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Research Article
Scoring System for Lesions Induced by Different Strains of Infectious Bursal Disease Virus in Chicken

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Abstract

Background and Objective: There are 3 pathogenic strains of infectious bursal disease virus (IBDV), which are classical, variant and very virulent strains. The objective of this study was to design a scoring system for lesions induced by different strains of infectious bursal disease virus in chicken. **Materials and Methods:** Three experiments were conducted. In experiments 1 and 2, chickens were divided into infected and control groups. Infected groups of experiments 1 and 2 consisted of 15 and 24 specific pathogen-free (SPF) chickens, respectively. Infection was induced by oral administration of $10^{7.5}$ 50% EID_{50}/0.1 mL of very virulent IBDV (vvIBDV). Infected chickens in experiment 1 were euthanised by cervical dislocation on days 1, 2, 3, 4 and 5 post-inoculation (pi). Infected chickens in experiment 2 were euthanised at hours (h) 2, 4, 6, 12 and days 1, 2, 4 and 6 pi. Control groups in experiments 1 and 2 consisted of 6 and 15 SPF chickens, respectively. Chickens of the control group in experiment 1 were euthanised on days 1 and 5 pi, whereas control group chickens in experiment 2 were euthanised on days 0, 1, 2, 4 and 6 pi. Then, 20 SPF chickens, in experiment 3, were divided into three groups; in the first group, 10 SPF chickens were infected with vvIBDV, in the second group, 5 SPF chickens were infected with classical IBDV (caIBDV) ($10^{3.0}$ EID_{50}/0.1 mL) and the third group of chicken was kept as a control group without infection. Five chickens from first group, five chickens of second group and five chicken of the third group were euthanised on day 4 pi. Another 5 chickens from first group were euthanized on day 7 pi. In all previous experiments, tissues of bursa of Fabricius, caecal tonsil, liver, kidney, spleen, junction of proventriculus and gizzard, intestine, muscle and thymus were collected, fixed in 10% buffered formalin, embedded in paraffin and sectioned. HS staining was applied. **Results:** When the strain of the virus was vvIBDV, the scoring was always greater in comparison with the strain caIBDV. The lesions induced in the bursa of Fabricius by vvIBDV strain, when the infection was chronic, were determined. **Conclusion:** A scoring system for the lesions induced by different strains of IBDV in 9 different tissues was expected to be beneficial in the field of histopathology.

Key words: Pathogenic strains, lesions, gumboro disease, young chickens, classical strain

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Infectious bursal disease (IBD) (Gumboro disease) is an extremely contagious infection affecting young chickens[1]. Its clinical signs appears solely in chickens (*Gallus gallus*) although turkeys, ducks, guinea fowl and ostriches may be infected[2].

The causative agent of the disease is infectious bursal disease virus (IBDV). There are two recognized serotypes of the virus. These serotypes are designated as serotype 1 and serotype 2. Cross-neutralisation assays are useful in the differentiation of serotypes. All commercial vaccines are manufactured to provide protection against serotype 1, because clinical disease is only associated with it[3]. The IBD virus has three strains, which are classical, variant and very virulent strains[2]. In another classification, serotype 1 comprises 4 pathogenic strains which are classical virulent strain, attenuated strain, antigenic variant strain and very virulent strain. Whereas, serotype 2 is a nonpathogenic strain[4].

In general, the diagnosis of poultry diseases necessitates consideration of the flock's history, clinical signs, gross postmortem lesions and microscopic lesions. Then for the confirmation of diagnosis, serological or molecular techniques are recommended[5]. But in case of suspicion of IBD, the histological lesion examination is very helpful in the diagnosis for the characteristic picture of the disease under light microscopy[6-7]. The histopathology of IBD also helps in differentiation between the strains and in determination of the vaccine efficacy. For diagnosis of IBD, it is better to use scoring system during histopathological examination. Thus, the comparison between different strains, the severity of the infection and the vaccine efficacy could be evaluated more accurately. The objective of this study was to design a scoring system for lesions induced by different strains of IBDV.

MATERIALS AND METHODS

Ethical approval: The study was carried out in accordance with the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM).

Chickens: Specific pathogen free chickens (SPF) were used for 3 experiments. At the onset of every experiment, the chickens were 6 weeks old. The breed was white leghorn.

