INTRODUCTION
Parasitic infections account for hundreds of millions of dollars in annual losses and medicated costs in the livestock and poultry industry throughout the world. The most costly parasites in terms of production losses are the gastrointestinal nematodes in ruminants and poultry (Gamble and Zarlenga, 1986). Studies have suggested that A. galli is the most common and most important parasite of chicks. Ascaridiasis is a gastrointestinal disease and is caused by an enteric nematode parasite, Ascaridia galli (Schrank, 1788). The parasites found in the small intestine of poultry belong to the general Ascaridida of these Ascaridia galli is the most common and most important parasite of chicks. Ascaridia is an intra intestinal worm found in chickens, turkeys, geese and a number of wild birds with direct life cycle, they develop to the next infective stage containing a second stage of larva in just 8-14 days under ordinary conditions but they may take a longer and shorter time depending on temperature availability. Reid (1960) found that A. galli reached the infective stage in 5 days at the optimum temperature of 30-34°C. The main objective is to investigate the immunological changes in Male White Leg Horn chicks due to nematode infection of Ascaridia galli parasite, enumerating their parasitological characteristics.

MATERIALS AND METHODS
Collection of Parasites and culturing of eggs
These female parasites were kept in petridish containing saline water for egg laying at 36°C in incubator. After 24-36 hours females layed large number of eggs which were collected in petridish having sterile solution. Eggs were also obtained by squeezing the uterus after dissecting the female parasites. The eggs were kept in normal saline solution at the 34°C for embryonation for 20 days. These embryonated infective eggs were used for given challenged infection in male WLH chicks.

Preparation of doses and counting of eggs
The fully embryonated infective eggs were prepared for inoculation at the time of infection. The dilution method was used for the counting of the eggs. The eggs were suspended in known volume of normal saline solution. With the graduated pipette the 0.2 ml of the suspended solution was sucked and placed on the clean and dry counting slide. The embryonated and infective eggs were counted with the help of stereo microscopic binocular microscope. Three values were taken by repeating the process. Mean of these three values was used for calculating the number of embryonated infective eggs of A. galli.

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ABSTRACT
The enteric nematode parasite Ascaridia galli modulate the immune system, of the hosts and eventually leads to malnutrition in the chicks which eventually results in the decreased return of products derived from poultry. The present work was carried out to investigate the immunological alteration of serum protein profiles, T-cells and B-cells, induced along with different doses of A. galli infective eggs. The percentage of T-lymphocytes showed highly significant (p<0.005) decrease as compared to control group indicating augmenting cellular immunity. The percentage of B-lymphocytes was observed to be highly significantly (p<0.005) increased as compared to control group showing elevated humoral immune response. The possible influence of the parasite induced alteration in immune responses in WLH Chicks is discussed in this paper.

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Administration of the embryonated infective eggs to the experimental male White Leg Horn (WLH) chicks

The male WLH chicks were grouped and labeled properly. The inocula with desired number of embryonated infective eggs (500 embryonated eggs as low dose and 1500 embryonated eggs as high dose) were administered orally to male white leg horn chicks. After infection, the male GLH chicks were kept separately in spacious wooden cages in the animal house. The food and water were given after four hour of administering the infection. The life cycle of Ascaridia galli was observed to be completed between 28-30 days and experimental results were taken after 25 days and 50 days. Four WLH chicks from each of the control group, infected with low dose(500 embryonated eggs), and high dose(1500 embryonated dose) groups were autopsied after 25 and 50 days for studying immunological study.

Estimation of serum protein profile of White Leg Horn Chicks (WLH)

The serum protein profiles were analyzed by agarose gel electrophoresis

Collection of blood and Preparation of serum

For the collection of blood, the male WLH chicks were sacrificed after 25 and 50 days of post infection. Blood was collected from the heart with the sterilized dry glass syringe by the cardiac puncture. Blood was taken in the vials that were sterilized by boiling in water and dried before using. This blood was used for the separation of T and B-lymphocytes and haematological studies. The blood was taken in the clean and dry centrifuge tube and centrifuged at 3000 rpm for 15 minutes. After centrifugation, the pale yellow serum was obtained and stored in deep freezer for analysis of serum protein profiles and further studies.

