Clinicopathological Effects of Infectious Bursal Disease on Broilers

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ABSTRACT

Clinicopathological studies on broiler chicks infected with infectious bursal disease revealed transient lymphocytosis followed by lymphopenia and heterophilia. With hypoproteinemia, hypoalbuminemia, hypergammaglobulinemia, In addition, increased of creatinine, uric acid and inorganic phosphorus with decrease in calcium were observed. Histopathological changes also occurred in bursa, spleen, liver and kidneys.

INTRODUCTION

Infectious bursal disease is an acute, highly contagious viral disease of young chickens between 3 to 7 weeks of age (*2*). The virus has double stranded RNA (dsRNA) and belongs to the Birnaviridae family (*11*). The disease (IBD) represents a great hazard for poultry industry as the causal agent adversely affects the defensive mechanism of the birds. It leads to immunosuppression and failure to develop a satisfactory immunity against several bacterial and viral diseases (*7*). The IBD vaccines had been categorized into mild, intermediate and hot vaccines *(16)*. The hot strain causes enlargement of the bursal size, three times than the normal. It is used in the endemic areas *(15)*. Although many vaccine programs are being used to control the spread of IBD, outbreaks of the disease are still recorded worldwide causing high mortalities (*22*). The aim of the present work was to study the clinicopathological, and histopathological effects of infectious bursal disease (IBD) on broilers.

MATERIAL AND METHODS

I-Material

[1] Chickens: one hundred and ten (one-day old) Hubbard broiler chicks maintained on commercial balanced non-medicated ration and clean water were provided throughout the period of the experiment.

[2] IBD virus: virulent IBD virus strain with $10^{5.5}EID_{50}$/0.1ml was kindly supplied by Dr. Ahmed Abd El-samih, Associate Professor and Head of the Virology Dept., Fac. of Vet. Med., Zag. Univ.

[3] Vaccines: a- Nobilis Gumboro D78: from Intervet Company, Holland. It is a live freeze-dried culture of D78 strain of IBDV. It is considered an intermediate strain by the producing company. (one ampoule, 1000 dose, dissolved in 35cc distilled water and each bird was given one drop via the ocular route).

. b-IBD BLEN: from Sanofi Company, USA. It is lyophilized vaccine consisting of a live strain of bursal disease virus of chicken embryo origin. It is considered a hot strain by the producing company. (one ampoule, 1000 dose, dissolved in 35cc distilled water and each bird was given one drop via the ocular route).

[4] Embryonated chicken eggs (ECEs): 9-12 day old ECEs were used for the propagation and titration of the IBDV.

II-Methods

Table 1. Experimental design.

Groups	No.	Type of vaccination	Time of vaccination in days	Time of Challenge in days
Gp.1 (Vaccinated non challenged)	20	D78 IBD BLEN	8 18	—
Gp.2 (Vaccinated then challenged)	30	D78 IBD BLEN	8 18	27
Gp.3 (Challenged)	40	Non vaccinated	—	27
Gp.4 (Negative control)	20	Non treated	—	—

2-Blood sampling

The blood samples were collected from the jugular vein on EDTA for the hematological examinations and without anticoagulant for serum samples for the biochemical assays *(3)*, four times from five chickens in each group, at the ages of 29, 31, 33 and 43 days.

3-Clinicopathological studies

a-The total leukocytic counts and absolute differential leukocytic number were calculated *(17)*.

b-The total protein *(9)*, albumin *(5)*, electrophoretic analysis *(4)*, creatinine *(10)*, uric acid *(18)*, calcium *(21)*and inorganic phosphorus *(8)* were determined.

4-Histopathology:

Specimens from the liver, kidneys, spleen, and bursa of Fabricius from five chickens in each group at the ages of 29, 31, 33 and 43 days were examined microscopically *(1)*.

5-Statistical analysis: The obtained data were statistically analyzed using the SAS/STAT user's guide *(20)*.

RESULTS

1-The result of virus titratation : After the virus was subjected to many successive passages in chicken embryos. It reached a titer of $10^{5.5}EID_{50}$/o.1 ml. It was given to chicks as a challenges virus via the ocular route.

