Immunoreactivity and morphological changes of bursal follicles in chickens infected with vaccine or wild-type strains of the infectious bursal disease virus

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ABSTRACT. Infectious bursal disease (IBD) is characterized by immunosuppression due to the depletion of lymphocytes in the atrophied bursa of Fabricius (BF). We have sometimes encountered contradictory findings: chickens infected with the vaccine IBD virus (IBDV) strain have sometimes exhibited a highly atrophied BF, but not immunosuppression. In this study, chickens administered vaccine or wild-type strains of IBDV were later vaccinated with the B1 strain of the Newcastle disease virus (NDV). Bursal changes were examined histologically with a focus on the bursal follicle. The immunoreactivity to NDV was also evaluated with the hemagglutination inhibition test. In gross examination, we observed a few chickens with a severely atrophied BF in vaccine strain-administered groups (vaccine groups), and the level of severity was the same as that in the wild-type strain-administered group (wild-type group). However, these chickens retained humoral antibody responses to NDV and were revealed to possess a higher number of bursal follicles than those of the wild-type group. These results indicated that macroscopic evaluation does not accurately reflect the immunoreactivity and degree of bursal damage in IBDV-administered chickens. We also found non-immunosuppressed chickens in the wild-type group. These non-immunosuppressed chickens retained a significantly higher number of normal follicles and total follicles according to our statistical analysis. Furthermore, a high correlation coefficient between the NDV-HI titer and the number of normal follicles was found in the wild-type group. These results implied that the retained number of normal follicles is important for the immunoreactivity of chickens infected with IBDV.

KEY WORDS: bursal follicle, infectious bursal disease, vaccine

Infectious bursal disease (IBD), an acute and highly contagious viral infection in young chickens, is of worldwide importance for the poultry industry [26]. IBD sometimes results in death and mortality depending on the virulence of the IBD virus (IBDV), which is the causative agent of IBD, and the mortality rate is up to 60% in very virulent IBDV infection [4, 15]. In addition to its mortality, immunosuppression is the most serious sequela of IBD, since immunosuppression is an inescapable outcome regardless of IBDV virulence [9]. After the acute phase of infection, surviving chickens exhibit suppressed humoral antibody responses to other vaccines [7] and become more susceptible to secondary infection, such as inclusion body hepatitis [5], coccidiosis [1], Marek’s disease [2, 25], hemorrhagic–aplastic anemia and gangrenous dermatitis [23], infectious laryngotracheitis [22], infectious bronchitis [18], chicken anemia agent [34], and salmonellosis and colibacillosis [33]. The immunosuppression of IBD is known to be caused by the depletion of lymphocytes in the bursa of Fabricius (BF) [10, 19, 26], which results in macroscopic BF atrophy, the most characteristic lesion of IBD [12]. Live vaccine strains of IBDV also cause BF atrophy to various extents [17, 21]. Nevertheless, these live vaccine strains are confirmed to exhibit effective antigenicity and to be safe, and they do not induce immunosuppression, because it is widely accepted that the extent of atrophy caused by live vaccine strains is slightly less compared with that caused by the wild-type strain [4]. On the other hand, we sometimes find live IBDV vaccine strain-vaccinated chickens showing an unexpectedly high extent of BF atrophy without immunosuppression. In addition, recent studies have shown that some chickens that survived IBD were immunosuppressed, though their BF were repopulated with B lymphocytes [31, 32]. These reports and our experience indicate that IBDV-induced immunosuppression may not be fully explained by atrophy of the BF and/or depletion of lymphocytes.

