HISTOPATHOLOGY AND TRANSMISSION ELECTRON MICROSCOPY OF THE BURSA OF FABRICIUS FOLLOWING IBD VACCINATION AND IBD VIRUS CHALLENGE IN CHICKENS

Histopatología y Microscopía Electrónica de Transmisión de la Bolsa de Fabricio en Pollos, después de la Vacunación y Desafío con el Virus IBD

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ABSTRACT

Two experiments were conducted in order to determine comparative severity of gross, histopathologic, and ultrastructural lesions among six lines and five cross-mating of chickens to infectious bursal disease virus (IBDV). In these experiments, 1,260 lines of chickens and 420 cross-mating of chickens and 200 Specific-Pathogen-Free (SPF) chickens were used. In the experiment one, chickens were vaccinated at 4 weeks of age by the oral route with a commercial intermediate live IBD vaccine. Samples of bursa of Fabricius at 4,8, and 11 weeks (wk) of age, were collected and fixed in 10% buffer formaldehyde for histophatologic study, or in a mixture of 2.5% glutaraldehyde, 1% paraformaldehyde, 0.05 M calcium chloride in 0.2 M sodium cacodylate-HCl buffer pH 7.2, 350 mOsm, for TEM study. Tissue samples were subsequently post-fixed in osmium and dehydrated through graded alcohol then embedded in epoxy resin. Ultrathin sections were doubly stained in uranyl acetate and lead citrate and observed using Hitachi H-7100 TEM. Seven wk after vaccination, histopathologic study showed bursae atrophy and reduced bursa:body weight (B:BW) ratios, which may imply a concurrent potential decrease in immune competence of chickens following IBD vaccination. Transmission electron microscopy study demonstrated IBD virions in cytoplasm of lymphocytes in bursa tissues from vaccinated lines and cross-mating of chickens and SPF chickens. In experiment two, 1,480 lines and 520 cross-mating of chickens and 210 SPF chickens were challenged with IBD standard challenge virus, at 4 weeks of age by the eye drop route. The mortality after challenge was 4% in lines and cross-mating chickens and 29% in SPF chickens. The histopatholgic and ultrastructural lesions were studied at 3, 7, and 24 days after challenge. The lesions were follicular lymphoid necrosis, depletion, and acute inflammation with edema, hemorrhage, and heterophils infiltration through the observation period of 24 days. The severity of bursal lesions were greater and more persistent in SPF chickens than in the lines and cross-mating chickens. Bursa samples from infected chicken were macerated and centrifuged, and the supernatants were directly applied to membrane supported grids for negative staining with 1% neutralized PTA. Ultrathin sections of bursal tissues from infected chickens revealed viral inclusions in the cytoplasm of lymphocytes and macrophages. In conclusion, these findings suggest that lines and cross-mating chickens had greater immune resistance to IBDV challenge than SPF chickens, which is possibly due to genetic factors.

Key words: Fabricius bursa, IBD virus, histopathology, TEM.

RESUMEN

Dos experimentos fueron realizados con el objeto de determinar la severidad de las lesiones macroscópicas, microscópicas y ultraestructurales causadas por la infección del virus de la bolsa de Fabricio (IBDV) en seis líneas y cinco cruces de pollos. En el primer experimento, se utilizaron 1.680 pollos de lí-

