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COMPARATIVE SUSCEPTIBILITY OF FAYOUMI, INDIGENOUS AND WHITE LEGHORN CHICKS TO INFECTIOUS BURSAL DISEASE

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ABSTRACT

A research study was undertaken in order to compare the susceptibility of Fayoumi, Indigenous and White Leghorn (WLH) chicks to infectious bursal disease (IBD). But indigenous chicks were naturally infected with IBD prior to experimental infection. Even though IBDV bursal homogenate [20 % (w/v)] (collected from a field sample) was prepared and virus challenge given @ 50 µl/bird on their 35th days (day 0) of age. After challenge, clinical signs, morbidity and mortality of birds were recorded and histopathology of lymphoid organs was studied either from dead or sacrificed birds on day 0 and days 3, 7 and 14 post challenge. Antibody levels were detected at different ages by using commercial IDEXX ELISA kit. Cumulative morbidity and mortality were found higher (88.46% and 80.77%, respectively) in Fayoumi birds than those of WLH birds (31.25% and 18.75%, respectively). Indigenous chicks were infected naturally with IBD at their 24th days of age and at that time 12 birds affected and 9 birds died. Indigenous birds did not show any morbidity and mortality after experimental challenge. In ELISA, antibody level at day-one was found lower (titer 1288) in Fayoumi than in WLH birds (titer 2293). On the other hand, it was lowest (titer 304) in indigenous chicks at day-one. Following virus challenge, antibody levels of Fayoumi birds increased in higher rate than WLH birds. Characteristic microscopic lesions, such as- hemorrhage, lymphoid depletion, cystic atrophy in bursa, increased bursal lesion scores, hemorrhagic and congested spleen, thymus and caecal tonsil were observed. In conclusion, it can be said that under confinement, indigenous birds are susceptible to infectious bursal disease. In between Fayoumi and WLH birds, Fayoumi are more susceptible to IBD than WLH.

Keywords: chicks, infectious bursal disease, susceptibility, fayoumi, indigenous, WLH.

INTRODUCTION

Infectious Bursal Disease (IBD), which is popularly known as, “Gumboro disease”, is caused by infectious bursal disease virus (IBDV). IBDV is a highly infectious agent which may cause 80% mortality in field outbreaks (Chowdhury *et al.*, 1996; Islam *et al.*, 1997; Hoque *et al.*, 2001). There are some reports (Biswas *et al.*, 2005; Biswas *et al.*, 2008) of IBD in chicks of either ‘Sonali’ (RIR X Fayoumi) or ‘Fayoumi’ reared under semi-scavenging system in the PLDP (Participatory Livestock Development Project) and SLDP-2 (Smallholder Livestock Development Project-2) areas of Bangladesh. In those reports, the author also suggested that the indigenous chicks were resistant against IBD. However, Okoye *et al.* (1999) reported that indigenous chickens could also be infected experimentally with IBD. If indigenous birds are found to be susceptible to IBD, there is possibility of dissemination of IBD to our backyard chicken-population. On the other hand, if indigenous birds found resistant to IBD, further studies can be undertaken to integrate its resistant gene of indigenous birds to higher egg producing birds. Among chickens, White Leghorn (WLH) breed was reported most susceptible by Lukert and Saif (1997). And, in case of Fayoumi birds, one report is available, wherein, the mortality rate was found 47% (Hassan *et al*., 2002).On this background, the present study was aimed to study the comparative susceptibility of Fayoumi, indigenous and WLH chicks by induced experimental infection with infectious bursal disease virus (IBDV), to observe microscopic lesions produced by the virus in the lymphoid tissues (bursa, spleen, thymus and caecal tonsils) and to measure the intensity of immune response (based on indirect ELISA) produced against IBDV in the virus induced chicks of three breeds.

MATERIALS AND METHODS

Preparing poultry sheds and purchasing chickens

The poultry sheds and equipment were properly cleaned, disinfected and fumigated. Among three types of chicks, 54 Fayoumi and 26 White Leghorn (WLH) day-old chicks were purchased from Central Poultry Farm, Mirpur, Dhaka, Bangladesh. In case of indigenous chicks, 150 fertile eggs of indigenous hens were purchased from nearby villages of Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh and then incubated at the Poultry Farm of BAU. At hatching, only 35 indigenous chicks were found apparently healthy.

