

*Full Length Research Paper*

## Bursa body index as a visual indicator for the assessment of bursa of Fabricius

A. A. Raji<sup>1\*</sup>, B. Mohammed<sup>2</sup>, S. B. Oladele<sup>2</sup>, L. Saidu<sup>3</sup>, A. H. Jibril<sup>4</sup> and C. Cazaban<sup>5</sup>

<sup>1</sup>Department of Veterinary Pathology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>2</sup>Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

<sup>3</sup>Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Nigeria.

<sup>4</sup>Department of Veterinary Physiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>5</sup>Innovation Strategy Department, Ceva Santé Animale, France.

Received 20 January, 2016; Accepted 11 July, 2016

The objective of the present study was to identify the significance of the Bursal-Body index (BB index) as a tool in assessing the health status of a chicken flock especially those under experimental condition. With the unending debate on the bursa of Fabricius as a diagnostic tool, a total of 135 day-of-hatch Dominant black Cockerel hybrid were housed in six separate houses with positive pressure and filtered airflow. Houses were assigned to the three vaccines (two intermediate (A, and C), and one intermediate plus (B) vaccine strain), a challenge group (D) and control group (E). The birds were vaccinated according to manufacturer's directives on day 17<sup>th</sup> post hatch; challenge group was inoculated with 0.05 ml of very-virulent isolate of the infectious bursal disease virus (vvIBDV) on the same day. On day(s) 1, 2, 3, 4, 5, 7, 10, 14 and 21 post vaccination/challenge (Dpv/c) three birds were humanely sacrificed from each group with Bursal-Body ratio (BB ratio) and Bursal-Body index (BB index) recorded. The values (>0.7, that is, no atrophy (mild vaccine); 0.3-0.7 (relative or transient atrophy (for intermediate or intermediate plus vaccine) and <0.3 for a strong atrophy (hot vaccine or infection with vvIBDV) were recorded conforming to the first described standard, and there was not a statistical difference ( $p>0.05$ ) observed between the groups. This indicating that BB index could be used as a tool in assessing the health status of a flock.

**Key words:** Avian, Bursa of Fabricius, bursal-body index (BB index), infectious bursal disease (IBD).

### INTRODUCTION

The bursa of Fabricius (BF) is an organ unique to birds. It BF undergoes striking changes in size during development, growing rapidly during late embryogenesis and for several weeks (8 to 10 weeks) after hatching before regressing in sexually matured adult chicken at age of 6 to 7 months (Fang and Peng, 2014; Olah and

Vervelde, 2012; Olah et al., 2014; Schat and Skinner, 2014). As a primary lymphoid organ, it plays a key role in the differentiation of B-lymphocytes (Cazaban et al., 2015:11). Stress related situations, that is, too low or too high temperature, too much or too little ventilation, other diseases (Marek's, Chicken infectious anaemia (CIA),

\*Corresponding author. E-mail: [abdullahi.raji@udusok.edu.ng](mailto:abdullahi.raji@udusok.edu.ng). Tel: 002348036354238.

infectious bursal disease (IBD), Newcastle disease and Avian influenza) (Jungbaeck and Nutolo, 2001), mycotoxins, management system (deep litter or battery cage) can directly impact BF size (van Herdeen et al., 2011).

With the absence of an ideal standard for the bursal size, it is difficult to evaluate and interpret bursa weight under field condition (Cazaban and Gardin, 2012). Although, Glick (1956) and Wolfe et al. (1962) addressed (BF) size and development in meat type and egg type chickens genetic lines kept in good "normal" conditions and considering bursa free from any infection, sex, age and husbandry influences in the bursa weight and Bursa Body weight ratio (B:B ratio), they fail to issue standards (van Herdeen et al., 2011). With a minimum bursa-to-body weight ratio standard of 0.11 proposed for broilers from 7 to 42 days of age by Cazaban et al. (2015), there is the need to update and device means of standardizing this published standard considering the genetic selection in the poultry today (Cazaban and Gardin, 2012).