2-Clinical signs: The chickens showed anorexia, pyrexia, watery-whitish diarrhea, prostration and death.

Table 2. Mortality and protection rates, pm picture and histopathology of experimental birds.

Groups	Mortality rate	Protection rate	Gross lesions	microscopic lesions
Gp.1	—	—	The liver, kidneys and spleen were normal.	Normal liver and kidneys tissues. With mild depletion of lymphocytes from the white pulp of the spleen.
			The bursa of Fabricius was slightly congested.	The bursa showed mild depletion of the lymphoid follicles with interfollicular fibrous tissue proliferation.
Gp.2	10%	90%	The liver was slightly congested and enlarged.	Hydropic degeneration of the hepatocytes with massive aggregations of round cells were detected among the hepatic cells.
			The kidneys were slightly enlarged and pale. The ureters were distended with urates.	The kidneys showed severe hemorrhages with increased hypercellularity of the glomeruler tufts.
			The spleen was enlarged and congested.	Moderate depletion and necrosis of the lymphocytes were seen in the white pulp of the spleen.
			The bursa of Fabricius was swollen and slightly congested. Petechial or ecchymotic hemorrhages were found on the mucosal surface of the bursa, at the age of 29 days. Bursal atrophy was seen at the age of 33 days.	The bursa of Fabricius showed moderate depletion of the lymphoid follicles with cyst formation in the mucous membrane.
Gp.3	17.5%	82.5%	The liver was congested and enlarged.	Perivascular aggregations of round cells and proliferation of fibroblasts, were associated with severe congestion of the liver. Moreover, hydropic degeneration of hepatocytes was encountered .
			The kidneys were enlarged, pale and the ureters were distended with urates.	Coagulative necrosis was encountered in the epithelial lining of the renal tubules. Congestion of the renal blood vessels with the presence of hyaline casts in the tubular lumens were observed.
			The spleen was enlarged and congested.	Severe depletion and necrosis of lymphocytes in the white pulp of the spleen were seen.
			The bursa of Fabricius at the age of 29 days was swollen and slightly congested. It showed petechial or ecchymotic hemorrhages on its mucosal surface. Moreover, bursal atrophy was also seen at the age of 33 days.	Severe depletion and necrosis of the lymphoid follicles of the bursa were associated with interfollicular fibrosis.
Gp.4	—	—	The liver, kidneys, spleen and bursa were normal.	the liver, kidneys, spleen and bursa were normal.

NB: The sacrificed chickens at the age of 43 days showed neither gross nor microscopic lesions in all groups.

4- Clinicopathological results

Table 3. Leukogram (x 10^3/µl) at the ages of 29,31,33&43 days (mean ±S.E.) (No=5).