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neas genéticas, 420 pollos de cinco cruces genéticos y 200 pollos libres de patógenos (SPF), que fueron vacunados a las 4 semanas de edad, por vía oral con una vacuna intermedia comercial a virus vivo. Las muestras de bolsa de Fabricio fueron colectadas a las 4 (previo a la vacunación), 8 y 11 semanas de edad (después de la vacunación) y fijadas en una solución formaldehído neutro al 10% para estudio histopatológico por microscopía óptica (MO), y/o fijadas en una mezcla de glutraldehído al 2,5%, paraformaldehído al 1%, 0,5 M Cloruro de calcio en solución tamponada de cacodilato de sodio al 0,2 M pH 7,2, 350 mOsm, para estudio por microscopía electrónica de transmisión (MET). Siete semanas después de la vacunación, la observación microscópica de las muestras de bolsa de Fabricio mostró atrofia y disminución de la relación bolsa:peso vivo, lo que puede sugerir que se produjo una disminución de la inmunidad en los pollos después de la vacunación. La observación por MET demostró el virus de IBD en el citoplasma de linfocitos de la bolsa de Fabricio provenientes de líneas y cruces genéticos de pollos, así como en pollos SPF, previamente vacunados. En el segundo experimento, se utilizaron 1.480 pollos de líneas genéticas, 520 pollos de cruces genéticos y 210 pollos SPF, que fueron desafiados a las 4 semanas de edad con el virus IBD estándar, mediante gota en el ojo. La mortalidad después del desafío fue del 29% en pollos SPF y 4% en los pollos de las líneas y cruces genéticos. El grado de las lesiones fue estudiada por MO y MET a los 3, 7 y 24 días después del desafío con IBDV. Las lesiones consistieron en necrosis folicular, depleción de linfocitos e inflamación aguda con edema, hemorragia e infiltración de heterófilos, durante el período de observación de 24 días. El grado de las lesiones de la bolsa de Fabricio fue mayor y más persistentes en los pollos SPF que en los pollos de las líneas y cruces genéticos. Muestras de bolsas de Fabricio de pollos infectados fueron maceradas y centrifugadas, y el sobrenadante fue recogido sobre rejillas con membrana de soporte y teñidas con tinción negativa con 1% de ácido fosfotungstico. Los cortes ultrafinos provenientes de pollos infectados revelaron la presencia de inclusiones virales en el citoplasma de linfocitos y macrófagos. En conclusión, estos hallazgos sugieren que los pollos de las líneas y cruces genéticos tuvieron una mayor resistencia al desafío con el virus de IBD que los pollos SPF, lo que pudiera deberse a factores genéticos.

Palabras clave: Bolsa de Fabricio, virus IBD, histopatología, ultraestructura.

INTRODUCTION

Infectious bursal disease (IBD) was described as a specific disease by Cosgrove in 1962. It is a highly contagious and immunosuppressive viral infection in young chickens which causes damage to lymphoid tissue with special predilection for the bursa of Fabricius [5, 19, 23]. Also, out breaks commonly appear due to lack of maternal antibodies or response to vaccine.

This disease has a short incubation period and appears suddenly in young chickens, fully susceptible to the clinical disease, between 3 and 6 weeks of age, usually the disease show high morbidity and low mortality rates and short duration of clinical signs. On the other hand, chickens less than 3 weeks of age usually do not exhibit clinical signs, but undergo immunosuppression [1, 8, 9, 10, 26, 27, 37, 40].

Clinical signs of IBD are not specific but gross and histopathologic bursal lesions in the initial stages are pathognomonic and include degeneration and necrosis of lymphocytes in the medulla of the bursal follicles. Later, there is infiltration of heterophils, pyknotic debris, and hyperplastic reticular cells. All lymphoid follicles are affected by 3 or 4 days after infection and the bursa of Fabricius increases in size and weight due to severe edema, hyperemia, and marked infiltration of hetrrophils. As the inflammation reaction declines, cystic cavities develop in medullary areas of follicles. Hyperplasia of epithelia is present, and necrosis and phagocytosis of heterophils and plasma cells occur, followed by atrophy and fibroplasia of the bursa [5, 6, 31, 38].

Immunization is the principal method used for the control of IBDV infection. Especially important is the immunization of breeder flocks in order to confer maternal immunity to the progeny. However, an universal vaccination program can not be suggested due to the variability in maternal immunity, genetic potential, and management [3, 4, 9, 17, 21, 24, 31, 36, 43, 45].

The purpose of this study was to determine, first, the comparative severity of gross, histopathologic, and ultrastructural lesions of the bursa of Fabricius in chickens to IBD virus, and second, the site of IBD virus replication and persistence.

MATERIALS AND METHODS

This study was conducted at the University of Florida, Poultry Research Buildings, Department of Large Animal Clinical Sciences, College of Veterinary Medicine, and at the University of Costa Rica, Electron Microscopy Laboratory.

