

## HAEMATOLOGICAL CHANGES FOLLOWING EXPERIMENTAL INFECTION OF BROILER CHICKS WITH FIELD ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS

A. GUPTA and P. C. SHARMA<sup>1</sup>

Department of Veterinary Epidemiology and Preventive Medicine

College of Veterinary Sciences

CCS Haryana Agricultural University, Hisar - 125 004

### ABSTRACT

Haematological changes (total leucocytic count and differential leucocytic count) following experimental infections of 26 days old broiler chicks with either of two field isolates, Hoshiarpur (Punjab) and Hisar-97 (Haryana) of infectious bursal disease virus (IBDV) on day 0, 1, 3, 5, 7, 10, 15 and 21 post inoculation were studied. Significant decrease in TLC was observed at 1 d.p.i followed by gradual increase till 7 d.p.i. The values returned to normal by 21 d.p.i. This pattern was almost similar in both the infected groups of chicks. Lymphocytopaenia followed by leucocytosis was observed in both the groups and the number returned to normal in terminal phase of the illness.

**Key words:** Infectious bursal disease virus, total leucocytic count, differential leucocytic count

Infectious bursal disease virus (IBDV) is a lymphotropic virus and infects pre-B lymphocytes in the bursa of Fabricius (BF). The latter is a primary lymphoid organ in chickens which is considered to be equivalent of bone marrow of mammals. The damage to lymphocytes of the BF results in immune deficiency. This complicates the vaccination programme against important diseases of chickens such as Ranikhet disease, Marek's disease, chicken anemia virus infection, coccidiosis etc. The present study describes some haematological changes observed in broiler chicks following inoculation of field isolates of infectious bursal disease virus.

### MATERIALS AND METHODS

**Experimental design:** Broiler chicks (26 days old) were divided into three groups viz. I, II and III comprising 21, 22 and 16 birds, respectively. Group I was inoculated with  $1 \times 10^5$  pfu of Hoshiarpur isolate of IBDV, group II with  $1 \times 10^4$  pfu of Hisar isolate and group III was kept as uninoculated control. The inoculum of each

viral isolate was introduced orally plus intraocularly. The blood samples were collected at 0, 1, 3, 5, 7, 10, 15 and 21 days post-inoculation (d.p.i.) for haematological studies. Pathological, virological and serological studies on samples/specimens obtained from chicks have earlier been described (Gupta and Sharma, 2005).

**Collection of blood samples:** Blood was drawn directly from the heart and analyzed for:

**(i) Total leucocytic count (TLC):** Blood samples collected in powdered sodium EDTA (@1.5mg/ml blood) were used for estimating TLC by standard procedure using Natt and Herrick stain (1952).

**(ii) Differential leucocytic count (DLC):** Methanol-fixed blood smears were stained with modified Wright stain in order to study DLC employing standard procedure (Lucas and Jamroz, 1974). The absolute count of lymphocytes, heterophils, monocytes and eosinophils was obtained by multiplying the per cent cells to the total leucocytic counts.

**Statistical analysis:** The data of TLC and DLC were analyzed by Duncan's multiple test and Fishers test (Snedecor and Cochran, 1967).

<sup>1</sup> Corresponding author

## RESULTS AND DISCUSSION

Total leucocytic counts are presented in Table 1. A marked decrease in TLC was observed at 1 d.p.i and 3 d.p.i. in group I followed by gradual increase. Leucocytosis was observed on 7 and 10 d.p.i., which peaked to maximum on 7 d.p.i. The number returned to normal by 15 d.p.i. An almost similar pattern was observed in group II. The difference in depression and increase in the number of leucocytes at the peak was not significant between the two groups (Table 1).

The absolute values of lymphocytes, monocytes, eosinophils and basophils on different days post inoculation are presented in Table 2. Lymphocytopenia was observed in chicks regularly till 5 and 10 d.p.i in groups I and II, respectively. Almost similar observations have been made by others (Cosgrove, 1962, Asdrubali and Muggetti, 1972, Mazuriewicz and Wachnik, 1972, Rao and Digraskar, 1998). Cho and Edgar (1972) reported a decrease in lymphocytes at 2 d.p.i, increase at 5 d.p.i but the changes were not significant. The lymphocytopenia in IBDV infection may be observed due to destruction of immature B lymphocytes in the bursa of Fabricius. Heterophilia was noticed on day 3 d.p.i to 15 d.p.i in both the groups (Table 2).