The objective of the study was to evaluate the changes in the bursa using BB index, a method developed to overcome the shortfalls of the earlier protocols used in assessing the bursa in some available vaccines in Nigeria and a Nigerian field isolate of the very virulent infectious bursal disease virus (vvIBDV) in cockerels.

## MATERIALS AND METHODS

### Birds

Two hundred (200) Dominant-Black hybrid commercial day-old cockerels were sourced from reputable hatchery (Terudee Hatchery, Oyo State, Nigeria). The chicks were hatched on the same day and came from the same breeder flock. The breeder flock was vaccinated against infectious bursal disease (IBD) with an intermediate live IBD vaccine at 4 and 10 weeks and inactivated IBD vaccine at 16 weeks. After quality sorting on arrival at the poultry experiment pens of the Avian medicine unit of Ahmadu Bello University, Zaria, and a total of 135 one-day-old chicks were used in the study.

### Housing

The birds were housed in the standard avian research facility that mimics field conditions. The birds were placed in a 30 m<sup>2</sup> naturally ventilated. They were raised in the 30 m<sup>2</sup> houses for 17 days (rearing period), and then, from 17<sup>th</sup> day of age onward they distributed into 5 houses at 3 birds/m<sup>2</sup>. They were fed starter from 1 to 21 days of age and grower-finisher feed from 22 days to the end of the study (38 days). Standard lighting period was observed throughout the period of the study.

### Vaccination and medication

As a tradition of the hatchery, the chicks were vaccinated against Marek's disease at the hatchery via subcutaneous injection and were vaccinated against infectious bronchitis by aerosol spray. Coccidiostat was incorporated in the grower feed. No other medications were given for the remainder days of the study.

## Experimental design and sampling

Guideline protocols of the Avian Medicine Unit standard research facility of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria was strictly adhered to. Observatory procedure of clinical signs and mortality was carried out daily throughout the period of the study. One hundred and thirty five (135) cockerels were randomly divided into five groups, with each group containing 27 birds each. Each of the group received different treatment; three were vaccinated with 2 intermediate vaccines, and 1 intermediate plus vaccine of the IBDV. The two remaining groups received 0.05 ml of vvIBDV isolate and 0.05 ml of distilled water respectively. The groups were housed in different houses and assigned A, B and C for the vaccinated (A, and C for intermediate and B for intermediate plus); D for the challenge and E for control groups. A total of 27 cockerels, 3 from each group were randomly sampled on day(s) 1, 2, 3, 4, 5, 7, 10, 14 and 21 post vaccination or challenged as follows: Necropsies were conducted at the avian pathology necropsy room of the veterinary pathology department postmortem room of Ahmadu Bello University, Zaria where all the measurements were taken.

Body weight and bursa weight were used to calculate the bursa-body (BB) ratio according to the following formula:

$$\text{BB ratio} = [\text{bursa weight (g)}/\text{body weight (g)}] \times 1000$$

Bursa-body (BB) index was calculated according to the following formula:

$$\text{BB index} = \text{BB ratio of infected (or vaccinated) birds} / \text{BB ratio of the controls}$$

Each of the BF was cut longitudinally into 2 parts and placed in a 20 ml bottle containing 10% neutral buffered formalin for histopathology and histopathological lesion score using Williams and Davison (2010) criteria with a scoring range of 0 to 5 (0, normal bursal follicle architecture and 5, complete loss of bursal).

## Statistical analysis

BB indexes were subjected to statistical analysis between the sampling groups over the period of study using the ANOVA test at a confidence level of 5% ( $P = 0.05$ ). Tukeys post hoc test was employed to determine differences between groups.