Experimental design

Birds	**No. of birds at one day of age**	**No. of birds at day 0** (35 days of age) [Date of infection]	**Birds in challenged group**	**Birds in control group**	**Dose of viral inocula** (bursal homogenate) [20%(w/v)]
Fayoumi	54	52	26	26	50 µl/bird (half intranasal and half intraocular)
WLH	26	24	16	8	-- do --
Indigenous	35	21	13	8	-- do --

Two birds from each breed were sacrificed at one day of age to collect blood sera. The experimental challenge was given on 35 days of age (day 0) at 5 p.m. The birds were closely observed at every 6 hours interval after experimental challenge for studying clinical signs, morbidity and mortality of birds. Three birds from challenged group and two birds from control group (either dead or sacrificed) were studied for histopathologic lesions in lymphoid organs (bursa, spleen, thymus, caecal tonsils) with bursal lesion scores on day 0 and day 3, 7 and 14 post challenge. The total experimental period was of 49 days.

Bursa/body weight ratio (B/BW ratio)
B/BW ratio was determined by the following formula:

$$\text{B/BW ratio} = \frac{\text{Bursal weight of individual bird in gm}}{\text{Body weight of individual bird in gm}} \times 1000$$

Histopathology

During postmortem examination the bursa, spleen, thymus and caecal tonsil of each sacrificed or dead birds were collected and fixed in 10% neutral buffered formalin. Formalin fixed tissue samples were processed and stained as per standard method (Luna, 1968) for histopathologic study.

Bursal lesion score

The scoring of the bursal lesions was done on the basis of the following criteria described by Raue *et al.* (2004).

Antibody level determination

Antibody level of 3 breeds of birds was detected from blood serum on day one, day 0 (35 days of age) and days 7 and 14 post challenge. Antibody titers of the collected serum samples were determined by a commercial ELISA kit (Infectious Bursal Disease Antibody Test Kit-IDEXX Laboratory, Inc. Westbrook, Main, 04092, USA). The IDEXX ELISA was performed according to the manufacturer's instruction using pre-coated plates and pre-diluted, ready to use, reagents and buffer. The titer was predicted from the absorbance value of 1:500 dilution of a serum using the formula supplied with the kit.

Statistical analysis

The data were analyzed by using SPSS package program using "t-test". The significance of differences between means was tested at the level of $P<0.01$.

RESULTS

Contraction of the vent region was the first clinical sign appeared in Fayoumi birds after 48 hours of vial challenge. On the other hand, same clinical sign appeared in WLH birds at 60 hours post challenge. The other signs gradually observed included - depression and drowsiness, drooling of saliva, bloody feces, severe prostration and death.

Tables 1 and 2 represent the morbidity and mortality on different days post challenge in Fayoumi and WLH chickens experimentally challenged with IBDV.

Table-1. Morbidity and mortality on different days post infection in Fayoumi chickens experimentally challenged with IBDV.

Days post challenge	**Total birds**	**Affected**	**Died**	**Cumulative morbidity** (%)	**Cumulative mortality** (%)
1	26	-	-	-	-
2	26	11	2	42.31	7.69
3	24	+7	+15	69.23	65.38
4	09	+4	+3	84.62	76.92
5	06	+1	-	88.46	76.92
6	06	-	+1	88.46	80.77
7	05	-	-	88.46	80.77
14	05	-	-	88.46	80.77

Table 2. Morbidity and mortality on different days post challenge in WLH chickens experimentally challenged with IBDV.

Days post challenge	Total birds	Affected	Died	Cumulative morbidity (%)	Cumulative mortality (%)
1	16	-	-	-	-
2	16	2	-	12.5	-
3	16	+3	1	31.25	6.25
4	13	-	+2	31.25	18.75
5	11	-	-	31.25	18.75
6	11	-	-	31.25	18.75
7	11	-	-	31.25	18.75
14	11	-	-	31.25	18.75

Higher cumulative morbidity (88.46%) was observed in Fayoumi birds in comparison to WLH birds (31.25%) and cumulative mortality rate was 80.77% in Fayoumi but only 18.75% in WLH (Tables 1, 2). Indigenous birds picked natural IBD infections by their 24th day of age, i.e., before giving experimental infection and then 12 birds were affected, 9 died out of 35 birds. Indigenous birds did not show any morbidity and mortality after experimental challenge at 35th days of age.