Specific-pathogen-free chickens (SPFAS Inc., Norwich, Con 06360) and chickens from six genetic lines and five cross-mating (Dekalb Poultry Research, Inc., Chicago, Illinois 0115) were used in these studies.

A commercial IBD intermediate live vaccine (D78, Intervet America Inc.) and Standard USDA field challenge virus (STC, 12/06/93, provided by the University of Delaware) were used in these experiments. The IBD challenge virus was propagated in 9 to 11-day-old SPF embryonated eggs. Tryptose-phosphate broth with antibiotics (0.61 gm penicillin, 1.31 gm streptomycin sulphate, and 25 gm amphotericin B in 100 mL) was used as the diluent. Chicken embryos were in-

oculated by the chorioallantoic membrane route with 0.1 mL of inoculum using a 1 mL syringe with a 20 gauge, 1 ½ inch needle. Infectious chorioallantoic fluid and tissues were pooled, the EID_{50} determined, and the virus was stored at – 70°c in 1 mL aliquots in sterile screw cap vials. Titers were determined by the method of Reed and Muench [35]. Thirty SPF chickens, three weeks old, were inoculated by eye drop route with $10^{4.5}$ EID_{50} [44]. Three days after infections, the 30 SPF chickens clinically affected were killed and their bursa of Fabricius were collected and stored at –70°C to harvest the IBD virus. All bursa tissues were homogenized and prepared using 20% bursa weight:volume in tryptose broth with antibiotics. The bursa homogenate was centrifuged at 1,500 xg for 20 minutes, and the supernate was collected and stored frozen at – 70°c until it was used as inoculum. Titration of IBD was performed using the chorioallantoic membrane (CAM) route of inoculation in 9 to 11 day-old SPF embryonated eggs. Each dilution, ranging from 10^{-3} to 10^{-6}, was inoculated into three eggs. All eggs were candled daily and deaths occurring within the first 48 hours were eliminated from the calculations. Seven days post-inoculation, the number of infected and dead embryos were recorded and the 50% endepoint determined by the method of Reed and Muench [36]. Each experimental chicken in the six groups received a dose of $10^{4.5}$ EID_{50} of the inoculum by eye drop route.

Before and after IBD vaccination and after IBDV challenge, samples of bursa of Fabricius from experimental chickens (lines and cross-mating) and SPF chickens were fixed in 10% buffered formaldehyde, embedded in paraffin, sectioned and stained with haematoxylin and eosin. Tissues were examined and scored for histopathologic changes on a scale of: 0, no lesion; 1, mild-necrosis in isolated follicles; 2, moderate-generalized lymphocyte depletion or isolated follicles with severe depletion; 3, marked-over 50% of follicles with severe lymphocyte depletion; 4, severe only outline of follicles remaining with few lymphocytes and increase in connective tissue, cysts and thickened corrugated epithelium; 5 atrophy-fibroplasia and loss of all follicular architecture [30]. Also, to determine degree of bursal atrophy bursa/body weight ratios were calculated as bursa of Fabricius weigth x 100/body weigth. Bursa of Fabricius samples were collected and prepared for electron microscopic study. These samples were fixed in a mixture of 2,5% glutaraldehyde, 1% paraformaldehyde in cacodylate buffer, and post-fixed in 1% osmium in cacodylate buffer [12]. The samples were embedded in low density resin (Spurr, Polyscience, Warrington, PA), sectioned at 60 to 90 nm and stained with uranyl acetate and lead monoxide [20]. This technique was performed to identify the presence of IBD virus and the associated ultrastructural changes in the bursa tissues. Fluid suspension of bursa samples from infected chickens were centrifuged at 10,000 xg and the supernatant prepared by negative staining technique with phosphotungstic acid for 2 minutes to observe the morphology of the IBD virus [20].

Isolation attempts were performed by homogenized selected tissues in 3.5 mL TPB broth plus antibiotics and maintained in sterile screw tubes at – 70°c until SPF embryos were inoculated. Three embryos per sample were inoculated and observed daily for 7 days for evidence of pathologic changes and death. Blood agar plates were used to detect bacterial contamination of the inoculum [45].