## RESULTS

### Clinical signs and mortality

Clinical signs were recorded in the challenge (infected) group at day one post challenge (1Dpc). Severe depression, ruffled feathers, anorexia and diarrhoea, characterised by whitish colour were observed in birds in the challenge group, with an increase in birds exhibiting clinical signs. Mortality began on 3Dpc, peaked on 4Dpc, and declined on 5Dpc. At 6Dpc, recovery was observed.

### Lesions of the BF

Gross lesions were observed as early as 1Dpc in the challenge group. Slight oedema, hyperaemia and gelatinous yellowish transudate covering the serosal



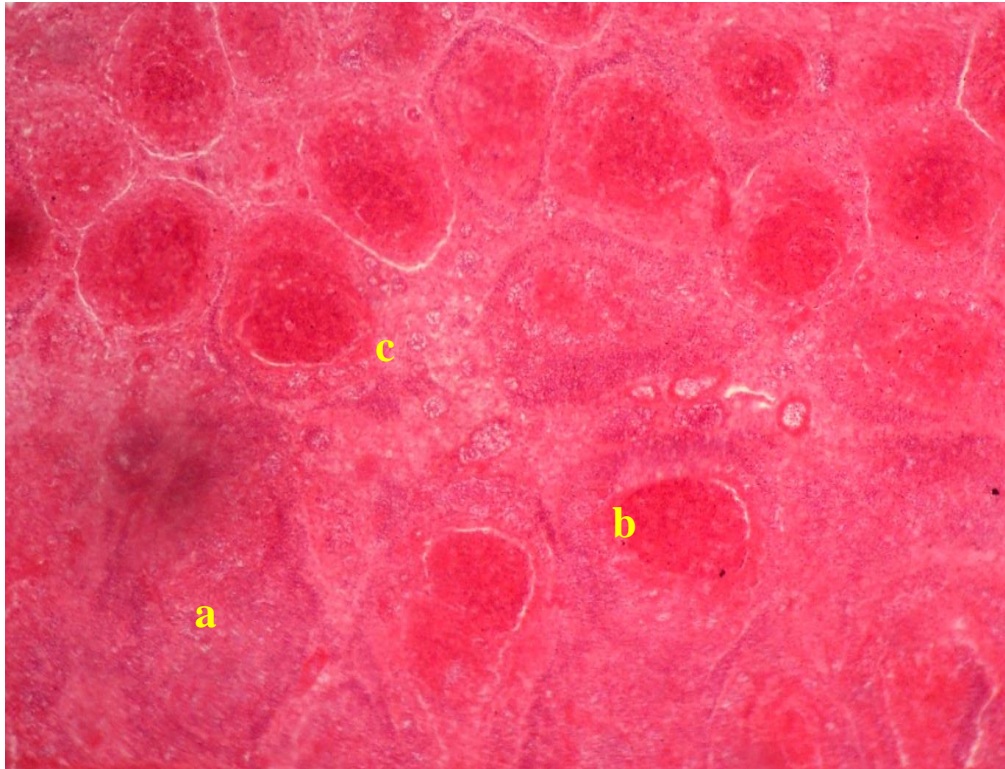
**Figure 1.** Severe bursal haemorrhage (white arrow) 3 Dpv/c in the challenge group.



**Figure 2.** Severe petechial and ecchymotic haemorrhages on the breast and thigh muscle (white arrows) 3 Dpv/c in the challenge group.

surface; on mucosal surfaces, the BF as well as the thigh and breast muscle showed mild petechial haemorrhages. The thymuses were also haemorrhagic on 1Dpc. Bursal haemorrhages; ecchymotic haemorrhages and severe BF congestion (Figure 1) were observed at 3Dpc in the

sacrificed and dead birds. At 5 Dpc, mild haemorrhages were observed in the bursae and thigh (Figure 2) of both sacrificed and dead birds, the spleen was also enlarged. The bursae have atrophied to about 1/3 of its size when compared with the control. No relevant gross pathology



**Figure 3.** Bursa of Fabricius from Challenge group 5 Dpv/c; lymphoid follicular necrosis (a); Follicular focal haemorrhages (b); marked oedema in the interstitial connective tissue (c). H&E stain  $\times 200$ .

was observed in birds in the control group.