Bursa/body weight (B/BW) **ratio**

Table-3 displays the bursa/body weight ratio of three breeds. The reduction of bursa/body weight ratio in challenge groups was highly remarkable in Fayoumi birds than WLH birds on day 3 post infection comparing with day 0 (35 days of age). On day 7 post challenge, the bursa/body weight ratios reduced at same rate in both breeds. But on day 14 post challenge, the B/BW ratio was found slightly increased in both types of birds. The B/BW ratios in control groups of both Fayoumi and WLH birds were decreased on day 14 post challenge. However, the B/BW ratios between challenged and age-matched unchallenge groups were found statistically insignificant except significant values on day 7 and day 3 post challenge in Fayoumi and WLH birds, respectively. On the other hand, the bursa/body weight ratios of indigenous birds did not maintain any pattern in terms of increase and decrease and were found statistically insignificant.

Table 3. B/BW ratio (Mean±SD) of Fayoumi, WLH and Indigenous birds.

Day	Fayoumi		WLH		Indigenous	
Day 0 (35 days of age)	7.93±1.93		5.64±2.18		1.01±0.15	
	Post challenge					
	Challenged	Control	Challenged	Control	Challenged	Control
Day 3	5.79±1.62	6.99±1.92**	5.29±2.28	2.92±0.08 **	1.32±0.18	0.76±0.06**
Day 7	1.52±0.55	4.63±0.91**	0.99±0.63	3.64±1.19 **	0.57±0.44	0.84±0.02**
Day 14	3.53±2.77	1.13±0.39**	1.03±0.22	1.17±0.22 **	0.98±0.37	1.07±0.93**

** P<0.01 % level of significance

Histopathology

Bursa

On day 3 post challenge, the most common lesions in bursa were hemorrhage and lymphoid depletion within the follicles in Fayoumi birds (Figure-1), less hemorrhage and more lymphoid depletion was observed in WLH birds (Figure-2), presence of necrotic cellular masses containing karyorrhectic nuclei along with intrafollicular edema, infiltration of neutrophils and macrophages. Severe lymphoid depletion (Figures 3, 4) and cystic atrophy of the lymphoid follicles were found on day 7 post challenge in both breeds; where as, on day 14 post infection, infolding of bursal epithelia and lymphoid regeneration (Figures 5, 6) were found. In case of Deshi birds, cystic atrophy (Figure-7) was found at 3 days post challenge (d.p.c.), infolding of bursal epithelia with lymphoid regeneration in bursa (Figure-8) was found after 7 and 14 days of challenge.

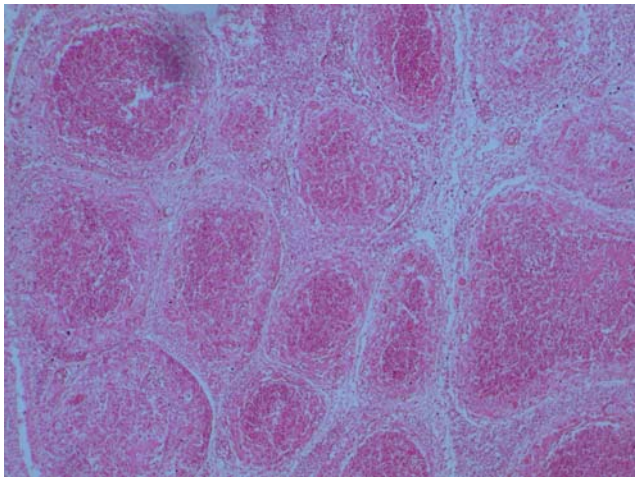

Figure-1. Hemorrhage and lymphoid depletion in bursa of Fayoumi birds on 3 d.p.c. (82.5x).

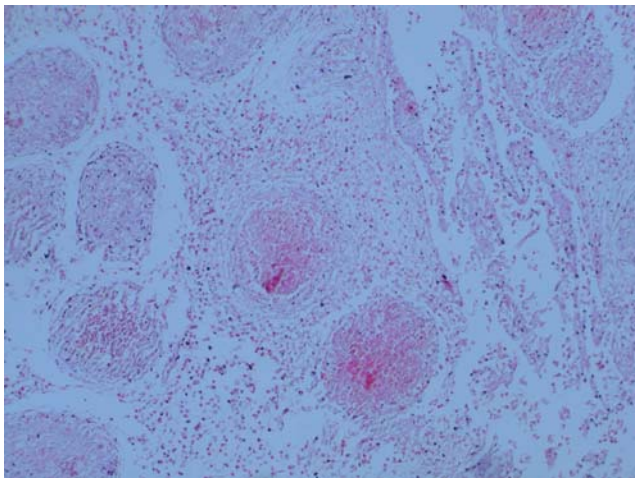

Figure-2. Hemorrhage and more lymphoid depletion in bursa of WLH birds on 3 d.p.c. (82.5x).