**Table 1**  
Total leucocyte counts on different days following IBDV infection by two field isolates (mean  $\pm$  S.D. in thousand/mm<sup>3</sup>)

d.p.i.	Control	Group I	Group II
0	31.0 $\pm$ 1.4 <sup>a</sup>	32.0 $\pm$ 1.1 <sup>a</sup>	31.0 $\pm$ 1.3 <sup>a</sup>
1	31.3 $\pm$ 1.3 <sup>a</sup>	27.0 $\pm$ 0.4 <sup>b</sup>	25.5 $\pm$ 0.6 <sup>b</sup>
3	32.0 $\pm$ 0.8 <sup>a</sup>	28.7 $\pm$ 0.5 <sup>b</sup>	27.2 $\pm$ 0.5 <sup>c</sup>
5	31.0 $\pm$ 2.5 <sup>a</sup>	33.8 $\pm$ 0.8 <sup>a</sup>	33.1 $\pm$ 1.8 <sup>a</sup>
7	32.5 $\pm$ 1.4 <sup>a</sup>	35.7 $\pm$ 1.0 <sup>b</sup>	36.2 $\pm$ 1.5 <sup>b</sup>
10	32.0 $\pm$ 0.5 <sup>a</sup>	34.5 $\pm$ 0.7 <sup>b</sup>	35.0 $\pm$ 0.5 <sup>b</sup>
15	32.0 $\pm$ 0.4 <sup>a</sup>	33.0 $\pm$ 1.2 <sup>a</sup>	33.5 $\pm$ 0.6 <sup>a</sup>
21	31.6 $\pm$ 0.8 <sup>a</sup>	32.2 $\pm$ 0.4 <sup>ab</sup>	33.0 $\pm$ 0.6 <sup>b</sup>

Similar letters in superscript within rows are not significant, d.p.i : days post inoculation, Group I : Hoshiarpur isolate inoculated birds, Group II : Hisar isolate inoculated birds.

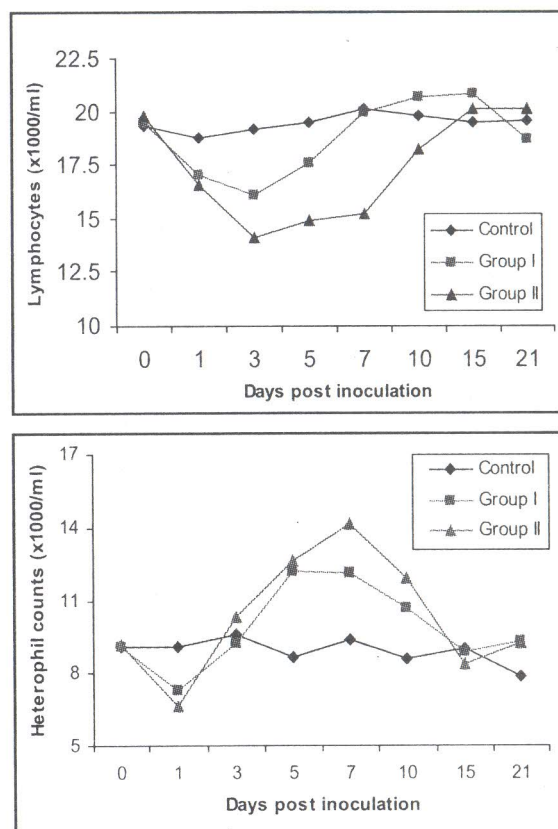


Fig.1. Absolute values of lymphocytes and heterophils following inoculation with field isolates of IBDV (Group I – Hoshiarpur isolate and Group II – Hisar isolate).

Earlier workers have also reported heterophilia (Cho and Edgar, 1972, Kumar and Rao, 1991). A significant decline at 1 d.p.i in both the groups might be due to infiltration of large number of heterophils in the bursa of Fabricius and other infected organs. We did not observe monocytosis. However, eosinophilia and slight basophilia were observed in group I (Table 2). Thus, both the field isolates caused leucocytopenia followed by leucocytosis, and significant variations in number of leucocytes at the peak were not observed. It can be concluded that following inoculation in chicks, both the isolates produced almost similar pattern of total leucocytic count and significant difference in peak values (leucocytosis or leucocytopenia) was not observed between the two groups. However, there were significant difference in the absolute count of lymphocytes and heterophils (Fig. 1).