Microscopically, lymphocytic depletion and haemorrhagic interfollicular interstitium in the medullary areas of the bursal follicles at 1Dpc were observed in the challenge group. Groups B and C presented haemorrhagic follicles at day-one post vaccination (1Dpv) (Figure 3). On days-three, post vaccination and challenge (3Dpv/c), challenge (D) group and group A presented necrosis and depletion of lymphocytes in the follicular medullar and cortex. At 5 Dpv/c, lymphoid necrosis was also observed in the challenge group, progressing to areas of coagulative necrosis within the follicles forming cystic areas (Figure 4) on 21Dpc. Moderate to marked atrophy of BF was observed in groups C and A respectively. Histopathologic lesions scores observed in the challenge group ranged from 4 to 5.

#### **Morphometric of the bursa of Fabricius**

Although the mean BF weight increased as birds grew older, however, the vaccinated groups were not able to reduce the bursal weight significantly when compared to the challenge and control groups. However, the challenge group when compared to the control group presented a significant variation in the BF weight on days 10, 14, and

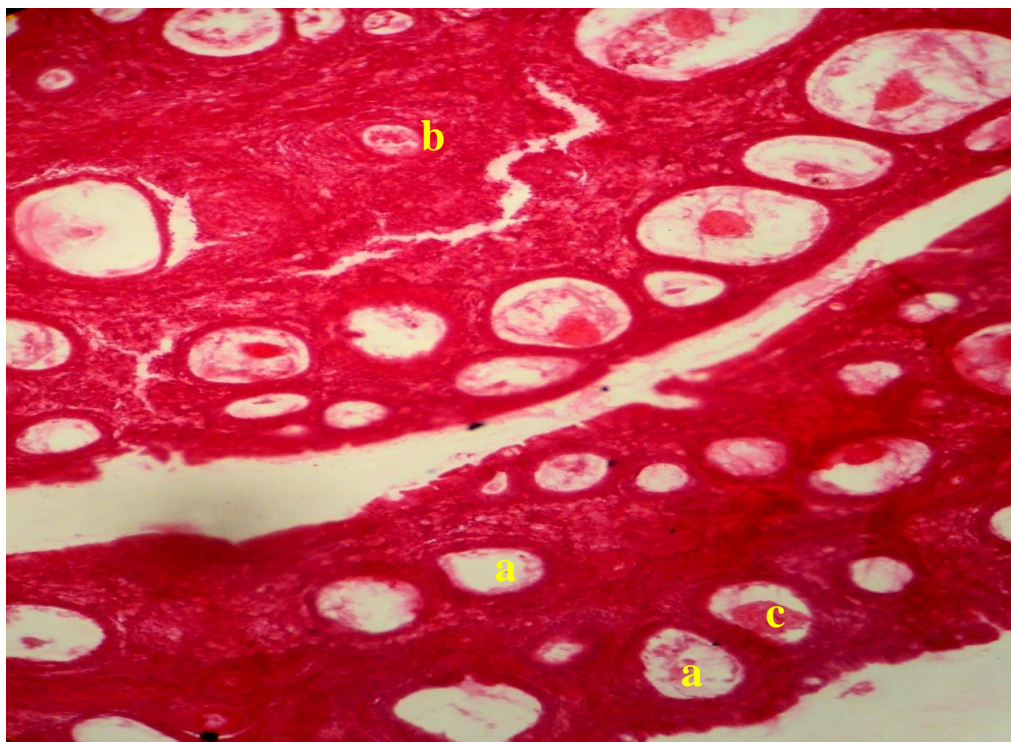
21pv/c.

The BB ration showed a non-significant difference ( $p>0.05$ ) between the groups (Table 1). On 21Dpv/c, all groups revealed a lower BB ratio, with the challenge group exhibiting an early reduction in the BB ratio on day 3 through days 21 post challenge.