Virus: In the first and second experiments, the chickens were inoculated orally with very virulent IBDV strain (vvIBDV) with a dosage of 0.1 mL of $10^{7.5}$ EID_{50} mL^{-1} of vvIBDV (UPM0081 isolate). While, in the third experiment, the chickens were inoculated orally and intraocularly with 0.1 mL (10 4.83 EID_{50}/0.1 mL) of vvIBDV (UPM0081 isolate) for one group. Anothor group was inoculated orally and intraocularly with 0.1 mL (103.0 EID_{50}/0.1 mL) of classical strain (caIBDV) (V877 isolate).

Experimental design

Experiment 1: Fifteen SPF chickens were infected with vvIBDV by oral administration of 0.1 mL of $10^{7.5}$ EID_{50}/0.1 mL (UPM0081 isolate). Feed and water were provided *ad libitum*. Chickens were monitored every day for the record of the clinical signs and gross lesions. Every day, 3 chickens were sacrificed by cervical dislocation on days 1, 2, 3, 4 and 5 post-infection (pi). The third group of 6 chicken was kept as a control group without infection. The chickens of the control group were sacrificed by cervical dislocation on days 1 and 5 pi. Chickens of the infected and control groups were sacrificed and the tissues of bursa of Fabricius, caecal tonsils, intestine, liver, spleen, thymus, junction of proventriculus, gizzard and kidney were collected and fixed in 10% buffered formalin. Formalin-fixed paraffin-embedded tissues were prepared. Tissues were sectioned, stained with HE, examined under light microscope and changes were recorded.

Experiment 2: Twenty-four SPF chickens were infected with vvIBDV by oral administration of $10^{7.5}$ EID_{50}/0.1 mL of vvIBDV UPM0081 isolate. A second group, of 15 SPF chickens, was kept as a control. Infected chickens were sacrificed by cervical dislocation at hours (hrs) 2, 4, 6, 12, days 1, 2, 4 and 6 pi. Control group chickens were sacrificed on days 0, 1, 2, 4 and 6 pi and the tissues of bursa of Fabricius, caecal tonsil, liver, kidney, spleen, junction of proventriculus and gizzard, intestine, muscle and thymus were collected, fixed in 10% buffered formalin. Formalin-fixed paraffin-embedded tissues were prepared. Tissues were sectioned, stained with HE, examined under light microscope and changes were recorded.

Experiment 3: One group of 10 SPF chickens were infected with vvIBDV by oral and intraocular administration of $10^{4.83}$ EID_{50}/0.1 mL of UPM0081 isolate (very virulent strain). A second group of 5 SPF chickens was infected by oral and intraocular administration of V877 isolate (caIBDV) ($10^{3.0}$ EID_{50}/0.1 mL) (classical strain). A third group of 5 SPF chickens was kept as a control group without infection. Five chickens from first group, 5 chickens of second group and 5 chickens of the third group were sacrificed on day 4 pi. The tissues of bursa of Fabricius, caecal tonsil, liver, spleen, junction of proventriculus and gizzard, muscle, kidney and thymus were collected.

Five chickens from the first group were also euthanized on day 7 pi for collection of bursa of Fabricius for detection of lesions in case of chronic infection. The collected tissues were fixed in 10% buffered formalin. Formalin-fixed paraffin-embedded tissues were prepared. Tissues were sectioned, stained with HE, examined under light microscopy and changes were recorded.

Scoring system for infected tissues: Scoring system, for the lesions of IBDV-infected-tissues, was modified according to the criteria described by Henry *et al*.[8], Hair-Bejo *et al*[9]. and Abu Tabeekh and Al-Mayah[10] . Briefly, in the bursa of Fabricius, caecal tonsil, thymus and kidney tissues, lesions were scored in 6 grades as 0 (normal), 1 (mild), 2 (mild to moderate), 3 (moderate), 4 (moderate to severe) and 5 (severe). In the junction of proventriculus and gizzard, the lesions were scored in 5 grades, which were 0, 1, 2, 3 and 4. In the liver, spleen and muscle tissues, the lesions were scored in 4 grades, which were 0, 1, 2 and 3. The procedure to score lesions in a tissue was described by Gaweco *et al*.[11]. Five random optical fields were examined, scored and then the mean of five fields was calculated. The mean for 3 tissues ± standard error of the mean (SEM) was determined using SPSS.