**Table 2**  
**Absolute values of different leucocytes on different days post-inoculation of IBDV isolates**  
**(mean  $\pm$  S.D. in thousand/mm<sup>3</sup>)**

Group	Days post inoculation							
	0	1	3	5	7	10	15	21
<b>Heterophils</b>								
Control	9.06 $\pm$ 0.06 <sup>a</sup>	9.07 $\pm$ 0.76 <sup>a</sup>	9.60 $\pm$ 0.08 <sup>a</sup>	8.70 $\pm$ 0.14 <sup>a</sup>	9.40 $\pm$ 0.12 <sup>a</sup>	8.60 $\pm$ 0.08 <sup>a</sup>	9.00 $\pm$ 0.16 <sup>a</sup>	7.90 $\pm$ 0.19 <sup>a</sup>
Group I	9.11 $\pm$ 0.7 <sup>a</sup>	7.29 $\pm$ 0.11 <sup>b</sup>	9.23 $\pm$ 0.08 <sup>c</sup>	12.22 $\pm$ 0.12 <sup>b</sup>	12.10 $\pm$ 0.15 <sup>b</sup>	10.71 $\pm$ 0.11 <sup>b</sup>	8.89 $\pm$ 0.18 <sup>a</sup>	9.30 $\pm$ 0.05 <sup>b</sup>
Group II	9.16 $\pm$ 0.07 <sup>a</sup>	6.63 $\pm$ 0.05 <sup>c</sup>	10.34 $\pm$ 0.12 <sup>b</sup>	12.60 $\pm$ 0.07 <sup>c</sup>	14.10 $\pm$ 0.22 <sup>c</sup>	11.90 $\pm$ 0.11 <sup>c</sup>	8.38 $\pm$ 0.09 <sup>b</sup>	9.24 $\pm$ 0.23 <sup>b</sup>
<b>Lymphocytes</b>								
Control	19.30 $\pm$ 0.03 <sup>a</sup>	18.80 $\pm$ 0.79 <sup>a</sup>	19.20 $\pm$ 0.27 <sup>a</sup>	19.50 $\pm$ 0.17 <sup>a</sup>	20.10 $\pm$ 0.23 <sup>a</sup>	19.80 $\pm$ 0.18 <sup>a</sup>	19.50 $\pm$ 0.08 <sup>a</sup>	19.60 $\pm$ 0.08 <sup>a</sup>
Group I	19.50 $\pm$ 0.17 <sup>ab</sup>	17.01 $\pm$ 0.15 <sup>b</sup>	16.07 $\pm$ 0.16 <sup>b</sup>	17.60 $\pm$ 0.08 <sup>b</sup>	20.00 $\pm$ 0.24 <sup>a</sup>	20.70 $\pm$ 0.10 <sup>b</sup>	20.8 $\pm$ 0.05 <sup>c</sup>	18.70 $\pm$ 0.17 <sup>c</sup>
Group II	19.79 $\pm$ 0.10 <sup>b</sup>	16.60 $\pm$ 0.08 <sup>b</sup>	14.10 $\pm$ 0.10 <sup>c</sup>	14.91 $\pm$ 0.08 <sup>c</sup>	15.20 $\pm$ 0.08 <sup>b</sup>	18.20 $\pm$ 0.11 <sup>a</sup>	20.10 $\pm$ 0.06 <sup>b</sup>	20.11 $\pm$ 0.09 <sup>a</sup>
<b>Monocytes</b>								
Control	2.29 $\pm$ 1.39 <sup>a</sup>	3.12 $\pm$ 0.70 <sup>a</sup>	2.54 $\pm$ 1.10 <sup>a</sup>	2.58 $\pm$ 2.19 <sup>a</sup>	2.35 $\pm$ 1.76 <sup>a</sup>	2.61 $\pm$ 1.04 <sup>a</sup>	3.18 $\pm$ 0.56 <sup>a</sup>	2.80 $\pm$ 0.72 <sup>ab</sup>
Group I	3.02 $\pm$ 1.04 <sup>a</sup>	2.16 $\pm$ 0.56 <sup>a</sup>	2.83 $\pm$ 0.77 <sup>a</sup>	3.30 $\pm$ 0.58 <sup>a</sup>	2.17 $\pm$ 1.22 <sup>a</sup>	2.74 $\pm$ 0.79 <sup>a</sup>	2.98 $\pm$ 1.17 <sup>a</sup>	3.24 $\pm$ 0.70 <sup>a</sup>
Group II	1.73 $\pm$ 1.08 <sup>a</sup>	1.98 $\pm$ 0.53 <sup>a</sup>	2.18 $\pm$ 0.53 <sup>a</sup>	3.90 $\pm$ 1.81 <sup>a</sup>	4.00 $\pm$ 1.50 <sup>a</sup>	2.77 $\pm$ 0.52 <sup>a</sup>	3.01 $\pm$ 0.51 <sup>a</sup>	1.34 $\pm$ 0.84 <sup>ab</sup>
<b>Eosinophils</b>								
Control	0.35 $\pm$ 0.01 <sup>a</sup>	0.31 $\pm$ 0.01 <sup>a</sup>	0.32 $\pm$ 0.01 <sup>a</sup>	0.31 $\pm$ 0.02 <sup>a</sup>	0.65 $\pm$ 0.01 <sup>a</sup>	0.64 $\pm$ 0.02 <sup>a</sup>	0.32 $\pm$ 0.01 <sup>a</sup>	0.95 $\pm$ 0.03 <sup>a</sup>
Group I	0.36 $\pm$ 0.04 <sup>a</sup>	0.54 $\pm$ 0.03 <sup>b</sup>	0.57 $\pm$ 0.02 <sup>b</sup>	0.34 $\pm$ 0.03 <sup>a</sup>	1.07 $\pm$ 0.07 <sup>b</sup>	0.35 $\pm$ 0.05 <sup>c</sup>	0.33 $\pm$ 0.03 <sup>a</sup>	0.64 $\pm$ 0.08 <sup>c</sup>
Group II	0.32 $\pm$ 0.02 <sup>a</sup>	0.26 $\pm$ 0.03 <sup>a</sup>	0.27 $\pm$ 0.03 <sup>c</sup>	1.66 $\pm$ 0.05 <sup>b</sup>	2.17 $\pm$ 0.09 <sup>c</sup>	1.75 $\pm$ 0.04 <sup>b</sup>	2.01 $\pm$ 0.03 <sup>b</sup>	1.65 $\pm$ 0.08 <sup>b</sup>
<b>Basophils</b>								
Control	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.33 $\pm$ 0.58 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.33 $\pm$ 0.58 <sup>a</sup>	0.00 <sup>a</sup>	0.33 $\pm$ 0.58 <sup>a</sup>
Group I	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.34 $\pm$ 0.03 <sup>b</sup>	0.36 $\pm$ 0.03 <sup>b</sup>	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.32 $\pm$ 0.33 <sup>b</sup>
Group II	0.00 <sup>a</sup>	0.00 <sup>a</sup>	0.27 $\pm$ 0.03 <sup>a</sup>	0.00 <sup>a</sup>	0.72 $\pm$ 0.03 <sup>a</sup>	0.35 $\pm$ 0.02 <sup>a</sup>	0.00 <sup>a</sup>	0.66 $\pm$ 0.06 <sup>a</sup>