The BB index values recorded on 7Dpv/c gave values of  $<0.3$  in the challenge group, indicating strong atrophy; 0.3 to 0.7 in the vaccinated groups, indicating relative transient atrophy (Table 2). The standard is presented in Table 3.

#### **DISCUSSION**

For over 50 years bursa of Fabricius has become a major organ (lymphoid organ) of debate since its discovery by Hieronymus Fabricius *ab aquapendente* in the late 16<sup>th</sup> to early 17<sup>th</sup> century (Madej et. al., 2012). There exists agreed physiologic pattern of development; colonisation and migration of bursal lymphocytes (B-lymphocytes) in the bursa of bursa of Fabricius, its usage as a tool in diagnosis of infectious bursal disease (IBD) especially bursal size remains unending. Bursal weight, bursal body ratio and bursal diameter were some of the parameters used by Glick in 1956 to study normal bursal regression.



**Figure 4.** Bursa of Fabricius from MB<sup>®</sup> group 21Dpv/c: Vacuolar degeneration of the epithelium (a); Follicular atrophy and vacuolar degeneration of the medullary area (b); Follicular necrosis (c). H&E stain  $\times 400$ .

**Table 1.** Mean bursa body weight ratio of different treatment and control groups.

Time	A	B	C	D	E
1Dpv/c	4.89 $\pm$ 0.53	5.62 $\pm$ 1.48	4.35 $\pm$ 0.83	5.98 $\pm$ 0.31	4.71 $\pm$ 1.38
2Dpv/c	6.85 $\pm$ 0.48	5.22 $\pm$ 1.36	5.80 $\pm$ 0.22	6.23 $\pm$ 0.91	6.06 $\pm$ 1.68
3Dpv/c	6.65 $\pm$ 0.93	5.31 $\pm$ 1.48	4.31 $\pm$ 0.57	2.49 $\pm$ 0.28	5.98 $\pm$ 1.55
4Dpv/c	4.69 $\pm$ 0.80	6.08 $\pm$ 1.56	4.37 $\pm$ 0.46	1.27 $\pm$ 0.13	1.27 $\pm$ 0.18
5Dpv/c	6.70 $\pm$ 0.22	5.60 $\pm$ 1.47	6.45 $\pm$ 1.06	1.82 $\pm$ 0.37	6.28 $\pm$ 1.53
7Dpv/c	2.34 $\pm$ 0.08	4.37 $\pm$ 1.09	6.19 $\pm$ 0.79	1.18 $\pm$ 0.09	5.90 $\pm$ 1.47
10Dpv/c	1.93 $\pm$ 0.38	2.49 $\pm$ 0.96	5.68 $\pm$ 1.86	0.86 $\pm$ 0.24	4.48 $\pm$ 1.06
14Dpv/c	1.44 $\pm$ 0.22	1.25 $\pm$ 0.34	5.73 $\pm$ 0.53	1.36 $\pm$ 0.15	6.20 $\pm$ 1.51
21Dpv/c	1.36 $\pm$ 0.16	1.25 $\pm$ 0.67	1.13 $\pm$ 0.29	1.39 $\pm$ 0.24	1.42 $\pm$ 0.28

Dpv/c, Day(s) post vaccination or challenge.

But this extensive study of his and that of Jolly in 1914 faced with varying challenging factors ranging from breed type, sex, housing, stress and disease.

Although specific pathogen free (SPF) pullets would have been better for the purpose of this study; however, the cockerels (chicks) used were acquired from an independent, reliable and reputable hatchery where vaccination programme against Marek's disease and infectious bronchitis are routinely administered to day-old-chicks (DOCs). These vaccines have no effect on the

integrity of the BF, and the use of the chicks reflected or mimics field conditions.