Days& gps.	TLC	Lymphocytes	Heterophils	Monocytes	Eosinophils	Basophils
29 Gp.1	28.70 ±0.20 ^{c}	13.43±0.35^{d}	14.35±0.18 ^{a}	0.35±0.15	0.51±0.20	0.06±0.05
Gp.2	44.00 ±0.35 ^{a}	34.50 ±0.57^{a}	8.02±0.77^{b}	0.44±0.11	0.62±0.14	·0.0± 0.00
Gp.3	39.60 ±0.24^{b}	30.10 ±0.51^{b}	8.55±0.14 ^{b}	0.40±0.05	0.56±0.26	·0.0±0.00
Gp.4	28.20 ±0.26 ^{c}	20.14 ±0.36^{c}	7.10±0.30 ^{b}	0.34±0.09	0.46±0.05	0.06±0.06
31 Gp.1	28.70±0.26 ^{c}	14.69±0.17 ^{b}	13.21±0.32 ^{c}	0.40±0.05	0.35±0.24	0.06±0.05
Gp.2	47.10 ±0.40^{b}	13.56±0.43 ^{b}	32.79 ±0.14^{b}	0.37±0.17	0.38±0.05	0.00±0.00
Gp.3	55.10 ±0.29^{a}	13.88±0.46 ^{b}	40.11±0.64 ^{a}	0.55±0.25	0.55±0.15	0.00±0.00
Gp.4	28.30±0.20 ^{c}	21.12±0.44 ^{a}	6.39±0.35^{d}	0.40±0.11	0.34±0.60	0.05±0.09
33 Gp.1	28.40±0.51^{c}	15.50±0.16 ^{b}	11.82 ±0.42^{c}	0.57±0.18	0.46±0.15	0.05±0.05
Gp.2	45.60±0.24 ^{b}	11.84 ±0.67^{c}	32.84 ±0.74^{b}	0.46±0.21	0.46±0.14	0.00±0.00
Gp.3	52.90±1.51 ^{a}	11.53±0.38 ^{c}	40.32 ±1.24^{a}	0.53±0.30	0.53±0.01	0.00±0.00
Gp.4	28.30±0.30 ^{c}	20.71±0.23 ^{a}	6.64 ±0.44^{d}	0.51±0.14	0.45±0.06	0.00±0.00
43 Gp.1	28.20±0.20	20.28±0.12	6.79±0.16	0.51±0.11	0.57±0.09	0.05±0.05
Gp.2	28.30±0.83	20.65±0.59	6.68±0.19	0.46±0.20	0.46±0.14	0.05±0.06
Gp.3	28.90±0.29	20.63±0.22	7.28±0.08	0.47±0.15	0.46±0.15	0.06±0.60
Gp.4	28.60±0.01	20.82±0.25	6.93±0.23	0.40±0.17	0.40±0.06	0.06±0.06

Rows with similar letters are not significantly different at ($p \leq 0.05$)
Each value represents the mean ± SD

Table 4. Proteinogram (gm/dl)at the ages of 29,31,33&43 days (mean ±S.E.) (No=5).

Days& gps.	T. prot.	Albumin	Globulins				
			T. glob.	α_1	α_2	β	δ
29 Gp.1	3.21±0.17 ^{a}	2.01±0.18 ^{a}	1.20±0.12^{b}	0.30±0.02	0.20±0.02	0.20±0.02	0.51±0.06 ^{a}
Gp.2	2.51 ±0.12^{b}	1.13±0.14^{b}	1.38±0.14 ^{a}	0.30±0.02	0.22±0.01	0.30 ±0.01	0.56±0.03 ^{a}
Gp.3	2.22 ±0.14^{b}	1.04±0.10 ^{b}	1.18±0.05^{b}	0.33±0.02	0.25±0.02	0.27 ±0.02	0.34 ±0.03^{b}
Gp.4	3.36 ±0.20^{a}	2.36±0.16 ^{a}	1.00±0.11^{c}	0.38±0.03	0.26±0.03	0.29±0.03	0.07 ±0.01^{c}
31 Gp.1	3.21±0.22^{a}	1.99±0.23^{a}	1.22±0.05^{a}	0.33±0.15	0.15±0.02	0.20±0.09	0.54 ±0.03^{a}
Gp.2	2.41±0.09 ^{b}	1.18 ±0.05^{b}	1.23±0.07^{a}	0.31±0.05	0.16±0.02	0.20±0.04	0.56±0.01^{a}
Gp.3	2.15±0.15 ^{b}	1.02 ±0.04^{b}	1.13±0.14^{a}	0.35±0.01	0.18±0.04	0.20±0.03	0.40 ±0.03^{b}
Gp.4	3.38±0.23 ^{a}	2.41±0.23 ^{a}	0.98±0.05^{b}	0.40±0.02	0.22±0.02	0.28±0.02	0.08 ±0.01^{c}
33 Gp.1	3.60±0.12 ^{a}	2.32±0.10 ^{a}	1.28±0.04 ^{a}	0.30±0.01	0.17±0.01	0.20±0.01	0.61±0.02^{a}
Gp.2	2.81±0.17 ^{b}	1.54±0.17 ^{b}	1.27±0.03 ^{a}	0.31±0.01	0.18±0.01	0.20±0.02	0.58±0.01 ^{a}
Gp.3	2.19±0.10 ^{c}	1.02±0.04 ^{c}	1.18±0.06 ^{a}	0.31±0.01	0.18±0.01	0.20±0.03	0.49±0.02 ^{b}
Gp.4	3.39 ±0.06^{a}	2.42±0.09 ^{a}	0.97±0.04 ^{b}	0.39±0.01	0.23±0.01	0.28±0.01	0.07±0.01 ^{c}
43 Gp.1	4.40±0.05 ^{a}	2.43±0.11	1.98±0.07 ^{a}	0.45±0.02	0.26±0.03 ^{ab}	0.32 ±0.82	0.95±0.02 ^{a}
Gp.2	3.49±0.08 ^{b}	2.14 ±0.04	1.35±0.04 ^{b}	0.45±0.01	0.21±0.09 ^{b}	0.32±0.01	0.37±0.01 ^{b}
Gp.3	4.35±0.05 ^{a}	2.28±0.07	2.07±0.03 ^{a}	0.47±0.06	0.29±0.02 ^{a}	0.34 ±0.07	0.97±0.04 ^{a}
Gp.4	3.37±0.08 ^{b}	2.37±0.09	1.00±0.03 ^{c}	0.39±0.06	0.25 ±0.04^{ab}	0.27±0.01	0.09±0.09 ^{c}