Similar letters in superscript within columns of each cell type are not significant  
 Group I - Hoshiarpur isolate inoculated birds, Group II - Hisar isolate inoculated birds

## REFERENCES

- Asdrubali, G. and Mughetti, L. (1972). Ricerche ematologiche nella mammata di Gumboro sperimentale. *Nuova Vet.* **48**: 210-225.
- Cho, Y. and Edgar, S.A. (1972). Characterization of infectious bursal disease. *Poult. Sci.* **51**: 60-69.
- Cosgrove, A.S. (1962). An apparently new disease of chickens – avian nephrosis. *Avian Dis.* **6**: 385-389.
- Gupta, A. and Sharma, P.C. (2005). Clinicopathological and serological characterization of two Indian field isolates of Infectious bursal disease virus. *Intl. J. Poult. Sci.* **4**: 408-413.
- Kumar, A. and Rao, A.T. (1991). Haematological and blood biochemical changes in experimental infectious bursal disease in 25-26 week old layers – a short note. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **5**: 123-124.
- Lucas, A.M. and Jamroz, C. (1974). Atlas of Avian Hematology. Oxford and IBH Publishing Co., New Delhi.
- Mazuriewicz, M. and Wachnik, Z. (1972). Peripheral blood picture, uric acid and some electrolyte levels in plasma of chickens with IBD. *Weterynaria Wroclaw.* **28**: 61-68.
- Natt, M.P. and Herrick, C.A. (1952). A new blood diluent for counting of erythrocytes and leucocytes in chickens. *Poult. Sci.* **31**: 735-738.
- Rao, B.C. and Digraskar, S. (1998). Haematological and biochemical alterations in chickens experimentally infected with IBD viral isolate. *Poult. Adv.* **31**: 13-16.
- Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. (6<sup>th</sup> edn.), The Iowa State University Press, Ames, Iowa.