Bursa of fabricius: 0 = normal, 1 = Medullary area in the lymphoid follicles showed mild degeneration and necrosis, 2 = Mild to moderate degeneration and necrosis in lymphoid cells of follicles in particular in medulla. Oedema and inflammatory cells infiltration were clear in interstitial connective tissue, 3 = Moderate necrosis. Cortex and medulla were involved. Follicles also showed scattering of pyknotic nuclei. Heterophils, macrophages, few red blood corpuscles and fibroblasts were also apparent in the interstitial space. Epithelial layer was thick and vacuolated in some parts, 4 = Follicles suffered from moderate to severe lymphoid cells depletion. Lymphoid cells aggregate in the cortex. In the medulla there were cysts and cells with necrosis. Inflammatory cells infiltrated the interstitial area which was piled with fibrous tissue. Hyperemia and haemorrhage were observed in intrafollicular and extrafollicular regions. Thickening, corrugation and vacuolation were seen in epithelial part, 5 = when it was acute infection: follicles revealed moderate to severe atrophy. Cellular degeneration and necrosis occurred in cortex and medulla. An observation of cysts filled with fibrous exudates debris of cells was clear. Inflammatory cells infiltration, oedema appeared in the interstitial tissue. Epithelium of bursa exposed thickening and it was vacuolated. Or 5 = In case of chronic infection: follicles suffer from moderate to severe atrophy, degeneration, necrosis and sometimes cysts. Infiltration of inflammatory cells and fibroblast formation in interstitial tissue, was obvious.

Junction of proventriculus and gizzard: 0 = Normal, 1 = Mild increase in lymphoid cell with some degeneration, 2 = Moderate increase in lymphoid cell with some degeneration and necrosis, 3 = Severe increase in lymphoid cell and necrosis, 4 = Severe increase in lymphoid cell, necrosis and haemorrhage.

Caecal tonsil: 0 = Normal, 1 = Lymphoid cell aggregation suffers from mild degeneration and necrosis, 2 = Lymphoid cells suffer from mild to moderate degeneration and necrosis, 3 = Lymphoid cells suffer from moderate degeneration and necrosis. Scattering of pyknotic nuclei in lymphoid cell aggregation, 4 = Lymphoid cell suffer from moderate to severe depletion. Appearance of pyknotic nuclei and haemorrhage, 5 = Lymphoid cells suffer from severe degeneration and necrosis and presence of haemorrhage.

Thymus: 0 = Normal, 1 = Cortex and medulla suffer from mild degeneration and necrosis in limited regions, 2 = Cortex and medulla suffer from mild to moderate degeneration and necrosis, 3 = Medulla suffers from moderate aggregation of debris cells, pyknotic nuclei and necrosis, 4 = Cortex and medulla suffer from moderate to severe sloughing. Vacuoles formation, 5 = Degeneration and necrosis are severe. Cortex and medulla lose morphology. Reduced size of medulla. Lots of vacuoles.

Kidney: 0 = Kidney and glomeruli appear normal. Tubules are attached together, 1 = Epithelial cells in tubules suffer from mild degeneration and necrosis. Mild proliferation in some glomeruli, 2 = Epithelial cells in tubules suffer from mild to moderate degeneration and necrosis. Glomeruli undergo atrophy, 3 = Moderate tubular degeneration and necrosis. Glomeruli undergo atrophy. Tubules and collecting ducts suffer from partial epithelial detachment, 4 = Some tubules suffer from moderate to severe epithelial sloughing and spread necrosis. Degeneration and necrosis in collecting ducts. Glomeruli undergo atrophy and detachment of tubular epithelium from basement membrane, 5 = Severe eodema between tubules and collecting ducts. Tubular epithelium suffers from fragmentation and sloughing from basement membrane. Atrophy of glomeruli with pyknotic cells.

Spleen: 0 = Normal, 1 = Observation of heterophil infiltration in the sinuses, 2 = Germinal centres undergo haemorrhage

and heterophil infiltration. Round aggregations of eosinophilic material scattered throughout and aggregates of dark staining nuclei near the capsule, 3 = Hyperaemia in the sinuses. Germinal centres infiltrated by heterophils and surrounded by round aggregations of eosinophilic material. Cell debris, pyknotic nuclei and eosinophilic material are also included in germinal centres.

Liver: 0 = Normal, 1 = Interstitial lymphocytic foci, 2 = Congestion and fatty degeneration, 3 = Hepatocellular necrosis.

Muscle: 0 = Normal, 1 = Mild appearance of haemorrhage, 2 = Scattered patches of haemorrhage, 3 = Large patches of haemorrhage.

Intestine: 0 = Normal, 1 = Mild to moderate infiltration of lymphocytic cells, 2 = Moderate degeneration of epithelial cell beside infiltration of mononuclear inflammatory cells usually in submucosal areas, 3 = Haemorrhagic foci accompanied by necrosis in mucosal lymphoid tissue.

