase contrast microscope after eosin staining.

Collected bursal cells were injected subcutaneously (SC) to recipient chicks for mast cells studies.

In present investigation, experiment was conducted in two phases.

PHASE I - Infected and Non-immunized Group

PHASE II - Infected and Immunized Group

Total numbers of groups were six. These are:-

GROUP 1- Control group (Infected and Non-immunized)

This group is further subdivided into three subgroups.

Ca- Control group at day 15 of infection (4 chicks)

Cb- Control group at day 30 of infection (4 chicks)

Cc- Control group at day 45 of infection (4 chicks)

GROUP 2- Infected with 300 embryonated eggs of *Ascaridia galli*

This group is further subdivided into three subgroups.

(C1)a- Autopsied after day 15 of infection (4 chicks)

(C1)b- Autopsied after day 30 of infection (4 chicks)

(C1)c- Autopsied after day 45 of infection (4 chicks)

GROUP 3- Infected with 1000 embryonated eggs of *Ascaridia galli*

This group is further subdivided into three subgroups

(C2)a- Autopsied after day 15 of infection (4 chicks)

(C2)b- Autopsied after day 30 of infection (4 chicks)

(C2)c- Autopsied after day 45 of infection (4 chicks)

GROUP 4- Control group (Immunized)

This group is further subdivided into three subgroups.

RCa- Control group at day 15 of immunization (4 chicks)

RCb- Control group at day 30 of immunization (4 chicks)

RCc- Control group at day 45 of immunization (4 chicks)

GROUP 5- Immunized with sensitised bursal cells and challenged with 300 embryonated eggs of *Ascaridia galli*

This group is again sub-divided into three groups according to the day of autopsying.

(RC1)a – Autopsied after 15 days (4 chicks)

(RC1)b– Autopsied after 30 days (4 chicks)

(RC1)c– Autopsied after 45 days (4 chicks)

GROUP 6- Immunized with sensitised bursal cells and challenged with 1000 embryonated eggs of *Ascaridia galli*

This group is again sub-divided into three sub-groups according to the day of autopsying

(RC2)a– Autopsied after 15 days (4 chicks)

(RC2)b– Autopsied after 30 days (4 chicks)

(RC2)c– Autopsied after 45 days (4 chicks)

Parameters of mast cells were studied in both infected and immunized groups.

III. RESULT AND DISCUSSION

In present studies, the WLH chicks immunized with bursal antigen revealed protection against ascaridiasis. It was observed that increase in the mast cell number was found to be dose dependent. Number of mast cells increased with increasing dose of infective eggs (Fig 1, 2 & 3) (Table 1). In immunized group, slight suppression of mast cell number was observed (Fig 4 & 5) (Table 2). The data obtained during mast cells studies were analysed by two way variance analysis (ANOVA) (Table 1a & 2a).

The counting of mast cells has been done in the following groups at 15th, 30th and 45th day of infection and immunization. The number of mast cells in intestinal section was counted in 20 microscopic fields in both groups.

Bursal antigen provides cellular immune response to the host against parasitic infection. Reason behind elevation in mast cell numbers could be that the biogenic substances released from mast cells would increase the permeability of the mucosa to antibodies and would have on effect on the expulsion of the parasites.

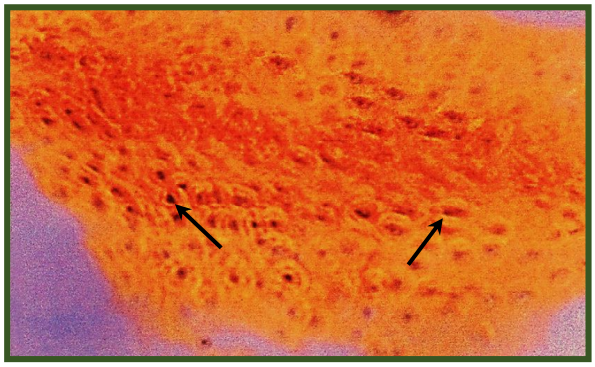

Figure 1: T.S. passing through the intestine of control chick at15th day of infection showing mast cells in infected group.

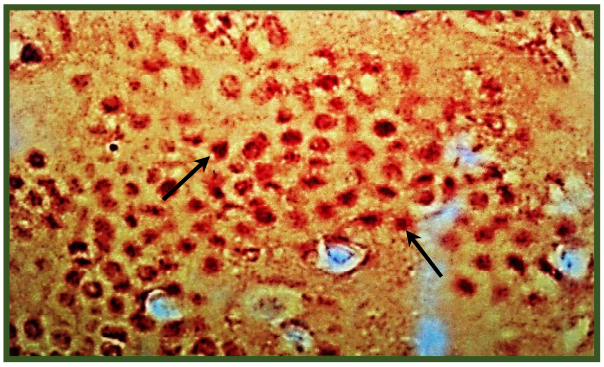

Figure 2: T.S. passing through the intestine of chick infected with low dose at 45th day of infection showing mast cells in infected group.