Analysis of serum protein profile (by Agarose gel electrophoresis)

Agarose gel electrophoresis was used for the analysis of various protein profiles of the serum of the control and different experimental group of male White Leg Horn chicks. Gel was prepared with the Agarose. The stock solution was made by dissolving barbituric acid (2.095 gms) and sodium barbitone (11.380 gms) in distilled water (11.5 litre). The electrophoretic apparatus were filled with the diluted buffer. Gel slabs were prepared using agarose (200 mg) dissolved in buffer solution (20 ml) for 6 sterilized microscopic slides. Agarose and buffer were taken in a clean glass tube for the preparation of gel. This tube was kept in beaker having the water and heated on the burner of gas. The solution of agarose and buffer was stirred until it became transparent. 2.5 ml of this transparent solution was poured per slide and spreaded very carefully and allowed to settle. These slides were kept in refrigerator at 4°C for proper setting of gel. The end point of each slide was connected to buffer chamber by strips of Whatman filter paper. Initially the apparatus was kept for one hour to set equilibrium at 350V voltage and 6 mA current. The serum was loaded on the slides about 1.5 cm from the cathode end. The 6 mA current was supplied for each slide for 90 minutes. After electrophoresis these slides were stained in freshly prepared 0.1 percent amido block (0.1 gm amido black in 7 ml glacial acetic acid and 93 ml distilled water) for 10 minutes. After 10 minutes these slides were kept in destain solution (15 ml methanol + 5 ml acetic acid + 80 ml distilled water) for 24 hours. The slides were kept in an incubator for drying. After drying the immunoglobulins, protein bands appeared clearly. These slides were photographed and scanned for determining the concentration of different protein bands of antibodies.

Counting of T-lymphocytes and B-lymphocytes

The blood of different groups of chicks such as control, infected (low and high dose) was collected from the heart by the cardiac puncture with the sterilized dry glass syringe. The blood was taken into the small glass vial which contained 10 units/ml preservation free heparin. 3.0 ml ficoll hypaque was taken to clean the plastic centrifuge tube. 1 ml blood was also added into this dry centrifuge tube through the pipet and centrifuged at 4000 rpm for 10 minutes. After centrifugation, the upper layer with 0.5 cm of the opaque interface containing mononuclear cells was discarded carefully with the help of pipet. Opaque interface was transferred into a neat and clean appendrof tube through the pipet and 10 ml phosphate buffer saline was added and mixed in a proper way and then centrifuged at 10,000 rpm for 10 minutes. Supernatant was discarded and pellet of cells was left into the bottom of the appendrof tube. 5 ml of PBS solution was added into appendrof tube and mixed gently by using glass road. Cell Pellet was washed with PBS three times to remove the ficoll hypaq, which could be toxic to the cultured cell. 1.0 ml trypans blue was added into this tube. After washing the cell pellet was dissolved after vortexing. Cells were counted through the slide according to size and density (Deys et al., 1996). T-cells and B-cells were identified. For counting of T-lymphocyte and B-lymphocyte 10 ml aliquot was kept on the counting slides. A cover slip was kept over the counting slide very carefully. The counting slide was kept under light microscope and T-cells and B-cells were counted. Counting of T-cells and B-cells were repeated three times and mean was taken.

Percentage of T-cells = \[
\frac{\text{Number of T-cells}}{\text{Total number of T-cells and B-cells}} \times 100
\]

Percentage of B-cells = \[
\frac{\text{Number of B-cells}}{\text{Total number of T-cells and B-cells}} \times 100
\]

RESULT

The accurate interpretation of avian plasma proteins is a very important diagnostic tool in host. Protein electrophoresis is a practical and useful test for diagnostic features of the host. Dramatic changes in protein fraction are important event in several diseases and may help in procuring a diagnosis. Gel electrophoresis is valuable in monitoring the response to therapy as well. Avian total proteins consist of albumin and globulin. Serum obtained from the control and infected groups were analyzed for the serum total protein profile concentration of antibodies after 25 and 50 days respectively.

T-Lymphocyte

- **Group-I**: Control Group-In control group of male WLH chicks the percentage of T-lymphocytes were counted to be 65 percent and 66.10 percent after 25 and 50 days respectively (Table I; Fig 1A, B).
Group-II: Low dose of 500 infective embryonated egg of *A. galli*- In infected group of male WLH chicks the percentage of T-lymphocytes were counted to be 62.40 percent and 61.10 percent after 25 and 50 days of Pi respectively. The percentage of T-lymphocytes were found to be significantly (p<0.005) decreased as compared to control group (Table-1; Fig-1 A, B).

Group-III: High dose of 1000 infective embryonated egg of *A. galli*- In infected group of male WLH chicks the percentage of T-lymphocytes were found to be 59.94 percent, 58.97 percent and 58.14 percent after 25 and 50 days of Pi respectively. The percentage of T-lymphocytes showed highly significant (p<0.005) decrease as compared to control group indicating augmenting cellular immunity (Table-1; Fig-1A, B).