RESULTS

Experiment 1: The bursa of Fabricius, thymus, caecal tonsil and kidney tissues had a scoring system of 6 grade (0-5). On day 1 pi, the scoring was more than 1. The scoring in the bursa of Fabricius, thymus and the caecal tonsil was 1.60±0.12, 1.40±0.12 and 1.27±0.07 respectively. On days 2, 3, 4 and 5, the scoring was higher in the bursa of Fabricius with relatively slight difference in thymus and relatively great difference in the caecal tonsil. In the kidney, lesions were not detected in tissue (Table 1). In the junction of proventriculus and gizzard tissue, which had scoring system of 5 grades (0-4), the scoring was 1.20±0.00 on day 1 pi. It increased gradually to reach 2.73±0.07 on day 5 pi (Table 2). Liver, spleen and muscle tissues had scoring system of 4 grades (0-3). On days 1, 2 and 3 pi, the scoring was higher in the liver and on days 4 and 5 pi, scoring of both liver and spleen was close to each other. But in muscle tissue, lesions were not detected. Also, lesions were not detected in tissues of control group, hence, 0 score was recorded (Table 3). It was worth noting that the lesions induced in one certain tissue were different from the lesions induced in other tissues.

Experiment 2

Appearance of lesions in vvIBDV-infected-tissues from hr 2 till day 6 pi: Lesions were recorded at successive times after examination of tissues. At hrs 2 and 4 pi, the lesions were not detected. At hr 6 pi, the lesions were detected in 3/3 tissues of junction of proventriculus and gizzard, intestine, caecal tonsil, thymus and spleen. At hr 12 pi, the lesions were recorded in

Table 1: Scoring of lesions (0-5) detected after HE staining in bursa of Fabricius, thymus, caecal tonsil and kidney tissues on days 1. 2, 3, 4 and 5 pi of vvIBDV

	Bursa of fabricius	Thymus	Caecal tonsil	Kidney
Day 1 pi	1.60±0.12 (3)	1.40±0.12 (3)	1.27±0.07 (3)	0.00±0.00 (3)
Day 2 pi	2.53±0.07 (3)	2.20±0.00 (3)	1.53±0.18 (3)	0.00±0.00 (3)
Day 3 pi	3.40±0.00 (3)	3.33±0.07 (3)	2.20±0.00 (3)	0.00±0.00 (3)
Day 4 pi	4.33±0.07 (3)	3.60±0.12 (3)	2.47±0.07 (3)	0.00±0.00 (3)
Day 5 pi	4.47±0.07 (3)	4.33±0.07 (3)	2.53±0.07 (3)	0.00±0.00 (3)

Scoring was expressed as Mean±SEM. Numbers in brackets indicate number of chickens. pi: Post-inoculation of virus. No lesions were detected in tissues of control group

Table 2: Scoring of lesions (0-4) detected after HE staining in junction of proventriculus and gizzard tissue on days 1. 2, 3, 4 and 5 pi of vvIBDV

	Junction of proventriculus and gizzard
Day 1 pi	1.20±0.00 (3)
Day 2 pi	1.27±0.07 (3)
Day 3 pi	2.33±0.07 (3)
Day 4 pi	2.47±0.07 (3)
Day 5 pi	2.73±0.07 (3)

Lesion scoring was expressed as Mean±SEM. Numbers in brackets indicate number of chickens. Lesions were not detected in tissues of control group. Scoring of 0 was recorded. pi indicates post-inoculation of virus

Table 3: Scoring of lesions (0-3) detected after HE staining of liver, spleen and muscle tissues on days 1, 2, 3, 4 and 5 pi of vvIBDV

	Liver	Spleen	Muscle
Day 1 pi	0.60±0.12 (3)	0.00±0.00 (3)	0.00±0.00 (3)
Day 2 pi	1.13±0.07 (3)	0.47±0.07 (3)	0.00±0.00 (3)
Day 3 pi	1.27±0.07 (3)	0.60±0.12 (3)	0.00±0.00 (3)
Day 4 pi	1.27±0.07 (3)	1.27±0.07 (3)	0.00±0.00 (3)
Day 5 pi	1.47±0.07 (3)	1.53±0.07 (3)	0.00±0.00 (3)

Lesion scoring was expressed as Mean±SEM. Numbers in brackets indicate number of chickens. Lesions were not detected in tissues of control group. pi: Post-inoculation of virus

previously mentioned tissues, beside (3/3) in bursa of Fabricius and (1/3) in liver. On day 1 pi, all tissues were (3/3) positive apart from kidney and muscle tissues which were not penetrated by lesions and so forth till day 6 pi (Table 4). Lesions were not induced in control group tissues.