Data were processed in a PC computer by using the SAS Program [41]. The data for body weight, bursa weight, and bursa:body weight ratios, were compared using Least square analysis of variance (ANOVA). Histophatological scores for bursa damage were analyzed by estimating proportions for each line, cross-mating and SPF chickens during the experiments [32].

RESULTS

Experiment 1: Microscopic Lesions

Neither clinical signs of IBD infection nor macroscopic lesions in the bursa of Fabricius were observed in the six lines, five cross-mating and SPF chickens in the experiment one. The total mortality during this experiment was 4.9% between to 2-3 weeks of age, due to diarrhea and cannibalism.

Least square (LS) analysis of variance for body weight (BW) showed statistical differences ($P<0.0001$) for the effect of lines, cross-mating, week, and the interaction cross-mating*week with a coefficient of variation of 18.29%. Also, least square analysis of variance for bursa:body weight (B:BW) ratios during experiment one showed significant differences ($P< 0,001$) for the effect of lines, cross-mating, week, and the interaction line*week, TABLE I.

At 4 weeks of age (before IBD vaccination), no cross-mating, line and SPF chickens showed evidence of bursal damage or atrophy in any of the 12 bursae evaluated from each group. The B:BW ratios did not show evidence of bursal atrophy in the experimental chickens.

At 8 weeks of age (4 weeks after IBD vaccination), microscopic evidence of bursal lesions was detected in lines, cross-mating, and SPF chickens (12 bursae sampled from each group), FIGS. 1 y 2. Results of histopathological examination were scored and had the following proportions: 1=69.11%, 2=29.42%, and 3=1.48% for lines and cross-mating chickens, and 1=25%, 2=80% for SPF chickens. The B:BW ratios decreased for all experimental groups with a range between 0.33 to 0.66. In addition, the B:BW ratios decreased at 8 weeks of age when these results were compared to the B:BW ratios at 4 weeks of age.

At 11 weeks of age (7 weeks after IBD vaccination) microscopic evidence of bursal degeneration and necrosis was detected in lines, cross-mating, and SPF chicken (12 bursae samples from each group). Results of histopathological examination were scored and had the following proportions:

TABLE I

EXP, 1, LEAST SQUARE (LS) MEANS AND STANDARD ERROR (SE) FOR BURSA:BODY WEIGHT RATIOS OF EXPERIMENTAL CHICKENS AND SPF CHICKENS BEFORE (4 WEEKS) AND AFTER (8 AND 11 WEEKS) IBD VACCINATION

Experimental	Weeks of age					
Group	4		8		11	
	MEAN[1] ± SE		MEAN[1] ± SE		MEAN[1] ± SE	
$CROSS_1$	0,707	0,038	0,661	0,054	0, 207	0,038
$CROSS_2$	0,590	0,038	0,499	0, 054	0,259	0,038
$CROSS_3$	0,516	0,038	0,324	0 054	0,133	0,038
$CROSS_4$	0,613	0,038	0,429	0,054	0,123	0,038
$CROSS_5$	0,640	0,038	0,483	0,054	0,134	0,038
LINE A_1	0,440	0,038	0,394	0,054	0,101	0,038
LINE A_2	0,431	0,038	0,331	0,054	0,110	0,038
LINE B	0,447	0,038	0,356	0,054	0,150	0,038
LINE C_1	0,640	0,038	0,490	0,054	0,135	0,038
LINE C_2	0,570	0,038	0,396	0,054	0,133	0,038
LINE C_3	0,553	0,038	0,347	0,054	0,148	0,038
LINE D_1	0,721	0,038	0,435	0, 054	0,186	0,038
LINE D_2	0,826	0,038	0,547	0,054	0,199	0,038
LINE E_1	0,671	0,038	0,479	0,054	0,132	0,038
LINE E_2	0,740	0,038	0,486	0,054	0,175	0,038
LINE E_3	0,742	0,038	0,486	0,054	0,170	0,038
LINE F	0,652	0,038	0,447	0,054	0,159	0,038
SPF	0,691	0,038	0,336	0,054	0,295	0,038

[1]bursa: body weight ratios (gm).