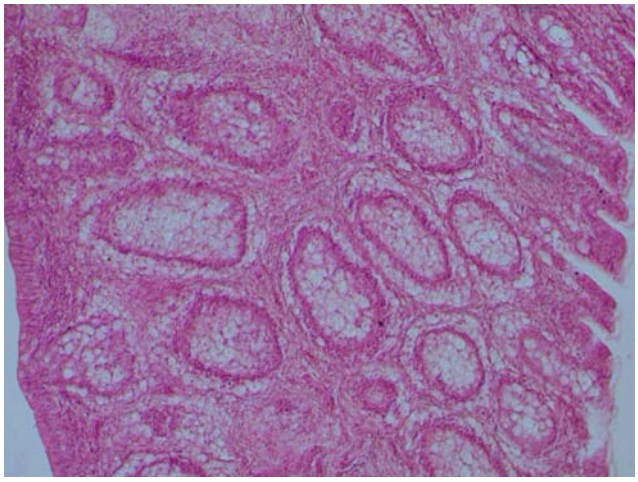

Figure 3. Severe lymphoid depletion in bursa of Fayoumi birds on 7 d.p.c. (82.5x).

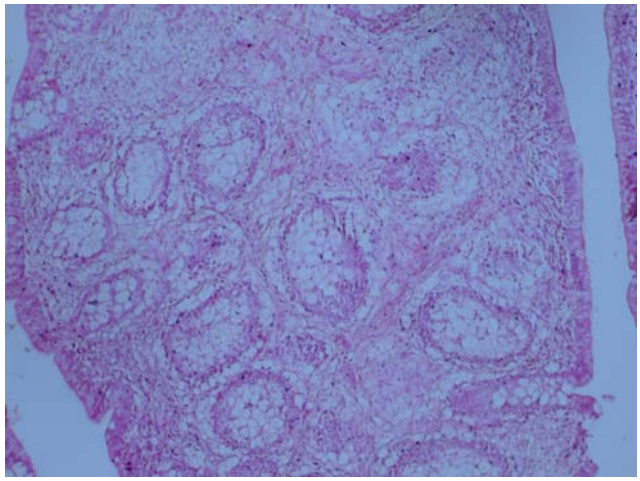

Figure-4. Severe lymphoid depletion in bursa of WLH birds on 7 d.p.c. (82.5x).

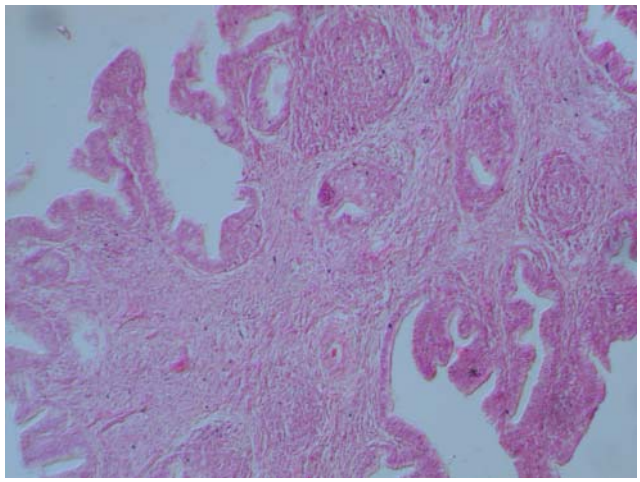

Figure-5. Infolding of bursal epithelia and lymphoid regeneration of Fayoumi birds on 14 d.p.c. (82.5x).

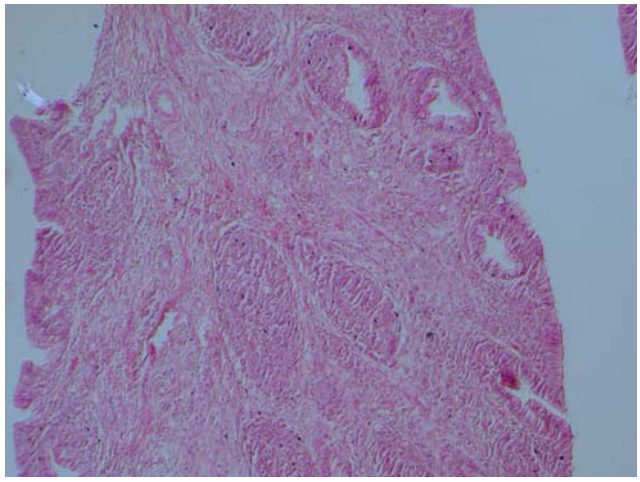

Figure-6. Infolding of bursal epithelia and lymphoid regeneration of WLH birds on 14 d.p.c. (82.5x).