The bursal follicle is the smallest component of the BF parenchyma, which consists of the medulla, the cortex and the follicular structure [8, 14, 24]. The follicular structure of the BF is composed of the follicle-associated epithelium, the basement membrane (BM) and the BM-associated epithelium [8, 14, 24]. It provides a microenvironment for the development of B lymphocytes and is important for immunoreactivity in chickens [6, 8, 16, 24]. In spite of the important role of the follicular structure, it has not been fully evaluated in the study of immunosuppression by IBDV. The aim of the present study was to assess the BF pathologically with a focus on the bursal follicle and immunoreactivity in chickens administered a vaccine strain or wild-type strain of IBDV.
MATERIALS AND METHODS

**Chickens**: Four-day-old specific pathogen-free White Leghorn chickens (Nisshiken Co., Ltd., Tokyo, Japan) were used. Chickens were bred in groups in a state of mixing males and females under *ad libitum* conditions. All procedures were in accordance with the guidelines of the Animal Research Committee of the National Veterinary Assay Laboratory and were approved by the committee (approval number O-034).

**Experimental design and virus**: Chickens (n=55) were divided into the following 5 groups: the Vac-Ch (n=9), Vac-IM (n=9), Vac-LC (n=10), IBDV wild-type strain-administered (n=15) and control groups (n=12). Chickens in the Vac-Ch group were administered the live IBDV vaccine strain for chicks (strain S706). Chickens in the Vac-IM group were administered the live IBDV vaccine strain for chicks (intermediate virulence type; strain 228E). Chickens in the Vac-LC group were administered the live IBDV vaccine strain for large chicks (strain MB-1). Chickens in the IBDV wild-type strain-administered group (wild-type group) were administered the IBDV wild-type strain (strain K-1). Chickens in the control group were not administered any viruses. The 3 vaccine strains are used in commercial vaccines in Japan. The IBDV wild-type strain was originally isolated from laying hens in 1992 in Niigata Prefecture, Japan. The titers of the administered viruses in the Vac-Ch, Vac-IM, Vac-LC and wild-type groups were 10^5.4, 10^4.5, 10^5.3 and 10^4.7 EID50/ml [50% embryo infectious dose (EID50)], respectively. Each group was kept in a separate isolator.

On day 1, all 4-day-old chickens, except for controls, were orally administered 0.2 ml of viral specimens using feeding needles. The control group was administered 0.2 ml of phosphate-buffered saline using feeding needles. At 7 days post infection (DPI), all chickens were vaccinated ocularly with one dose of the commercial live vaccine of Newcastle disease virus (NDV) containing the B1 strain. Chickens in the Vac-Ch group were administered the live IBDV vaccine strain for chicks (intermediate virulence type; strain 228E). Chickens in the Vac-LC group were administered the live IBDV vaccine strain for large chicks (strain MB-1). Chickens in the IBDV wild-type strain-administered group (wild-type group) were administered the IBDV wild-type strain (strain K-1). Chickens in the control group were not administered any viruses. The 3 vaccine strains are used in commercial vaccines in Japan. The IBDV wild-type strain was originally isolated from laying hens in 1992 in Niigata Prefecture, Japan. The titers of the administered viruses in the Vac-Ch, Vac-IM, Vac-LC and wild-type groups were 10^5.4, 10^4.5, 10^5.3 and 10^4.7 EID50/ml [50% embryo infectious dose (EID50)], respectively. Each group was kept in a separate isolator.

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**Histopathological examination**: Fixed BFs were transected at the point of maximum cross section to evaluate each BF under the same conditions. The specimens were em-bedded in paraffin, sectioned (4 μm thick) and stained with hematoxylin and eosin and the periodic acid–Schiff reaction.

**Immunofluorescence**: For antigen retrieval, the deparf-inized sections were heated at 98°C in an immunosaver (Nisshin EM Corp., Tokyo, Japan) for 45 min. The sections were then incubated with an anti-keratin AE1/AE3 antibody (1 in 200 dilution; Dako Denmark A/S, Glostrup, Denmark) or anti-chicken Bu-1 antibody (clone AV20; 1 in 100 dilution; SouthernBiotech, Birmingham, AL, U.S.A.) at 4°C overnight and visualized with an Alexa Fluor 488-conjugated goat anti-mouse IgG antibody (1 in 1,000 dilution; Life Technologies Corp., Gaithersburg, MD, U.S.A.). The fluorescent signals in the sections were observed with a fluorescence microscope (FSX100, Olympus Corp., Tokyo, Japan). The follicle-associated epithelium and B lymphocytes in the BF of a control chicken were used as a positive control for the anti-keratin AE1/AE3 antibody and anti-chicken Bu-1 antibody, respectively. Negative controls were obtained by omitting the primary antibody.