1=2.41%, 2=9.64%, 3=60.24%, and 4=27.71 for lines and cross-mating chickens, and 2=80%, and 3=20% for the SPF chickens. B:BW ratios showed evidence of bursa atrophy with range of 0.10 to 0.29, which would be considered atrophy for chickens at 11 weeks of age.

Histopathological results at 4 and 7 weeks after IBD vaccine administration and the B:BW ratios demonstrated that live intermediate IBD vaccine could cause mild to marked lesions in the bursae of Fabricius, FIGS. 1 y 2.

Ultrastructural Lesions

At 4 weeks of age (before IBD vaccination) lines, cross-mating chickens and SPF chickens showed no evidence of ultrastructural lesions.

At 8 and 11 weeks of age (4 and 7 weeks after vaccination) lines, cross-mating chickens and SPF chickens presented ultrastructural lesions in the bursae of Fabricus. Transmission electron microscopy (TEM) indicated the presence of viral particles in the cytoplasm of lymphocytes and macrophages in the bursae of Fabricius from samples at 4 weeks after IBD vaccine administration. The average diameter of virus particles was 50 nm and failed to show a crystalloid pattern or a surrounding membrane, FIG. 3.

Ultrastructurally, lymphocytes were shrunken and contained lipid vacuoles and mild to moderate disorganization of cytoplasmic organelles. Nuclear chromatin was aggregated. Degenerative and necrotic lymphocytes appeared as masses of electron-dense material and was usually observed within the cytoplasm of macrophages. Conversely, at 7 weeks after IBD vaccine administration, no evidence of viral particles was detected in lymphocytes or any other cell in the bursae of Fabricius.

Experiment 2: Microscopic Lesions

In the experiment two, clinical signs were first observed in SPF chickens 3 days after IBDV challenge and 4 days after IBDV challenge in lines and cross-mating chickens. Clinical signs were depression, ruffled feathers, green diarrhea, anorexia, postration and death. Macroscopically, 3 days after challenge, the bursae of infected chickens showed an increase in size and weight, edema, and hypremia. In the chickens which died during the course of disease, a marked hemorrhagic diathesis. In addition severe bursae edema and necrosis were observed, FIG. 4. At 7 and 24 days after challenge the bursae were decreased in size and weight, sometimes with yellow to gray coloration. After IBDV challenge the total daily mortality

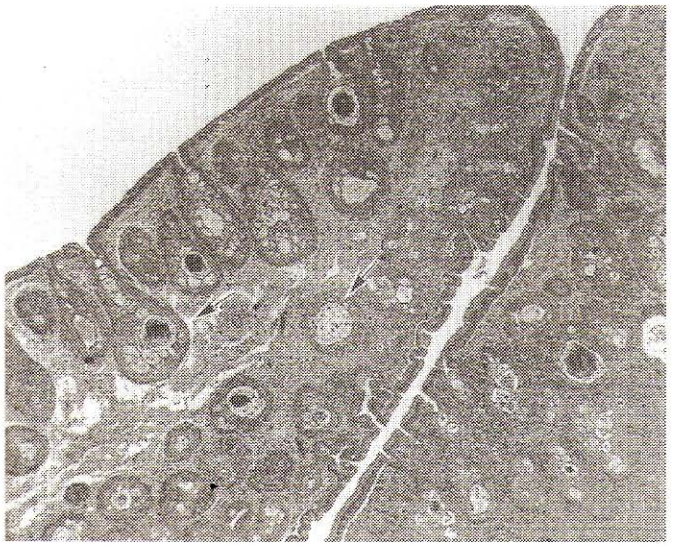

FIGURE 1. MICROGRAPH OF THE BURSA OF FABRICIUS FROM 8 WK OLD CHICKEN, 4 WK AFTER IBD VACCINE ADMINISTRATION. BURSITIS SHOWING LYMPHOID FOLLICLES WITH LYMPHOCYTIC DEPLETION (ARROW). HE X 40.

was 4% on lines and cross-mating chickens and 29% on SPF chickens.