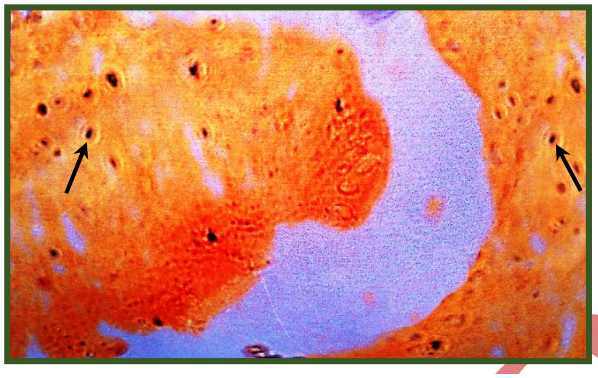

Figure 3: T.S. passing through the intestine of chick infected with high dose at 45th day of infection showing mast cells in infected group.

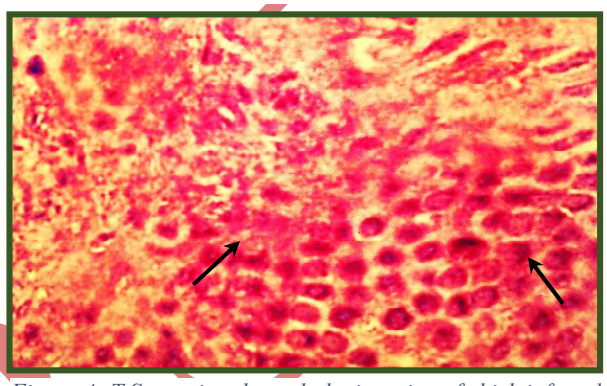

Figure 4: T.S. passing through the intestine of chick infected with low dose at 45thday of infection and immunization showing mast cells in immunized group.

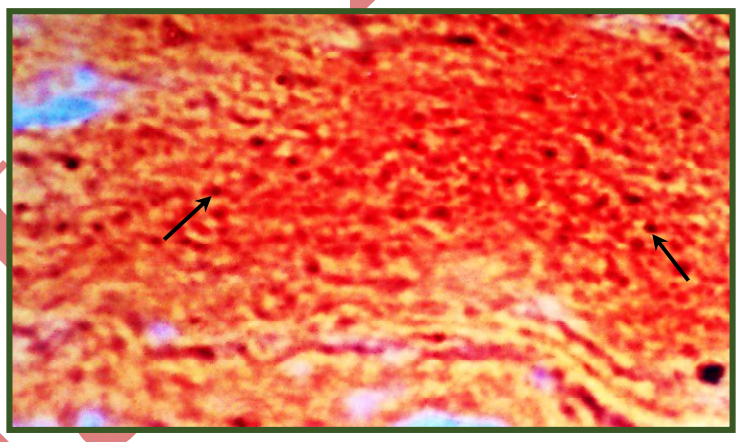

Figure 5: T.S. passing through the intestine of chick infected with high dose at 45th day of infection and immunization showing mast cells in immunized group.

The significant increase in the number of mast cells may be associated with an inflammatory reaction in the intestine during the development of partially protective immune responses to this parasitic nematode which contributes to the dynamic equilibrium between the strength of the intestinal response and the worm biomass.

Present investigations are in favour of findings of Monika and Lal (2011).They worked on mast cell responses in female somatic antigen non-sensitized and sensitized albino rats during experimental ascariasis. They observed the numbers of mast cells were increased during experimental ascariasis. Findings are also in agreement to Winter *et al*., (1997).They reported mast cell and eosinophil response of young lambs to primary infection with *Nematodirus battus.* They observed elevation of mast cell in infected lambs.

Table 1: Parameters of mast cells of White Leg Horn chicks infected with the low and high doses of embryonated eggs of Ascaridia galli in infected group.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	94	112.8	132.6
		S.D.	± 1.87083	± 1.30384	± 4.50555
		S.E.	± 0.83666	± 0.5831	± 2.01494
2	30	Mean	93.4	130.8	148
		S.D.	± 1.67332	± 2.04939	± 1.41421
		S.E.	± 0.74833	± 0.91652	± 0.63246
3	45	Mean	95.6	133.8	154
		S.D.	± 1.14018	± 5.21536	± 1
		S.E.	± 0.5099	± 2.33238	± 0.44721

Table 2: Parameters of mast cells of White Leg Horn chicks infected with the low and high doses of embryonated eggs of Ascaridia galli in immunized group.