**Quantitative analysis of microscopic findings**: For the quantitative evaluations, histological scoring evaluations of follicular lesion were performed according to previous reports [12, 13, 20, 21, 26, 27, 29]. In brief, the scoring evaluations were based on the percentage of affected follicles, i.e. those showing lymphocyte depletion, in all the follicles: 0 ≤1%, 1=1–25%, 2=26–50%, 3=51–75% and 4 ≥75%.

In addition to the scoring evaluation based on previous reports, we classified and counted the number of bursal follicles. The BM of the bursal follicle was stained with the periodic acid–Schiff reaction, and the associated epithelium was positive for cytokeratin. According to the population of lymphocytes and state of the follicular BM-associated structures, namely the BM and BM-associated epithelium, we classified the bursal follicle of the present chickens as follows: a follicle that retained lymphocytes and the BM-associated structures was classified as a normal follicle, and that exhibiting depletion of lymphocytes and a lack of a discernible cortex and medulla but retained the BM-associated structures was classified as a small follicle. We counted the numbers of normal follicles and small follicles and calculated the number of total follicles by summation of the numbers of normal and small follicles. In the case of inappropriate specimens in which the mucosal folds on the luminal surface of the BF were not transected vertically, the specimens and chickens were excluded from the following statistical analyses (Nos. 8 and 9 in the Vac-Ch group and No. 15 in the Vac-IM group).

**Statistical analysis**: The data are expressed as the arithmetic mean ± standard deviation (SD), except for the HI titers, which were assessed by the geometric mean and expressed as the geometric mean ± geometric SD. For the HI titer, undetectable values were calculated as 1. The HI titer was expressed on a base-2 logarithmic scale for the following statistical procedures. Statistical significance was determined with the Student’s *t*-tests or one-way analysis of variance (ANOVA), which was followed by Tukey–Kramer post hoc tests for multiple comparisons. Spearman’s rank correlation coefficients (r) between the HI titer and other
parameters were calculated. The analyses were conducted with GraphPad Prism ver. 5 (GraphPad Software, Inc., La Jolla, CA, U.S.A.). In addition to the group statistical comparisons, chickens in the wild-type group were subdivided into 2 subgroups according to the presence or absence of an anti-NDV HI antibody response and subjected to the same statistical analysis as described above: the chickens with detectable NDV-HI antibody production (n=6, partly immunoreactive subgroup) and the chickens without antibody production (n=7, immunosuppressed subgroup).

RESULTS

Clinical manifestations: No clinical signs were observed in the vaccine strain-administered groups (vaccine groups). In the wild-type group, 2 chickens died at 3 DPI, and the others showed depression and ruffled feathers at 3–7 DPI.

Macroscopic findings of the BF and HI titers: At necropsy, we observed macroscopically mild to severe atrophy in the vaccine groups (Fig. 1A–1C) and severe BF atrophy in the wild-type group (Fig. 1D). In the Vac-IM and Vac-LC groups, unexpectedly severe BF atrophy was sometimes observed (Fig. 1B). The mean F/B ratios were significantly decreased in the vaccine groups compared with the control group (Table 1). The mean F/B ratios were significantly decreased in the wild-type group compared with the other groups. The F/B ratios of the severely BF-atrophied chickens in the vaccine groups were less than 0.10 (0.05, Nos. 7 and 13 in Vac-IM; 0.07, No. 15 in Vac-LC; 0.08, No. 25 in Vac-LC) and fell within the mean F/B values ± 2SD of the wild-type group.