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Each value represents the mean ± SD

Table 5. The kidney function at the ages of 29, 31, 33&43 days (mean ±S.E.) (No=5).

Days& gps.	creatinine	uric acid	calcium	inorganic phosphorus
29 Gp.1	1.33±0.06^{bc}	6.81±0.19^{b}	10.57±0.27^{a}	5.24±0.06^{c}
Gp.2	2.18±0.18^{ab}	7.87±0.52^{ab}	9.66±0.62^{b}	6.53±0.34^{b}
Gp.3	2.66±0.63^{a}	8.67±0.65^{a}	8.55±0.36^{c}	7.40 ±0.40^{a}
Gp.4	1.12±0.07^{c}	6.75 ±0.44^{b}	10.74±0.27^{a}	5.26±0.16^{c}
31 Gp.1	1.40±0.12^{c}	6.53±0.33^{b}	10.53±0.26^{a}	5.24±0.13^{b}
Gp.2	2.14±0.05^{b}	7.47±0.31^{ab}	9.57±0.32^{b}	6.54±0.30^{a}
Gp.3	2.75±0.23^{a}	8.33±0.63^{a}	8.35±0.44^{b}	7.15±0.32^{a}
Gp.4	1.13±0.08^{c}	6.43±0.25^{b}	10.52 ±0.19^{a}	5.53±0.31^{b}
33 Gp.1	1.28±0.08^{c}	6.59±0.35	10.38±0.17	5.39±0.10^{b}
Gp.2	1.99±0.09^{b}	7.76±0.42	9.51±0.42	6.65±0.22^{a}
Gp.3	2.71±0.21^{a}	7.55±0.56	9.65±0.25	6.92±0.28^{a}
Gp.4	1.11±0.05^{c}	6.42±0.60	10.38±0.38	5.46±0.07^{b}
43 Gp.1	1.32 ±0.08	5.82±0.23	10.26±0.33	5.39 ±0.10
Gp.2	1.34 ±0.08	5.71±0.22	10.28±0.24	5.30 ±0.12
Gp.3	1.33 ±0.11	5.90±0.21	10.32±0.23	5.31±0.05
Gp.4	1.11±0.09	6.24±0.30	10.33±0.20	5.37±0.13

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DISCUSSION

IBD treated birds showed different degrees of leukocytosis, observed at the age of 29 days. This was due to an early antigenic stimulation *(12)*, while the leukocytosis, observed at the ages of 31 and 33 days, was due to heterophilia due to tissue destruction *(3)*. lymphopenia which may be due to the immunosuppressive effect of the virus leading to degeneration and necrosis of the lymphoid follicles in the bursa and spleen *(6)*. The histopathological studies showed moderate to severe depletion and necrosis of the lymphocytes in the follicles of the bursa of Fabricius and in the splenic white pulp. Similar results were reported *(6)* which showed a significant leukocytosis on the 1st and 2nd day PI which continued till the 7th day PI, but at a lesser magnitude with a significant increase in WBCs in the vaccinated group 7 day PI. The leukocytosis was always associated with heterophilia and lymphopenia from the 3rd to 7th day PI or vaccination. On countrary, it has been cited that lymphopenia and heterophilia on the 2nd day PI followed by lymphocytosis and heteropenia on the 6th day PI of 24 day old chickens with IBDV intranasally *(13)*. This may be due to the difference in the route of inoculation and the time of infection.