Numbers and percentages of different vvIBDV-infected tissues manifesting lesions: From hr 2 pi till day 6 pi, among 24 infected chickens, different collected tissues were stained with HE and examined; the lesions appeared in 18 out of 24 caecal tonsil tissues (75.00%), 18 out of 24 junction of proventriculus and gizzard tissues (75.00%), 18 out of 24 intestine tissues (75.00%), 18 out of 24 spleen tissues (75.00%), 18 out of 24 thymus tissues (75.00%), 15 out of 24 bursa of Fabricius tissues (62.50%),13 out of 24 liver tissues (54.17%), 0 out of 24 kidney tissues (0.00%), 0 out of 24 muscle tissues (0.00%) (Table 5). The lesions were not detected in control tissues.

Lesions scoring (0-5) of different HE stained tissues since hr 2 till day 6 pi: During hrs 2 and 4 pi, lesions were not detected in the bursa of Fabricius, caecal tonsil, thymus and kidney tissues which had the scoring system of 6 grades (0-5). At hr 6 pi, the scoring was the highest in caecal tonsil (0.40±0.12) and then in thymus (0.20±0.00). At hr 12 pi and onwards, bursa of Fabricius always obtained the highest scoring. It increased gradually till day 6 pi to be 5.00±0.00 (bursa of Fabricius) followed by 2.73±0.07 (caecal tonsil) and 2.67±0.07 (thymus) (Table 6). In the junction of proventriculus and gizzard which had a scoring system of 5 grades (0-5), the lesions were not detected at hr 2 and 4 pi. At hr 6 pi, scoring was 0.53±0.07 and then it increased gradually to become 3.20± 0.12 on day 6 pi (Table 7). Among the liver, spleen, intestine and muscle tissues which had scoring system of 4 grades (0-3), at hr 2 and 4 pi, the lesions were not induced. At hrs 6, 12, days 1, 2, 4 and 6 pi, the scoring was always the highest in the spleen tissue. It was 2.47±0.18 on day 6 pi (Table 8).

Table 4: Appearance of lesions induced by vvIBDV in different HE-stained tissues from hr 2 till day 6 pi

Oral inoculation of vvIBDV	Number of tissues, out of 3 examined tissues, that underwent lesion induction after application of HE staining
Hour 2 pi	No lesion was detected
Hour 4 pi	No lesion was detected
Hour 6 pi	(3/3) in junction of proventriculus and gizzard, intestine, caecal tonsil, thymus and spleen
Hour 12 pi	(3/3) in bursa of Fabricius, junction of proventriculus and gizzard, intestine, caecal tonsil, thymus and spleen (1/3) in liver
Day 1 pi	(3/3) in bursa of Fabricius, junction of proventriculus and gizzard, intestine, caecal tonsil, thymus, liver and spleen
Day 2 pi	(3/3) in bursa of Fabricius, junction of proventriculus and gizzard, intestine, caecal tonsil, thymus, liver and spleen
Day 4 pi	(3/3) in bursa of Fabricius, junction of proventriculus and gizzard, intestine, caecal tonsil, thymus, liver and spleen
Day 6 pi	(3/3) in bursa of Fabricius, junction of proventriculus and gizzard, intestine, caecal tonsil, thymus, liver and spleen

Values in the brackets before slash (/) indicate number of a certain tissue in which lesions were detected. While, values between brackets after slash (/) indicate whole number of a certain tissue collected from chicken pi of vvIBDV. Every time (hr 2 pi, hr 4 pi, hr 6 pi…etc.), 3 tissues of bursa of Fabricius, 3 tissues of caecal tonsil and 3 tissues of liver…etc. were collected. Then HE staining was applied. Lesions were not induced in control group tissues

Table 5: Numbers and percentages of different vvIBDV-infected tissues stained with HE in which lesions appeared from hr 2 pi till day 6 pi

	Caecal tonsil	Junction of proventriculus and gizzard	Intestine	Spleen	
No. of positive tissues*	18 (24)	18 (24)	18 (24)	18 (24)	
Percentage of positive tissues*	75.00%	75.00%	75.00%	75.00%	
	Thymus	Bursa of fabricius	Liver	Kidney	Muscle
No. of positive tissues*	18 (24)	15 (24)	13 (24)	0 (24)	0 (24)
Percentage of positive tissues*	75.00%	62.50%	54.17%	0.00%	0.00%