The LS analyses of variance for BW of line, cross-mating chickens and SPF chickens showed significant differences ($P<0.0001$) for the effect of line, cross-mating and day; and did not show significant differences for the interaction cross-mating, with a coefficient of variation of 15.2%, TABLE II.

At 33 days of age (3 days after IBDV challenge) lines, cross-mating chickens and SPF chickens demonstrated microscopic evidence of bursal lesion. Histopathological examination of bursa tissues from lines and cross-mating chickens were scored with the following proportion: 1=19.59%, 2=37.55%, 3=35.51%, 4=7.35% and 4=100% for SPF chickens. Histopathological results of bursa tissues from SPF chickens showed stronger evidence of bursal lesions than the bursal lesions in lines and cross-mating chickens. Least square analysis of variance for B:BW ratios showed significant differences ($P>0.0001$) for the effect of line, cross-mating and day, the interaction cross-mating*day, with a coefficient of variation of 43.46% B:BW ratios increased at 3 days after IBDV challenge probably due to acute inflammation in the bursa tissues.

At 37 days of age (7days after IBDV challenge) in lines, cross-mating chickens and SPF chickens microscopic evidence of bursal lesions increased. Histopathological results showed the following occurrence: 1=0.83%, 2=6.2%, 3=29.63%, 4=63.37% for lines and cross-mating chickens and 4=100% for SPF chickens. Histopathological results from bursa tissues of SPF chickens showed more bursal damage than lines and cross-mating chickens. Bursa:body weight ratios were decreased at 7 days after IBDV challenge probably due to atrophy in the bursa tissues, FIGS. 5 y 6.

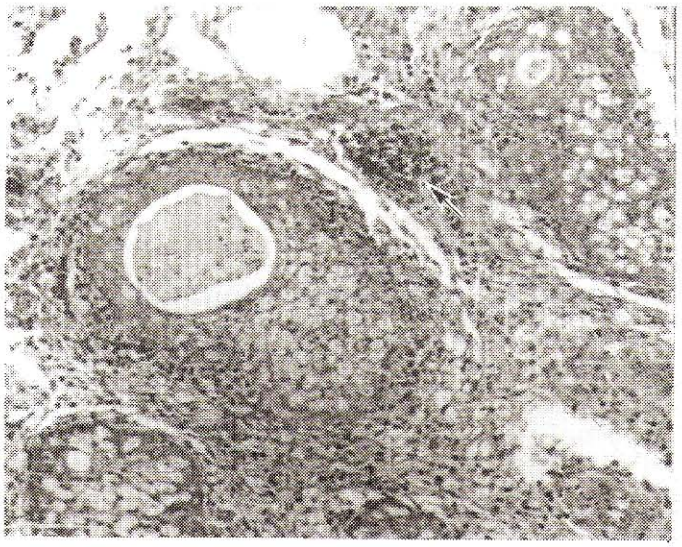

FIGURE 2. HIGHER MAGNIFICATION FROM FIG. 2 ONE FOLLICLE (CENTER) WITH NECROSIS AND NECROTIC DEBRIS IN THE MEDULLA (ARROW). INTERSTITIAL EDEMA AND INFILTRATION OF INFLAMMATORY MONONUCLEAR CELLS (ARROW). HE X 200.