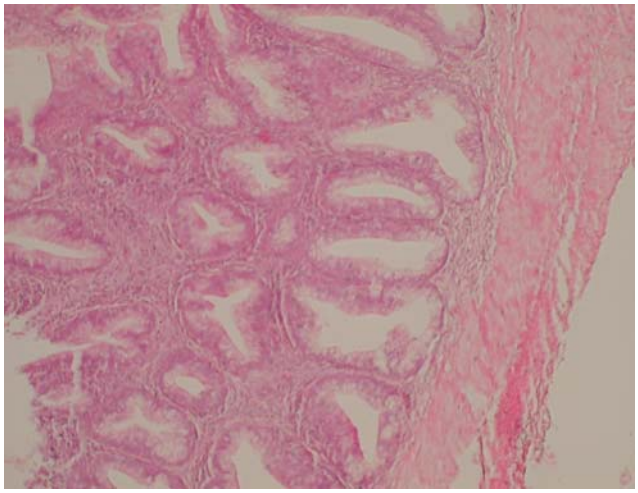

Figure-7. Cystic atrophy of bursa of indigenous birds on 3 d.p.c. (82.5x).

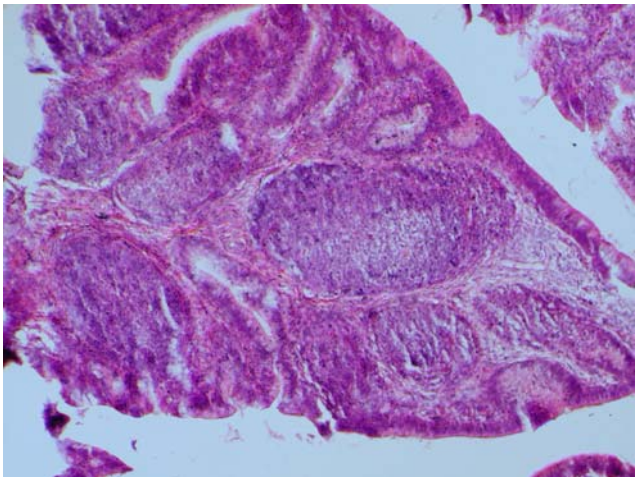

Figure-8. Infolding of bursal epithelia and lymphoid regeneration in the bursa of indigenous birds on 7 and 14 d.p.c. (82.5x).

Bursal lesion score

Significant bursal lesions were observed in all breeds of birds. The results are presented in Table-4.

Table 4. Bursal lesion scores in three breeds of birds on different days post infection.

Bird	Bursal lesion scores						
	0 d.p.i (35 days of age)	Post challenge					
		3 d.p.c.	Control	7 d.p.c.	Control	14 d.p.c.	Control
Fayoumi	0,0,0	3, 2, 2	0,0	4, 3,4	0,0	4, 4, 4	0,1
WLH	0,0,0	3, 2, 2	0,0	3, 4, 4	0,0	4, 4, 4	1,0
Indigenous	4,3,3	4, 4, 4	3,4	4, 4, 4	4,4	4, 4, 4	4,4

Spleen, thymus and caecal tonsils

Distinctive histopathologic lesions were not found in spleen, thymus and caecal tonsils at different days post challenge. Marked hemorrhages (Figures 9, 10) and lymphocytic depletion were found in spleen. Other lesions were hyaline degeneration, eosinophilic tissue debris containing karyorrhectic nuclei of necrotic lymphocytes. In thymus, moderate to severe lymphoid depletion, hemorrhages (Figure-11), presence of tissue debris and interlobular edema were the most common lesions. In caecal tonsils, characteristic lesions were congestion and lymphocytic depletion (Figure-12) at different days post challenge.