Values in brackets "()" represents whole number of SPF chickens which were infected with vvIBDV from hour 2 pi till day 6 pi. Positive tissue* indicates tissue in which lesions appeared. Lesions were not induced in control group tissues

Table 6: Lesions scoring (0-5) of HE stained tissues of bursa of Fabricius, caecal tonsil, thymus, and kidney collected from SPF chickens inoculated with vvIBDV

	Hour 2 pi	Hour 4 pi	Hour 6 pi	Hour 12 pi	Day 1 pi	Day 2 pi	Day 4 pi	Day 6 pi
Bursa	0.00±0.00 (3)	0.00±0.00 (3)	0.00±0.00(3)	0.80±0.12(3)	1.33±0.07(3)	2.87±0.18(3)	4.27±0.07 (3)	5.00±0.00 (3)
C tonsil	0.00±0.00 (3)	0.00±0.00 (3)	0.40±0.12(3)	0.73±0.07(3)	1.00±0.12(3)	1.47±0.07(3)	2.27±0.07 (3)	2.73±0.07 (3)
Thymus	0.00±0.00(3)	0.00±0.00(3)	0.20±0.00(3)	0.27±0.07(3)	0.87±0.07(3)	1.27±0.07(3)	2.13±0.07 (3)	2.67±0.07 (3)
Kidney	0.00±0.00 (3)	0.00±0.00 (3)	0.00±0.00 (3)	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00(3)

pi means post-inoculation of virus. Values in the brackets "( )" indicate number of chickens. Bursa means bursa of Fabricius, C Tonsil means caecal tonsil. Control group tissues were scored 0

Table 7: Lesions scoring (0-4) of HE stained tissues of junction of proventriculus and gizzard collected from SPF chickens inoculated with vvIBDV

	Hour 2 pi	Hour 4 pi	Hour 6 pi	Hour 12 pi	Day 1 pi	Day 2 pi	Day 4 pi	Day 6 pi
junction of proventriculus and gizzard	0.00±0.00(3)	0.00±0.00 (3)	0.53±0.07 (3)	0.93±0.07(3)	1.20±0.12 (3)	1.20±0.12 (3)	2.40±0.12 (3)	3.20±0.12 (3)

pi: Post-inoculation of virus. Values in the brackets "( )" indicate number of chickens. Control group tissues were scored 0