**Table 1** Percentage of T and B lymphocytes in male WLH chicks treated with low and high doses of *A. galli*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Day</th>
<th>Control</th>
<th>Low dose infection</th>
<th>High dose infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-lymphocytes (%)</td>
<td>25</td>
<td>65.00 ± 0.1254</td>
<td>62.40± 0.3123</td>
<td>59.94± 0.5644</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>66.10 ± 0.2564</td>
<td>61.10± 0.5465</td>
<td>58.14± 0.4641</td>
</tr>
<tr>
<td>B-lymphocytes (%)</td>
<td>25</td>
<td>30.00± 0.3312</td>
<td>33.90± 0.8741</td>
<td>35.94± 0.5554</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>30.51± 0.1631</td>
<td>35.08± 0.4641</td>
<td>38.12± 0.4654</td>
</tr>
</tbody>
</table>

B-Lymphocyte

Group-I: Control Group-In the above group of male WLH chicks the percentage of B-lymphocytes was found to be 30 and 30.51 after 25 and 50 days respectively (Table-1; Fig-2 A, B).

Group-II: Low dose of 500 infective embryonated eggs of *A. galli*-In the above group of male WLH chicks the percentage of B-lymphocytes was counted to be 33.90 and 35.08 percent after 25 and 50 days of Pi respectively. A significant (p<0.005) rise was observed in percentage of B-lymphocytes in comparison to control group depicting increased humoral immunity (Table-1 Fig-2 A, B).

Group-III: High dose of 1000 infective embryonated eggs of *A. galli*-In the above group of male WH chicks the percentage of B-lymphocytes was counted to be 35.94 and 38.12 percent after 25 and 50 days of Pi respectively. The percentage of B-lymphocytes was observed to be highly significantly (p<0.005) increased as compared to control group showing elevated humoral immune response (Table-1; Fig-2A, B).

**Figure 1 A & B**: Values of T-lymphocytes in male WLH chicks in control and infected with low and high doses of *A. galli* eggs after 25 days & 50 days respectively.

**Figure 2 A, B**: Values of B-lymphocytes in male WLH chicks in control and infected with low and high doses of *A. galli* eggs after 25 days & 50 days respectively.
DISCUSSION

Analysis of T and B-lymphocytes

T and B-lymphocytes play important role in cellular humoral immune responses. The numbers of B-lymphocytes were found increased in infected group of WLH male chicks. The number of B-lymphocytes were found highly increased in group infected with high dose of infective eggs of (1000 eggs) Ascaridia galli increased in comparison to control group during the present investigation. Pedras (1996) reported that the infection with S. mansoni in mice lead to the development of concurrent Th-2 and type-1 CD8+ cell responses. Decrease in the mean value of T4+ and T4+/T8+ ratio was found in Schistosomiasis compared with healthy control. The B cell percentages peaked in MLN of T. muris infected BALB/C mice at 21st day of Pi. Total IgG and total IgA producing cells from pooled MLN cells also peaked at day 14 PI, the B cells activation and proliferation in the mesenteric lymph nodes of BALB/C infected with T. muris and concluded that B cell responses must be involved in resistance to this parasite (Koyama et al., 1999). Babu et al., (1999) reported a more significant role of B-cells in resistance to the early phase of experimental murine filariasis than previously envisaged. Whatever the mechanism by which B-cells mediate their effect the understanding of the role of B-cells as an important effectors of resistance to B. malayi infection becomes significant in developing vaccine molecule. Paciorkowski et al., (2000) found that functional activity of B-cells played a critical role in host protection against lymphatic filarial parasites. Lowered immune responses during bovine onchocerciasis have been reported in both in vivo and in vitro assay systems. The fourth stage larval (L4) soluble extract caused slight but not statistically significant decrease in the percentage of T-cells and increase in B-cell percentage (Gomez et al., 2004).

CONCLUSION

During the present investigation the percentage of T-lymphocytes and B-cells were found to be approximately same in both infected groups of male WLH chicks. The modulations of T-lymphocytes and B-lymphocytes were observed in experimental host in all groups. Suppression of T-lymphocytes was found in all groups. In infected group (high dose and low dose) the percentage of T-lymphocytes was found to be highly decreased slightly fall in percentage of T-lymphocytes. A high rise in percentage of B-lymphocytes was observed in infected group. In conclusion, the study on the alteration in lymphocyte Responses in White Leg Horn Chicks with Experimental different doses of infection of Ascaridia galli and the data obtained would be extremely helpful in designing the ant parasitic agents against nematodes infection in chicks. This investigation would be extremely helpful in the development of vaccine against ascaridiasis in chicks.

References


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