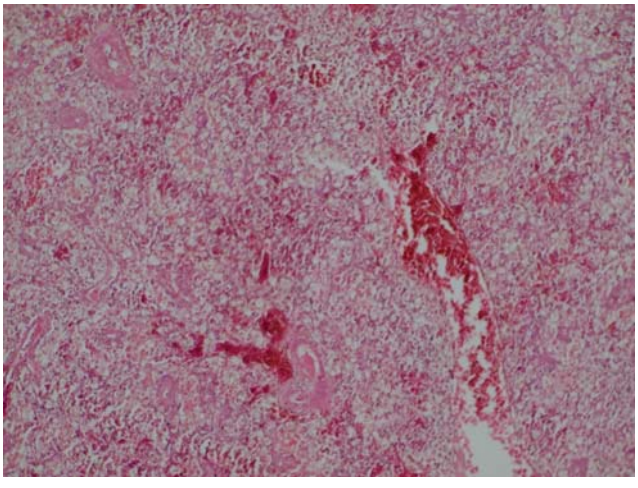

Figure-9. Hemorrhage and congestion in spleen of Fayoumi birds (82.5x).

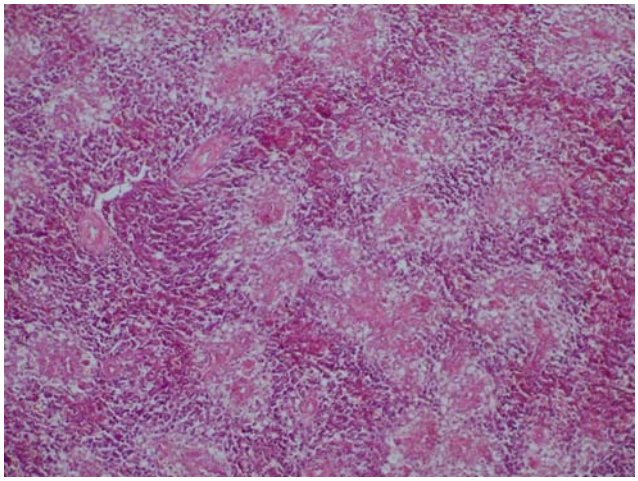

Figure-10. Hemorrhage in spleen of WLH birds (82.5x).

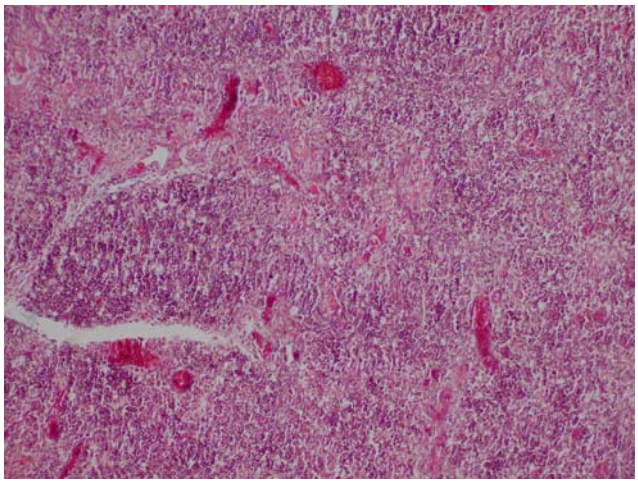

Figure-11. Hemorrhage in thymus of Fayoumi birds (82.5x).

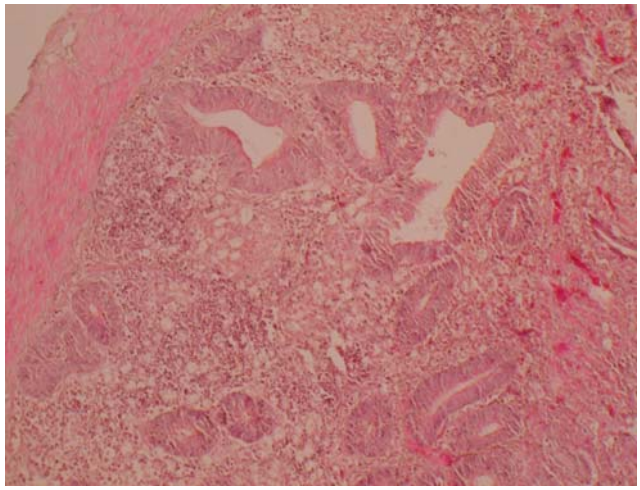

Figure-12. Congestion and lymphoid depletion in caecal tonsil of WLH birds (82.5x).