Vaccine selection was based on commonly available vaccine and as used on poultry farm with little modification. Of the two types of vaccines used in this study, one of the intermediate vaccine produced a moderate to marked reaction on the BF, whereas, the intermediate plus vaccine produced a non-to-mild reactions on the BF. The lesions observed were expected when live IBD vaccine is administered leading to impact

**Table 2.** Mean ( $\pm$  SEM) value of Bursal Body Index (BB index) of different treatment.

Groups	Bursa Index								
	1Dpv/c	2Dpv/c	3 Dpv/c	4 Dpv/c	5 Dpv/c	7Dpv/c*	10Dpv/c	14Dpv/c	21Dpv/c
A	1.04 $\pm$ 0.60	1.13 $\pm$ 0.65	1.11 $\pm$ 0.64	3.69 $\pm$ 2.13	1.07 $\pm$ 0.62	0.40 $\pm$ 0.23	0.43 $\pm$ 0.25	0.23 $\pm$ 0.13	0.96 $\pm$ 0.55
B	1.19 $\pm$ 0.69	0.86 $\pm$ 0.50	0.89 $\pm$ 0.51	4.79 $\pm$ 2.76	0.89 $\pm$ 0.52	0.74 $\pm$ 0.43	0.55 $\pm$ 0.32	0.20 $\pm$ 0.12	1.61 $\pm$ 0.93
C	0.92 $\pm$ 0.53	0.96 $\pm$ 0.55	0.72 $\pm$ 0.42	3.44 $\pm$ 1.99	1.03 $\pm$ 0.59	1.05 $\pm$ 0.61	1.27 $\pm$ 0.73	0.92 $\pm$ 0.53	0.79 $\pm$ 0.46
D	1.27 $\pm$ 0.73	1.03 $\pm$ 0.59	0.42 $\pm$ 0.24	1.00 $\pm$ 0.58	0.29 $\pm$ 0.17	0.20 $\pm$ 0.12	0.19 $\pm$ 0.11	0.22 $\pm$ 0.13	0.98 $\pm$ 0.56

SEM = Standard error mean; N=3; Dpv/c= Day(s) post vaccination or challenge. A and C = intermediate vaccines; B = intermediate plus vaccine; D = challenge (Infected) group; 7Dpv/c\*= BB index calculation day.

**Table 3.** Standards of the BB index used in classifying IBD viruses, or conventional live IBD vaccines.

BB index	Conclusion
>0.7	Physiological variability = no atrophy Mild vaccines
0.3-0.7	Relative and transient atrophy Intermediate vaccines
<0.3	Intermediate plus vaccines Strong atrophy; Hot vaccines

BB index = BB ratio of infected (or vaccinated) birds / BB ratio of the controls.

on the BF size (Jungbaeck and Nutolo, 2001). Mazeriegos et al. (1990) showed that intermediate vaccine varied in their pathogenicity. They divided intermediate vaccines into 3 pathogenic categories based on bursal damage, bursal B/W and histopathological findings; low or mild pathogenic, moderate pathogenic and highly pathogenic. In line with this result, intermediate vaccine used in group "A" showed to be highly pathogenic.

Furthermore, no clinical signs and mortality were observed in the vaccinated and non-vaccinated (control group (group E)); however the challenge group (D) presented clinical signs exhibited by birds infected with vvIBD virus.

The presence of histopathological lesions on the BF confirmed the pathogenicity of the field IBD virus field isolate used. Although the scoring pattern followed that of Williams and Davison, (2010); it was compared with the European pharmacopoeia lesion scoring because of the live attenuated IBD vaccines used in this study, with lesion of 4 and 5 recorded in the challenge group.

With values of BF weight and size steadily increasing prior to vaccination and challenge as a result of variables due to continues genetic selection through output in egg and carcass. This variability as described by Cazaban and Gardin (2012) was observed during the course of this study.