HI antibody responses ranged from 1:5 to 1:160 in the control group and vaccine groups. The mean HI titers of the vaccine groups did not significantly differ compared with the control group or among vaccine groups (Table 1). Notably, the chickens with very low F/B ratios in the vaccine groups showed antibody responses (HI titers of 1:40, 1:5, 1:20 and 1:40 for Nos. 11, 13, 15 and 25, respectively). The mean HI titer of the wild-type group was significantly lower than those of the other groups. However, 6 chickens showed HI antibody responses.

Histological findings of the BF: Histologically, the bursal tunica intimae in the vaccine groups were occupied by normal and small follicles (Fig. 2A–2C). In the wild-type group, a few small follicles and a few normal follicles were interspersed with slight to moderate fibrosis in the lamina propria.
mucosae (Fig. 2D). In the control group, no significant lesions were observed, and normal follicles were retained (Fig. 2E). In the vaccine groups, lymphocytes and normal follicles were decreased to various extents, and the lesion scores were higher than that of the control group (Table 1). The chickens with very low F/B ratios in the vaccine groups showed a similar number of total follicles regardless of the F/B ratios, number of normal follicles or lesion scores (Table 1). The chickens in the wild-type group had a significantly smaller number of follicles compared with the control and vaccine groups.

Correlation coefficients between NDV-HI titers and other parameters: To identify the factors that correlated with immunoreactivity, the correlation coefficients between the NDV-HI titer and the following observational data were calculated: F/B ratio, number of normal follicles or lesion scores (Table 1). The chickens with very low F/B ratios in the vaccine groups had average numbers of total follicles, and the numbers were higher compared with that in the wild-type group (total number of follicles, 591, 590 and 437 for Nos. 11, 13 and 25, respectively). The chickens in the wild-type group had a significantly smaller number of follicles compared with the control and vaccine groups.

Correlation coefficients between NDV-HI titers and other parameters: To identify the factors that correlated with immunoreactivity, the correlation coefficients between the NDV-HI titer and the following observational data were calculated: F/B ratio, number of normal follicles, total number of follicles and lesion scores (Table 2). In all the chickens, correlations were found for NDV-HI titer with the F/B ratio ($r=0.39$, $P<0.05$), the number of normal follicles ($r=0.53$, $P<0.0005$) and the total number of follicles ($r=0.49$, $P<0.0005$). No correlation was found between the NDV-HI titer and the number of normal follicles in chickens in the vaccine strain and control groups ($r=0.04$, $P>0.05$; Table 2). On the other hand, within the wild-type group, a high cor-
relation was found with the number of normal follicles ($r=0.71$, $P<0.05$; Table 2).

**Immunoreactivity and bursal follicles in the wild-type group:** We divided the wild-type group into 2 subgroups: the partly immunoreactive subgroup ($n=6$) and the immunosuppressed subgroup ($n=7$) (Table 3). Although both subgroups had severe lymphocyte reductions and severe follicular lesion scores, the partly immunoreactive subgroup had a larger number of normal follicles and total number of follicles compared with the immunosuppressed subgroup ($t$-test, $P<0.05$).

### DISCUSSION

In gross examination, we observed severe atrophy of the BF in the chickens of the vaccine groups, and the level of severity was the same as in the wild-type group. However, these chickens retained immunoreactivity to exogenous antigens and were found to possess a higher number of total follicles than the wild-type group histologically and statistically. These results indicated that macroscopic evaluation does not accurately reflect the immunoreactivity and the degree of bursal damage in IBDV-administered chickens.