Determination of antibody level

Antibody levels of three breeds of birds before and after viral challenge are presented in Table-5. At one day of age, the antibody level in WLH birds was higher than Fayoumi birds. These levels decrease substantially at 35 days of age. After experimental challenge, the antibody levels in challenged groups of both Fayoumi and WLH birds increased but the rate of increase was higher in Fayoumi than in WLH birds. Antibody levels were also increased in control groups.

Table-5. Antibody titer of three breeds of birds at different age before and after viral challenge (Mean ± SD).

Bird	Day-one of age	Day 0 (35 days of age)	Day 7 post challenge		Day 14 post challenge	
			Challenged	Control	Challenged	Control
Fayoumi	1288 ± 1100	223 ± 166	1642 ± 367	743 ± 426**	2605 ± 841	2385±590**
WLH	2293 ± 2155	680 ± 520	1332 ± 427	1011± 914**	1997 ± 971	1449±244**
Indigenous	304 ± 292	1581 ± 892	2989 ± 914	2410±1048**	3413 ± 986	3348±1132**

** P< 0.01 level of significance

DISCUSSIONS

This study was undertaken to determine the comparative degree of susceptibility of Fayoumi, Indigenous and White Leghorn birds to infectious bursal disease. But the indigenous birds were infected naturally with Gumboro disease 11 days prior to the scheduled date of experimental infection i.e. at 24th day of age. So, comparison was mostly limited between the other two types of birds. However, in PLDP and SLDP-2 areas, indigenous birds were found resistant against Gumboro disease reared under semi-scavenging systems (Biswas *et al.*, 2005; Biswas *et al.*, 2008). Low virus load in semi-scavenging system of rearing may be the cause of less susceptibility of Deshi birds. According to the present study, when 'susceptibility of indigenous birds' is on discussion, it can be said that under confined condition indigenous birds are susceptible to infectious bursal disease and they are also not genetically resistant to IBD. This early infection of indigenous birds might have occurred due to lower level of maternally derived antibody (MDA) titer (304) at day one than the other two breeds of

birds (titer in Fayoumi was 1288 and titer in WLH was 2293) (Table-5).

Susceptibility to Gumboro disease may vary from breed to breed. Lukert and Saif (1997) reported that among chickens, all the breeds are affected but White Leghorn chicks exhibited the most severe reactions and had the highest mortality rate. In the present experimental infection, first clinical sign appeared in Fayoumi birds after 48 hours of infection. On the other hand, same clinical sign appeared in WLH birds after 60 hours. Higher morbidity (88.46%) was observed in Fayoumi birds in comparison to WLH birds (31.25%) (Tables 1-2). WLH birds also showed less mortality (18.75%) than Fayoumi (80.77%) that indicates the higher susceptibility of Fayoumi to IBD than WLH (Tables 1, 2). However, highest susceptibility (about 80% mortality) in a Brown Leghorn line was reported by Bumstead *et al*. (1993). On the other hand, Hassan *et al*. (2002) observed higher susceptibility to Gumboro infection with mortality of 47% in Fayoumi birds than that of Leghorn birds (20% mortality) which keeps with observation of the present findings. This higher mortality of Fayoumi birds, in present experiment, may be due to lower antibody level (titer 223) before infection at 35 days of age in comparison to WLH (titer 680) (Table-5).

In IBD affected birds, the bursa/body weight ratio become lower than the normal birds (Rosales *et al.*, 1989c; Thangavelu *et al.*, 1998). In this experiment, the reduction of bursa/body weight ratio in Fayoumi birds (from 7.93 to 5.79) was noteworthy on day 3 post challenge than those of WLH birds (from 5.64 to 5.29) comparing with day 0 (35 days of age) (Table-3). Higher rate of morbidity and mortality in Fayoumi birds may be an indication of decreased bursa/body weight ratio on day 3 post challenge. On day 7 post challenge, remarkable reduction in bursa/body weight ratio occurred in both types of birds. On day 14 post challenge the bursa/body weight ratio was found slightly increased in both Fayoumi and WLH birds than that of day 7 post challenge (Table-3). This may be due to somewhat lymphocytic regeneration in the bursa on day 14 post challenge. Bursa/body weight ratios were also reduced in birds of control groups of both Fayoumi and WLH at 14 days post challenge (Table-3). These results indicate that the birds of control groups also picked up some infection. In case of indigenous birds, the bursa/body weight ratios did not maintain any pattern in terms of increase or decrease at 3 days post challenge (Table-3). This might happened as they picked previous natural infection and did not show any morbidity or mortality after experimental challenge.