S. No.	Day of post infection		Control	Infected with low dose	Infected with high dose
1	15	Mean	95.6	102.8	107.6
		S.D.	± 1.67332	± 2.77489	± 1.81659
		S.E.	± 0.74833	± 1.24097	± 0.8124
2	30	Mean	96	97.2	109.6
		S.D.	± 1.41421	± 1.30384	± 2.07364
		S.E.	± 0.63246	± 0.5831	± 0.92736
3	45	Mean	96.6	100	97.4
		S.D.	± 1.14018	± 1.22474	± 1.81659
		S.E.	± 0.5099	± 0.54772	± 0.8124

Table 1(a): Two way ANOVA of mast cell given in Table 1

Source of Variation	***Sum of Squares***	***Degree of Freedom***	***Mean Square***	***F***	***P-value***	***Frequency***
T	1742.933333	2	871.4666667	122.55	8.59E-17	3.259446306
R	19536.53333	2	9768.266667	1373.663	1.03E-34	3.259446306
RT	778.5333333	4	194.6333333	27.37031	1.76E-10	2.633532094
P,0.050 > 1.76E-10						

Table 2(a): Two way ANOVA of mast cell given in Table 2

Source of Variation	***Sum of Squares***	***Degree of Freedom***	***Mean Square***	***F***	***P-value***	***Frequency***
T	128.7111111	2	64.35555556	20.75986	1.01E-06	3.259446306
R	582.9777778	2	291.4888889	94.02867	5.09E-15	3.259446306
RT	380.3555556	4	95.08888889	30.67384	3.78E-11	2.633532094
P, 0.050 > 3.78E-11						

Mast cells are closely associated with rejection of helminths from the alimentary tract (Woodbury *et al*., 1984) and numbers of these cells rise rapidly if animals are repeatedly infected (Huntley *et al*., 1992).

Askenase (1977) reported the role of mast cells and basophils in inflammatory responses and in most, generally appreciates in immediate hypersensitivity reactions, such as allergic responses mediated by immunoglobulins (IgE) antibodies.

Several studies imply functional role for mast cell proteases in the expulsion of some gastrointestinal helminth (Wastling *et al*., 1997; McLauchalan *et al*., 1999). Mucosal mast cells are responsible for protection against intestinal helminth infection (Rothwell, 1989; Nawa *et al*., 1994).

Intestinal mastocytosis is observed in certain intestinal helminth infections and these rapidly generated mast cells in the intestinal mucosa are thought to play an important role in the mucosal defence against intestinal parasites (Miller, 1984). Apart from IL-3, repetitive administration of recombinant rat stem cell hyperplasia (Tsai *et al*., 1991).Helminth infections of animals which have been shown to cause an increase of mast cells produce factors that stimulate the production of mast cells (Fernex and Fernex, 1962; Murray *et al*., 1971). Taliaferro and Sarles (1939) reported an increase of intestinal connective tissue basophils thought to be different from mast cells in rats during infection with *Nippostrongylus brasiliensis.* Jarrett and Miller (1982) and Ishih (1992) also suggested that marked worm expulsion was associated with the increased mucosal mast cell number. Mastocytosis occurred in the intestinal mucosa of rats infected with *H. diminuta* (Hindsbo *et al*., 1982; Featherston and Copeman 1990; Ishih, 1992). It has been suggested that intestinal changes associated with mastocytosis or with other inflammatory cell populations may contribute to anti-worm effects (Novak *et al*., 1990; Hopkins and Andreassen, 1991).

One of the most marked features of a gastrointestinal nematode infection is the recruitment and hyperplasia of mucosal mast cells (MMCs). Mucosal mastocytosis, including the presence of intraepithelial globule leucocytes, is invariable associated with gastrointestinal helminthiasis (Huntley *et al*., 1992), suggesting that type-I immediate hypersensitivity reactions are important in worm expulsion (Miller, 1984).

The significant increase in the number of mast cells may be associated with an inflammatory reaction in the intestine during the development of partially protective immune responses to this parasitic nematode, which contribute to the dynamic equilibrium between the strength of the intestinal response and the worm biomass. Mast cells are closely associated with rejection of helminths from the alimentary tract (Woodbury *et al.*, 1984) and numbers of these cells rise rapidly if animals are repeatedly infected (Huntley *et al.*, 1992).

A complete study of helminth parasitic antigen is a prerequisite for control programmes based on proper immunochemical diagnosis and immunity by vaccination and immune modulation of diminish pathological alterations. In the present studies, the chicks immunized with bursal antigen revealed protection against the ascaridiasis. The above discussion supported that parasitic infections and immunization too modify or modulate the immune complexes of the host. Various experimental evidences proved that bursal antigen and infected eggs induced immunomodulations in experimental WLH chicks. Outcome of above study on the basis of experimental findings revealed that the bursal antigen could be adopted as conventional vaccine to immunize WLH chicks against *Ascaridia galli*.

IV. CONCLUSION

On the basis of various experimental evidences, it was observed that mast cell number was increased with increasing dose of infective eggs. Slight suppression in mast cell number was observed in immunized group. The above studies proved beyond doubt that bursal antigen and infected eggs induced modulations in mast cell number in experimental WLH chicks and hence bursal antigen could be adopted as conventional vaccine against Ascaridiasis.

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