In the histological examination, we observed the emergence of small follicles, which lost almost all lymphocytes and lacked the cortical area, but retained their BM-associated structures. Similar small follicles had been reported in previous reports of IBDV-infected chickens and had been thought not to be fully functional [31, 32]. In the present study, almost equal numbers of small follicles were observed in the wild-type group, regardless of the immunoreactivity of the chickens. This result also demonstrated that the small follicles were not functional and not associated with immunoreactivity of IBDV-infected chickens. On the other hand, the emergence of the small follicles was a pathological change due to IBDV administration, because these findings were not detected in the control chickens but significant increases were detected in the vaccine-administered chickens. B lymphocytes in the BF are the main target of IBDV, but the bursal stromal components also exhibit susceptibility to IBDV [11]. Taking into account the observation that the small follicles retained BM-associated structures, the small follicles might represent mild lesions in IBDV infection that were enough to damage lymphocytes in bursal follicles but not too severe to damage BM-associated structures. Small follicle-like structures have also been reported in cyclophosphamide-treated chickens [3, 28]. Since cyclophosphamide is a selective toxicant to avian and mammalian B lymphocytes [30], the resemblance of the small follicles also implied the small follicles were mild pathological lesions of IBDV that were enough to damage lymphocytes without damaging stromal cells. All the chickens in the vaccine groups, including the chickens with macroscopically severe BF atrophy, retained larger total numbers of follicles, which were calculated by summation of the numbers of normal and small follicles, than that in the wild-type group. In other words, they retained larger numbers of BM-associated structures. Although it is necessary to investigate using larger numbers of wild-type IBDV and long-term experiments for a proper evaluation, preservations of BM-associated structures may be related with the virulence of IBDV and an important key to analysis of the effect of IBDV on chickens.

We also found non-immunosuppressed chickens in the wild-type group, despite the fact that all the chickens in the wild-type group showed severe BF atrophy in gross examination, a decreased F/B ratio and the worst follicular lesion score. These non-immunosuppressed chickens retained a significantly higher number of normal follicles and total number of follicles. Furthermore, a high correlation coefficient between the NDV-HI titer and the number of normal follicles was found in the wild-type group. These results suggested that the retained number of normal follicles is important for immunoreactivity in chickens infected with IBDV, in addition to a previously reported factor, namely the extent of depletion of lymphocytes in the BF [10, 19, 26].

In addition to the small follicles, we observed small nests of PAS-positive membranous structures only in the wild-type groups. It is reported that highly virulent IBDV infects stromal cells more frequently than a moderately virulent IBDV wild-type strain [27]. It is suspected that the PAS-positive membranous structures might be due to the destruction of BM-associated structures resulting from infection of stromal cells of bursal follicles after administration of IBDV, but the detailed mechanisms remain unclear. It is necessary to analyze histopathological effects in chickens infected with a larger number of immunosuppressive strains of IBDV to determine the relationship between state of immunoreactivity and histopathological findings.

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### REFERENCES

1. Anderson, W. I., Reid, W. M., Lukert, P. D. and Fletcher, O. J. Jr. 1977. Influence of infectious bursal disease on the development of immunity to Eimeria tenella. *Avian Dis.* 21: 637–641. [Medline] [CrossRef]

2. Cho, B. R. 1970. Experimental dual infections of chickens with
infectious bursal and Marek’s disease agents. I. Preliminary observation on the effect of infectious bursal agent on Marek’s disease. Avian Dis. 14: 665-675. [Medline] [CrossRef]

3. Corrier, D. E., Elissalde, M. H., Ziprin, R. L. and DeLoach, J. R. 1991. Role of immunosuppression with cyclophosphamide, cyclosporin, or dexamethasone on Salmonella colonization of broiler chicks. Avian Dis. 35: 40–45. [Medline] [CrossRef]

4. Eterradossi, N. and Saif, Y. M. 2008. Infectious bursal disease. pp. 185–208. In: Diseases of Poultry, 12th ed. (Saif, Y. M., Fadly, A. M., Glisson, J. R., McDougald, L. R., Nolan, L. K., and Swanye, D. E. eds), Blackwell Publishing, Ames.