Bursal lesion score is considered as an accurate indication of the pathogenicity of IBD virus (Rosales *et al.*, 1989c; Tsukamoto *et al.*, 1995b). In the present study, at day 3 post infection, the scores were limited between 2 and 3 in challenged groups of both Fayoumi and WLH birds (Table-4). On day 7 post challenge, the scores were between 3 and 4 and on day 14 post challenge, the scores were 4 in both breeds (Table-4). Two birds of control groups of both Fayoumi and WLH at day 14 post challenge showed mild lymphoid depletion and were scored 1 (Table-4). This may be due to some infection in control groups. In case of indigenous birds, higher scores were found bursal lesions got in both challenged and control groups as they were infected previously (Table-4).

Birds of control groups in both Fayoumi and WLH showed higher level of antibody titer (Table-5). It appears that the control birds also became mildly infected in the course of experiment as indicated by the increase in antibody level, reduction in bursa/body weight ratios and higher bursal lesion scores.

An important limitation of this study was that the indigenous birds were infected naturally before the stipulated day of infection. So, their accurate morbidity, mortality rates and immunity level could not be studied properly, thus could not compare with other two types of birds. But in spite of having some shortfalls, from the present study it can be concluded that Fayoumi birds are more susceptible to IBD than WLH; indigenous birds under confinement can be considered as 'susceptible' to infectious bursal disease. It may also be concluded that MDA level and management system (viral load in the premises) are the main determinants of the susceptibility to infectious bursal disease in different breeds of chickens.

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REFERENCES

Biswas PK, Biswas D, Ahmed S, Rahman A and Debnath NC. 2005. A longitudinal study of the incidence of major endemic and epidemic diseases affecting semi-scavenging chickens reared under the Participatory Livestock Development Project areas in Bangladesh. Avian Pathology. 34: 303-312.

Biswas PK., Uddin G.M N, Barua H, Roy K, Biswas D, Ahad A and Debnath NC. 2008. Survivability and causes of loss of broody-hen chicks on smallholder households in Bangladesh. Preventive Veterinary Medicine. 83: 260-271.

Bumstead N, Reece RL and Cook JKA. 1993. Genetic differences in susceptibility of chicken lines to infection with infectious bursal disease virus. Poultry Science. 72: 403-410.

Chowdhury EH, Islam MR, Das PM, Dewan ML and Khan MSR. 1996. Acute infectious bursal disease in chickens: pathological observation and virus isolation. Asian-Australian Journal of Animal Science. 9: 465-469.

Hassan MK, Afify M and Aly MM. 2002. Susceptibility of vaccinated and unvaccinated Egyptian chickens to very virulent infectious bursal disease virus. Avian Pathology. 31: 149-156.

Hoque MM, Omar AR, Chong LK and Aini I. 2001. Pathogenicity of Sspl-positive infectious bursal disease virus and molecular characterization of hypervariable region. Avian Pathology. 30: 369-380.

Islam MR, Chowdhury EH, Das PM and Dewan ML. 1997. Pathology of acute infectious bursal disease virus in chickens induced experimentally with a very virulent isolate. Indian Journal of Animal Science. 67: 7-9.

Lukert PD and Saif YM. 1997. Infectious bursal disease. In: B. W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif [Editors] Diseases of Poultry. 10th Ed. Iowa State University Press, Ames. pp.721-738.

Luna LG. 1968. Manual of Histopathologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Ed. McGraw-Hill Book Company, London. pp. 32-46.

Raue R, Islam MR, Islam MN, Islam KM, Badhy SC, Das PM and Müller H. 2004. Reversion of molecularly engineered, partially attenuated very virulent infectious bursal disease virus during infection of commercial chickens. Avian Pathology. 33: 181-189.

Rosales AG, Villegas P, Lukert PD, Fletcher OJ, Brown J, Mohammed MA and Brown J. 1989c. Isolation, identification and pathogenicity of two field strains of infectious bursal disease virus. Avian Diseases. 33: 35-41.

Thanagavelu A, Raj GD, Elankumaran S, Murali B, Manohar BM, Koteeswaran A and Venugopalan AT. 1998. Pathogenicity and immunosuppressive properties of infectious bursal disease virus field isolates and commercial vaccines in India. Tropical Animal Health and Production. 30: 167-176.

Tsukamoto K, Tanimura N, Mase M and Imai K. 1995b. Comparison of virus replication efficiency in lymphoid tissues among three infectious bursal disease virus strains. Avian Diseases. 39: 844-852.