The normal TLC, lymphopenia and heterophilia in Gp.1 at the ages of 29, 31 and 33 days, get along with the histopathological picture in the spleen and bursa of Fabricius. The mild depletion of the lymphocyte from the splenic white pulp and in the follicle of the bursa of Fabricius reflected the encountered lymphopenia which has been observrd with the potant vaccine *(3)*. Similarly leukocytosis, heterophilia and lymphopenia were recorded from the 3rd to the 7th day post vaccination. besides transient lymphocytosis on the 1st and 2nd day post-vaccination in 31 day old chickens experimentally infected with virulent field isolate of IBDV via eye instillation *(6)*.

The hypoproteinemia, in gps.2 &3, at the ages of 29, 31 and 33 days may be due to the hypoalbuminemia. This may result from glomerular or liver damage *(19)*. The gps.1,2&3 did not show any significant changes in the α and β-globulin concentrations at the ages of 29, 31 ,33 and 43 days. No significant changes in the serum levels of α_1, α_2 and β-globulins were observed in 4 week old chicks *(6,14)*. The hypergammaglobulinemia in gps.1,2&3 at the

ages of 29, 31, 33 and 43 days may be attributed to the enhancing action of the virus on the B-cells to proliferate and produce the immunoglobulins *(12)*. The serum creatinine showed a highly significant increase at the ages of 29, 31 and 33 days in gps 2&3. The serum creatinine may become elevated in birds with renal damage *(3)*. Results has been proved by the histopathological findings which showed severe hemorrhage, increased hypercellularity of the glomerular tufts, necrosis in the epithelial lining of the renal tubules and congestion of the renal blood vessels with presence of hyaline casts inside the tubular lumens (in gps.2&3). A significant increase in the serum uric acid was encountered in gp.3 with 0.1 ml of IBDV at a titer of $10^{5.5}$ EID_{50} at the ages of 29& 31 days. Hyperurecemia, in birds, occurs with starvation, gout, massive tissue destruction and renal diseases *(3)*. The calcium level was significantly decreased, at the ages of 29 and 31 days in gps.2&3. The hypocalcaemia may be attributed to reduced renal calcium reabsorption, decreased calcium absorption from the intestine and/or hypoalbuminemia *(3)*. A highly significant increase was found in the phosphorus level of the gps.2&3 at the ages of 29,31and 33 days. The hyperphosphatemia may be due to a decrease in the serum calcium level. Moreover, the damaged kidneys are unable to eliminate the inorganic phosphorus *(3)*.

It could be concluded that the vaccination ameliorated the damaging effect of the disease, particularly on the liver, kidneys and bursa. Consequently, the signs, the leukogram and blood chemistry are improved, in the presence of mild side-effects of the vaccine.

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الملخص العربي

التأثيرات الباثولوجية الإ كلينيكية لمرض التهاب كيس فابريشيس المعدى على بدارى التسمين

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أظهرت الدراسات الباثولوجية الإ كلينيكية لمرض التهاب كيس فابريشيس المعدى على بدارى التسمين زيادة مؤقتة فى الخلايا الليمفاوية تلاها نقص فى هذه الخلايا و زيادة فى الخلايا متعادلة الصبغة كما وجد انخفاض فى نسبة البروتين الكلى والزلال فى الدم مع زيادة فى الجاما جلوبيولين بالإضافة إلى زيادة فى معدل الكرياتنين و حمض البوليك و الفسفور مع نقص فى مستوى الكالسيوم فى الدم. كما أوضحت الصفة التشريحية للأعضاء الداخلية وجود تغيرات فى الكبد و الكليتين والطحال و حافظة فابريشيس.