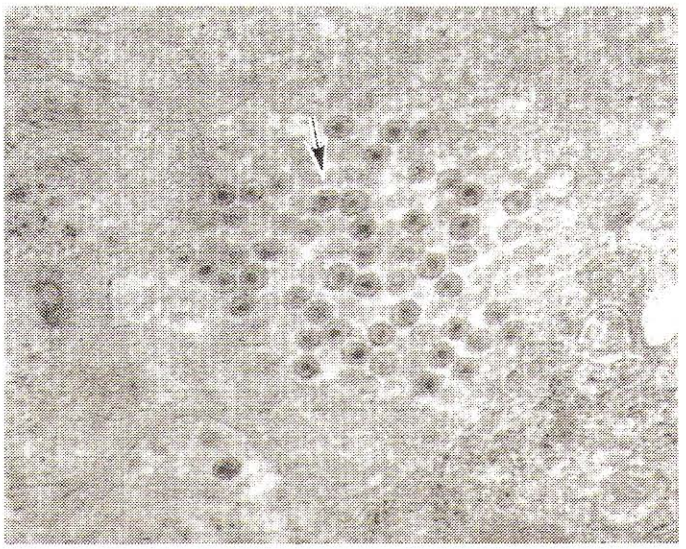

FIGURE 3. TRANSMISSION ELECTRON MICROGRAPHS (TEM) OF THE BURSA OF FABRICIUS FROM 8 WK OLD CHICKEN, 4 WEEKS AFTER IBD VACCINE ADMINISTRATION. IBD VIRUS PARTICLES (ARROW) NON-MEMBRANE–BOUND WITH CENTRAL ELECTRON-DENSE CORE IN THE CYTOPLASM OF LYMPHOCYTE CELL. X 30,000.

At 58 days of age (24 days after IBDV challenge) lines, cross-mating chickens and SPF chickens presented microscopic lesions in the bursa of Fabricius at the following proportions: 3=7.32%, 4=45.12%, 5=47.56% for lines and cross-mating chickens and 4=33.33%, 5=66.66% for SPF chickens. Histopathological results of bursa tissues from SPF chickens showed increased bursal damage compared to lines and cross-mating chickens. Bursa:body weight ratios showed evi-

dence of bursa atrophy 24 days after IBDV challenge in lines, cross-mating chickens and SPF chickens, FIGS. 7 y 8.

Ultrastructural Lesions

The first ultrastructural change was indentified in the medullary area of the bursal follicles 3 days after IBDV challenge. Degeneration and necrosis of lymphocytes in the follicles with condensation and margination of nuclear chromatin of lymphocytes were observed. Bursal cells were widely separated due to accumulation of intercellular edema fluid and infiltration of macrophages and heterophils. Viral particles were observed in the cytoplasm of lymphocytes and macrophages. The degree of degeneration and necrosis of lymphocytes was greater in the SPF chickens than lines and cross-mating chickens throughout experiment two.

At 7 days after challenge, ultrastructural changes observed in the bursae follicles were similar to those described previously, but lesions were more severe and disseminated. Frequently, lipid droplets were seen in the cytoplasm of macrophages, lymphoid cells, and reticular cells, also in phagolysosomes of macrophages. Virions were seen in the cytoplasm of lymphocytes and macrophages. The virions were 50 to 55 nm in diameter with an electron-dense core. Virions appeared in a

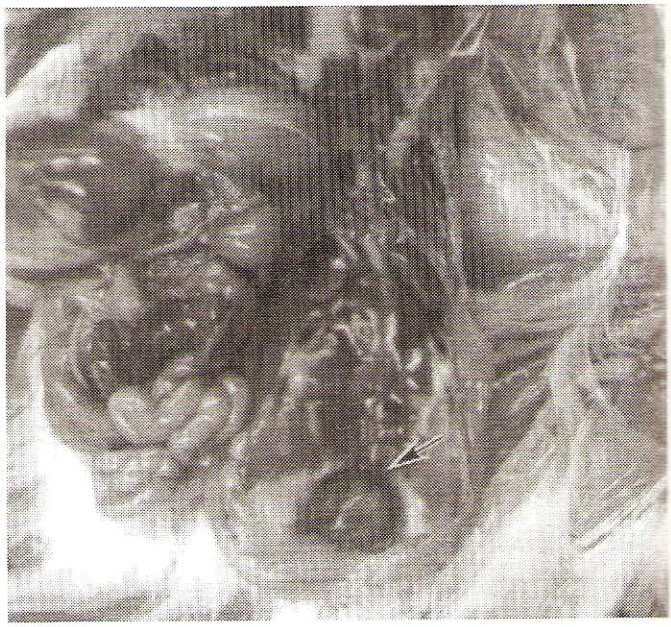

FIGURE 4. PICTURE OF 4 WK OLD SPF CHICKEN, 3 DAYS AFTER IBD STANDARD VIRUS CHALLENGE BY EYE ROUTE. PATHOGNOMONIC LESIONS OF THE BURSA OF FABRICIUS INCLUDING SWELLING AND HEMORRHAGE (ARROW).