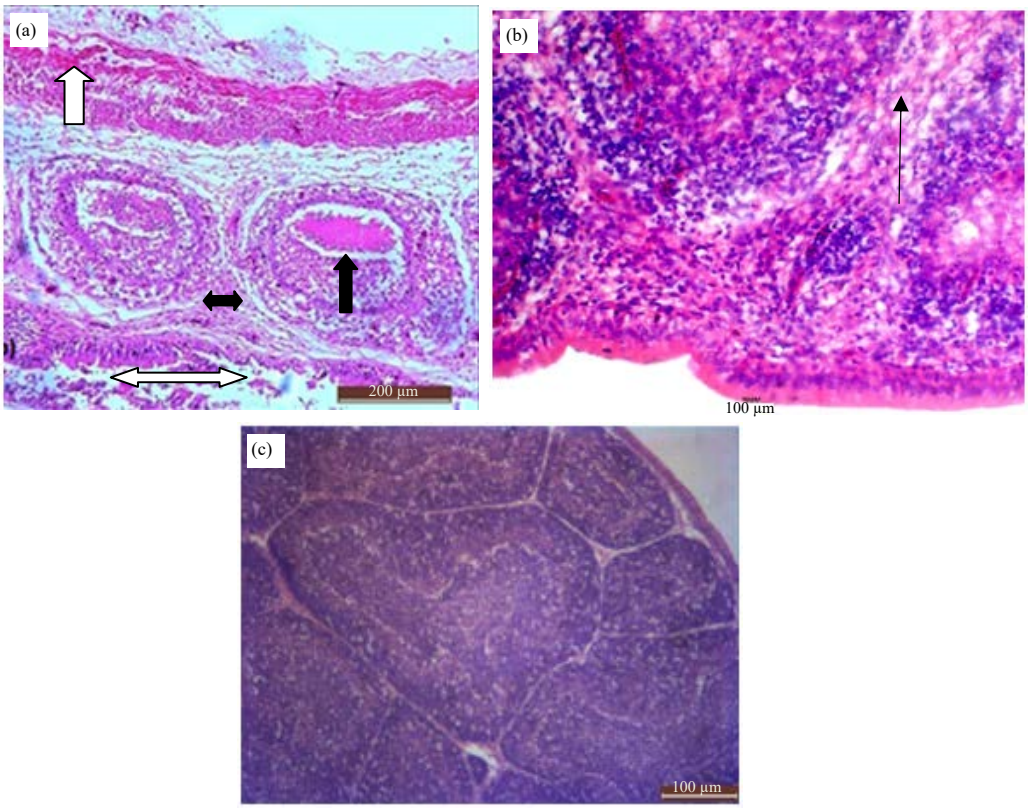

Fig. 1 (a-c): Bursa of Fabricius of SPF chickens. (a) Day 4 pi of vvIBDV, acute infection, severe depletion of lymphoid cells in follicles, necrotic cells and cysts in follicles (black arrow), infiltration of inflammatory cells and fibrous connective tissue in interstitial space (black double arrow), haemorrhage (white arrow), folded and thickening of epithelium (white double arrow) lesion scoring of 5.00. scale bar: 200 µm, (b) Day 7 pi, chronic infection, inflammatory cell infiltration, fibroblast formation (arrow), absence of cysts, lesion scoring of 5. Scale bar: 100 µm, (c) Control group, lesion scoring of 0. Scale bar: 50 µm. HE

Table 8: Lesions scoring (0-3) of HE stained tissues of liver, spleen, intestine and muscle collected from SPF chickens inoculated with vvIBDV

	Hour 2 pi	Hour 4 pi	Hour 6 pi	Hour 12 pi	Day 1 pi	Day 2 pi	Day 4 pi	Day 6 pi
Liver	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00(3)	0.33±0.33(3)	0.47±0.07(3)	0.33±0.07(3)	0.73±0.07(3)	2.00±0.23 (3)
Spleen	0.00±0.00(3)	0.00±0.00(3)	0.33±0.07 (3)	0.47±0.07(3)	0.80±0.12(3)	1.40±0.12(3)	2.07±0.07(3)	2.47±0.18 (3)
Intestine	0.00±0.00(3)	0.00±0.00(3)	0.27±0.07 (3)	0.33±0.07(3)	0.33±0.07(3)	0.47±0.07(3)	1.27±0.07(3)	2.33±0.24 (3)
Muscle	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00 (3)	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00(3)	0.00±0.00 (3)

pi: Post-inoculation of virus. Values in the brackets "( )" indicate number of chickens. Control group tissues were scored 0

Among all tissues, bursa of Fabricius was single to obtain full scores (5.00±0.00) on day 6 pi. The lesions were not detected in the tissues of control group. And 0 score was recorded. It was worth noting that lesions induced in certain tissue were different from lesions induced in other tissues. (Table 6-8).

Experiment 3

Comparison of vvIBDV-infected tissues with caIBDV-infected tissues: In the group of bursa of Fabricius, caecal tonsil, thymus and kidney tissues, which had a scoring system composed of 6 grades (0-5), the scoring of lesions, induced by vvIBDV, was always greater than 3. While, the scoring of lesions, induced by caIBDV, was always less than 2. The lesions were not detected in the control tissues (Table 9). Lesions were different between acute and chronic infection in the bursa of Fabricius tissue (Fig. 1). It was worth noting that the lesions induced in one tissue were different from the lesions induced in other tissues.

In the tissue of junction of proventriculus and gizzard which had a scoring system composed of 5 grades (0-4), when the strain was vvIBDV, the scoring was 4.00 ± 00. While,

Table 9: Lesions scoring (0-5) induced by very virulent and classical IBDV strains in HE stained bursa of Fabricius, caecal tonsil, thymus and kidney tissues on day 4 pi

	vvIBDV	calBDV	Control
Bursa of Fabricius	Acute* 5.00±00 (5)	1.84±0.41 (5)	00.0±00 (5)
	Chronic** 5.00±00 (5)		
C Tonsil	4.16±0.19 (5)	0.67±0.12 (5)	00.0±00 (5)
Thymus	3.24±0.17 (5)	1.80±0.20 (5)	00.0±00 (5)
Kidney	3.36±0.52 (5)	0.60±0.13 (5)	0.0±00 (5)

*Bursa of Fabricius was examined when infection was acute on day 4 pi. **Bursa of Fabricius was examined when infection was chronic on day 7 pi. Values between brackets indicate the number of examined tissues, vvIBDV indicate very virulent strain, calBDV indicates classical strain