5. Fadly, A. M., Winterfield, R. W. and Olander, H. J. 1976. Role of the bursa of Fabricius in the pathogenesis of inclusion body hepatitis and infectious bursal disease viruses. Avian Dis. 20: 467–477. [Medline] [CrossRef]

6. Hansell, C., Zhu, X. W., Brooks, H., Sheppard, M., Withanage, S., Maskell, D. and McConnell, I. 2007. Unique features and distribution of the chicken CD83+ cell. J. Immunol. 179: 5117–5125. [Medline] [CrossRef]

7. Hirai, K., Shimakura, S., Kawamoto, E., Taguchi, F., Kim, S. T., Chang, C. N. and Iritani, Y. 1974. The immunodepressive effect of infectious bursal disease virus in chickens. Avian Dis. 18: 50–57. [Medline] [CrossRef]

8. Houssaint, E., Diez, E. and Hallet, M. M. 1986. The bursal microenvironment: phenotypic characterization of the epithelial component of the bursa of Fabricius with the use of monoclonal antibodies. Immunology 58: 43–49. [Medline]

9. Ingrao, F., Rauw, F., Lambrecht, B. and van den Berg, T. 2013. Infectious Bursal Disease: a complex host-pathogen interaction. Dev. Comp. Immunol. 41: 429–438. [Medline] [CrossRef]

10. Ivan, J., Nagy, N., Magyar, A., Kacskovics, I. and Mészáros, J. 2001. Functional restoration of the bursa of Fabricius following in ovo infectious bursal disease vaccination. Vet. Immunol. Immunopathol. 79: 235–248. [Medline] [CrossRef]

11. Käufer, I. and Weiss, E. 1976. Electron-microscope studies on the pathogenesis of infectious bursal disease after intrabursal application of the causal virus. Avian Dis. 20: 483–495. [Medline] [CrossRef]

12. Kim, I. J., Jagoe, M. and Sharma, J. M. 1999. Recovery of antibody-producing ability and lymphocyte repopulation of bursal follicles in chickens exposed to infectious bursal disease virus. Avian Dis. 43: 401–413. [Medline] [CrossRef]

13. Kim, I. J., You, S. K., Kim, H., Yeh, H. Y. and Sharma, J. M. 2000. Characteristics of bursal T lymphocytes induced by infectious bursal disease virus. J. Virol. 74: 8884–8892. [Medline] [CrossRef]

14. Madej, J. P., Chrząstek, K., Piaszecki, T. and Wieliczko, A. 2013. New insight into the structure, development, functions and popular disorders of bursa Fabiceii. Anat. Histol. Embryol. 42: 321–331. [Medline] [CrossRef]

15. Mahgoub, H. A., Bailey, M. and Kaiser, P. 2012. An overview of infectious bursal disease. Arch. Virol. 157: 2047–2057. [Medline] [CrossRef]

16. Morimura, T., Miyatani, S., Kitamura, D. and Goitsuka, R. 2001. Notch signaling suppresses IgH gene expression in chicken B cells: implication in spatially restricted expression of Serrate2/Notch1 in the bursa of Fabricius. J. Immunol. 166: 3277–3283. [Medline] [CrossRef]

17. Muskett, J. C., Hopkins, I. G., Edwards, K. R. and Thornton, D. H. 1979. Comparison of two infectious bursal disease vaccine strains: efficacy and potential hazards in susceptible and maternally immune birds. Vet. Rec. 104: 332–334. [Medline] [CrossRef]

18. Pejkovska, C., Davila, F. G. and Kouwenhoven, B. 1979. Immunosuppressive effect of infectious bursal disease virus on vaccination against infectious bronchitis. Avian Pathol. 8: 95–106. [Medline] [CrossRef]

19. Pejkov, P., Linne, H. G., Kapczynski, D. R. and Sellers, H. S. 2009. Identification and characterization of two distinct bursal B-cell subpopulations following infectious bursal disease virus infection of White Leghorn chickens. Avian Dis. 53: 347–355. [Medline] [CrossRef]