Following infection or vaccination, the BF goes through several stages; (1) Acute inflammation stage, where bursa is getting larger, and lasts for about 4 days post infection (dpi); (2) Sub-acute stage where BF quickly

regresses and gets back to its original size at around 5 dpi and the final stage; (3) Which is the relevant stage to record bursal atrophy, and calculate BB ratio and BB index (which is the main target of this study). As in the previous and only study on BB index by Cazaban and Gardin (2012), the relevant schedule to start recording BB index is from 7 dpi onwards (and say up to 14dpi). The sizes recorded in this study presented the picture of the vaccine virus strain used and likewise the strain of the field virus. The challenge virus isolate gave value of <0.3 BB index indicative of a hot vaccine or vvIBDV infection, whereas the intermediate and intermediate plus vaccines gave a value of between 0.3 to 0.7 which is indicative of the administration of either of the vaccines used.

Conclusively, this study has confirmed that BB index can be used in assessing the BF status (in vaccination or infection) as the BF histopathologic lesion scoring scale is used in designing the safety of live IBD vaccines.

### Conflict of Interests

The authors have not declared any conflict of interests.

### REFERENCES

- Cazaban C, Gardin Y, Kaewpoowat N (2012). The BB Index Assists on Assessing Flock Health (Part 2). Chick Program Online 41:5.  
Cazaban C, Gardin Y (2012). Bursa of Fabricius is a visual indicator (Part 1). Chick Program Online 40:5.

- Cazaban C, Masferrer NM, Pascual RD, Espadamala MN, Costa T, Gardin Y (2015). Proposed bursa of fabricius weight to body weight ratio standard in commercial broilers. *Poult. Sci.* 94(9):2088-2093.
- Fang J, Peng X (2014). Developmental changes in cell proliferation and apoptosis in the normal duck bursa of Fabricius. *J. Vet. Sci.* 15(4):465-474.
- Glick B (1956). Normal growth of the bursa of Fabricius in chickens. *Poult. Sci.* 35:843-851.
- Jungbaeck C, Nutolo S (2001). The degree of bursa damage as a possible parameter to define the virulence of IBDV vaccine strain. *Proc. II. International symposium on infectious bursal disease and chicken infectious anaemia.* Rauschholzhaus, Germany. pp. 460-474.
- Madej JP, Chrzazstek K, Piasecki T, Wieliczka A (2012). New Insight into the Structure, Development, Functions and Popular Disorders of Bursa Fabricii. *Anat. Histol. Embryol.* 42:321-331.
- Mazeriegos LA, Lukert PD, Brown J (1990). Pathogenicity and immunosuppressive properties of infectious bursal disease "Intermediate" strains. *Avian Pathol.* 9:395-405.
- Olah I, Vervelde L (2012). Structure of the avian lymphoid system. In: *Avian Immunology.* Davison F, Kaspers B, Schat KA (Eds.). London: Academic Press. pp. 13-50.
- Olah I, Nagy N, Vervelde L (2014). Structure of the Avian Lymphoid System. in *Avian Immunology, 2nd Edition.* Schat KA, Kaspers B, Kaiser P (Eds.). Elsevier Ltd. Publishing, San Diego, CA. pp. 11-44
- Schat KA, Skinner MA (2014). Avian Immunosuppressive Diseases and Immune Evasion. in *Avian Immunology, 2nd Edition.* Schat KA, Kaspers B, Kaiser P (Eds.). Elsevier Ltd. Publishing, San Diego, CA. pp. 275-297
- Van Herdeen K, Cazaban C, Alva B (2011). The Relevance of Bursa Size in Modern Poultry Production. August 2011. *Pluimvee Poult. Bull.* pp. 24-26.
- Williams AE, Davison TF (2010). Enhanced immunopathology induced by very virulent infectious bursal disease virus. *Avian Pathol.* 34(1):4-14.
- Wolfe HR, Sheridan SA, Bilstad NM, Johnson MA (1962). The growth of lymphoid organs and the testes of chickens. *Anat. Rec.* 142:485-493.