Table 10: Lesions scoring (0-4) induced by very virulent and classical IBDV strains in HE stained junction of proventriculus and gizzard tissues on day 4 pi

	vvIBDV	calBDV	Control
junction of proventriculus and gizzard	4.00±00 (5)	1.40±0.17 (5)	00±00 (5)

Values between brackets indicate the number of examined tissues, vvIBDV: Very virulent strain, calBDV: Classical strain

Table 11: Lesions scoring (0-3) induced by very virulent and classical IBDV strains in HE stained liver, spleen and muscle tissues on day 4 pi

	vvIBDV	calBDV	Control
Liver	1.80±0.50 (5)	0.80±0.26 (5)	00±00 (5)
Spleen	3.00±0.00 (5)	1.60±0.41 (5)	00±00 (5)
Muscle	0.00±0.00 (3)	0.00±0.00 (3)	00±00 (3)

Values between brackets indicate the number of examined tissues

when the strain was calBDV, the scoring was less than 2. The lesions induced in the control tissues were 00±0 (Table 9).

In the tissues of liver, spleen and muscle, which had a scoring system composed of 4 grades (0-3), the scoring of lesions induced by vvIBDV, was always greater than the scoring of lesions induced by calBDV, in liver and spleen tissues. The lesions were not detected in the muscle tissues. The lesions were not detected in the control tissues, too. It was worth noting that lesions induced in a certain tissue were different from lesions induced in other tissues (Table 11).

DISCUSSION

The present study was different from previous ones because it comprised 9 different tissues. However, in a previous study, Henry *et al*.[8] determined the scoring system for lesion for 6 different tissues. While, Hair-Bejo *et al*.[9]; Abu Tabeekh and Al-Mayah[10] applied the lesion scoring technique only on the tissue of bursa of Fabricius. A novelty was implicated in this study. It was the first study which determined the lesions induced in the bursa of Fabricius when the infection was chronic (7 days or more than 7 post-infection). Moreover, in the scoring system of Henry *et al*.[8], the scores ranged from 0-3 for the thymus and kidney. In this study, the scores ranged from 0-5 for the thymus and kidney. Also, in the study of Abu Tabeekh and Al-Mayah[10], score 6 was added to the lesions induced in the bursa of Fabricius tissue when the atrophy of the follicles was very severe till the follicles disappeared.

The score of lesions, when the strain was very virulent IBDV, in the bursa of Fabricius tissue collected on the last days of experiments 1, 2 and 3 in this study were 4.47±0.07, 5.00±0.00 and 5.00±0.00. However, Hair-Bejo *et al*.[9] found that the score was 5 on day 7 post-infection with UPM 94283 isolate. The score in the bursa of Fabricius in the present study, when the chickens were infected with calBDV, was 1.84±0.41. However, it was 2-3, when embryonated broiler eggs were inoculated with *in ovo* IBD vaccine (UPM 93273) with different doses. But in the study of Abu Tabeekh and Al-Mayah[10], the lesions were not detected till day 14 post-vaccination with some IBDV vaccines [an intermediate (Bursine®-2) and an intermediate-plus vaccine (Bursine® Plus of Fort Dodge Animal Health, Fort Dodge, Iowa, USA)].

In the present study, some lesions were detected in the liver, spleen, thymus and caecal tonsil tissues when chickens were infected with classical strain. However, Ignjatovic[12] mentioned that the microscopic lesions were almost absent in classical or variant IBDV infection. In the present study, the haemorrhage was not detected in both gross and microscopic examination of muscle tissues. But haemorrhage cases were observed in 1% of IBDV infected chickens in field cases. However, experimentally, it has no significance[1,9].

The findings of experiment 2 were helpful in understanding the pathogenesis of IBD and the tissue tropism of the causative agent. It seemed that after oral inoculation of vvIBDV, the virus was transported from the oral cavity to the junction of proventriculus and gizzard, intestine and caecal tonsil. Probably it was transported via digestive tract lumen through direct contact or it might be transported via the viraemia. Then probably it reached the spleen, thymus and the bursa of Fabrius. After inoculation, the tissues that were collected earlier showed lower lesion score. While the tissues that were collected later, showed higher lesion score.

CONCLUSION

A scoring system was designed for the lesions induced by different strains of IBDV and, accordingly, the lesions were

scored. It was the first study which determine the lesions induced in the bursa of fabricius tissue in case of chronic infection of vvIBDV.

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