20. Rautenschlein, S., Yeh, H. Y. and Sharma, J. M. 2003. Comparative immunopathogenesis of mild, intermediate, and virulent strains of classic infectious bursal disease virus. Avian Dis. 47: 66–78. [Medline] [CrossRef]

21. Rautenschlein, S., Kraemer, C., Vanmarcke, J. and Montiel, E. 2005. Protective efficacy of intermediate and intermediate plus infectious bursal disease virus (IBDV) vaccines against very virulent IBDV in commercial broilers. Avian Dis. 49: 231–237. [Medline] [CrossRef]

22. Rosenberger, J. K. and Gelb, J. Jr. 1978. Response to several avian respiratory viruses as affected by infectious bursal disease virus. Avian Dis. 22: 95–105. [Medline] [CrossRef]

23. Rosenberger, J. K., Klop, S., Eckroade, R. J. and Krauss, W. C. 1975. The roles of the infectious bursal agent and several avian adenoviruses in the hemorrhagic-aplastic-anemia syndrome and gangrenous dermatitis. Avian Dis. 19: 717–729. [Medline] [CrossRef]

24. Sayegh, C. E., Demaries, S. L., Pike, K. A., Friedman, J. E. and Ratcliffe, M. J. 2000. The chicken B-cell receptor complex and its role in avian B-cell development. Immunol. Rev. 175: 187–200. [Medline] [CrossRef]

25. Sharma, J. M. 1984. Effect of infectious bursal disease virus on protection against Marek’s disease by turkey herpesvirus vaccine. Avian Dis. 28: 629–640. [Medline] [CrossRef]

26. Sharma, J. M., Kim, I. J., Rautenschlein, S. and Yeh, H. Y. 2000. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Dev. Comp. Immunol. 24: 223–235. [Medline] [CrossRef]

27. Taniguma, N., Tsukamoto, K., Nakamura, K., Narita, M. and Maeda, M. 1995. Association between pathogenicity of infectious bursal disease virus and viral antigen distribution detected by immunohistochemistry. Avian Dis. 39: 9–20. [Medline] [CrossRef]

28. Toivanen, P., Toivanen, A. and Good, R. A. 1972. Ontogeny of bursal function in chicken. I. Embryonic stem cell for humoral immunity. J. Immunol. 109: 1058–1070. [Medline] [CrossRef]

29. Williams, A. E. and Davison, T. F. 2005. Enhanced immunopathology induced by very virulent infectious bursal disease virus. Avian Pathol. 34: 4–14. [Medline] [CrossRef]

30. Wilmer, J. L., Colvin, O. M. and Bloom, S. E. 1992. Cytogenetic mechanisms in the selective toxicity of cyclophosphamide analogs and metabolites towards avian embryonic B lymphocytes in vivo. Mutat. Res. 268: 115–130. [Medline] [CrossRef]

31. Withers, D. R., Davison, T. F. and Young, J. R. 2006. Diversity of bursal medullary B cells survive and expand independently after depletion following neonatal infectious bursal disease virus infection. Immunology 117: 558–565. [Medline] [CrossRef]

32. Withers, D. R., Young, J. R. and Davison, T. F. 2005. Infectious bursal disease virus-induced immunosuppression in the chick is associated with the presence of unidentified follicles in the recovering bursa. Viral Immunol. 18: 127–137. [Medline] [CrossRef]

33. Wyeth, P. J. 1975. Effect of infectious bursal disease on the response of chickens to S typhimurium and E coli infections. Vet. Rec. 96: 238–243. [Medline] [CrossRef]

34. Yuasa, N., Taniguchi, T., Naguchi, T. and Yoshida, I. 1980. Effect of Infectious Bursal Disease Virus Infection on Incidence of Anemia by Chicken Anemia Agent. Avian Dis. 24: 202–209. [CrossRef]