TABLE II

EXP. 2. LEAST SQUARE (LS) MEANS AND STANDARD ERROR (SE) FOR BURSA:BODY WEIGHT RATIOS OF EXPERIMENTAL CHICKENS AND SPF CHICKENS AFTER (3, 7, AND 24 DAYS) IBDV CHALLENGE AT 4 WEEKS OF AGE

Experimental	Days after challenge					
Group	3		7		24	
	LS MEAN[1] ±	SE	LS MEAN[1] ±	SE	LS MEAN[1] ±	SE
$CROSS_1$	0,59	0,031	0,24	0,031	0,11	0,031
$CROSS_2$	0,55	0,031	0,25	0,031	0,11	0,031
$CROSS_3$	0,51	0,031	0,26	0,031	0,09	0,031
$CROSS_4$	0,61	0,031	0,35	0,031	0,10	0,031
$CROSS_5$	0,50	0,031	0,25	0,031	0,11	0,031
LINE A_1	0,41	0,031	0,22	0,031	0,10	0,031
LINE A_2	0,37	0,031	0,21	0,031	0,10	0,031
LINE B	0,37	0,049	0,26	0,049	0,10	0,035
LINE C_1	0,49	0,031	0,21	0,031	0,10	0,031
LINE C_2	0,50	0,032	0,28	0,031	0,10	0,031
LINE C_3	0,45	0,031	0,23	0,031	0,10	0,031
LINE D_1	0,60	0,031	0,31	0,031	0,18	0,031
LINE D_2	0,64	0,031	0,43	0,031	0,24	0,031
LINE E_1	0,59	0,031	0,31	0,031	0,11	0,032
LINE E_2	0,82	0,031	0,45	0,031	0,15	0,033
LINE E_3	0,75	0,031	0,37	0,031	0,10	0,031
LINE F	0,61	0,031	0,31	0,031	0,14	0,031
SPF	0,48	0,025	0,20	0,025	0,10	0,025

[1]burga: body weight ratios (gm).

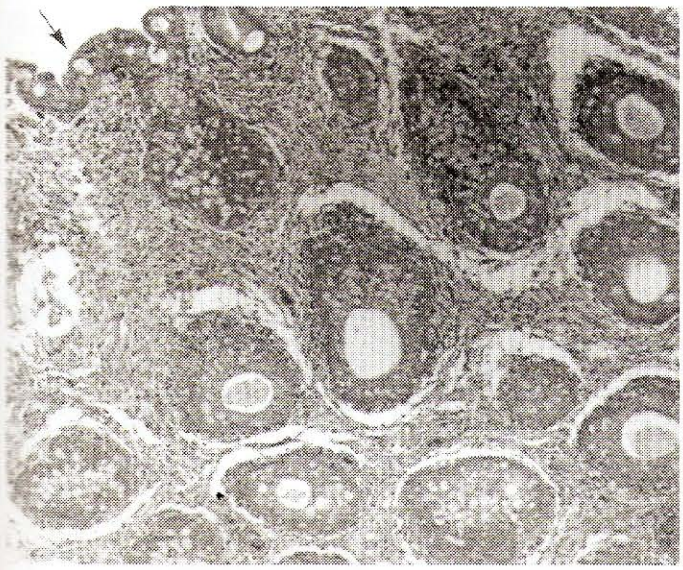

FIGURE 5. MICROGRAPHS OF THE BURSA OF FABRICIUS FROM 5 WK OLD CHICKEN, 7 DAYS AFTER IBD STANDARD VIRUS CHALLENGE. LYMPHOID FOLLICLES WITH SEVERE ACUTE NECROSIS AND LYMPHOID DEPLETION, EDEMA, AND CYSTIC EPITHELIA (ARROW). HE X 80.

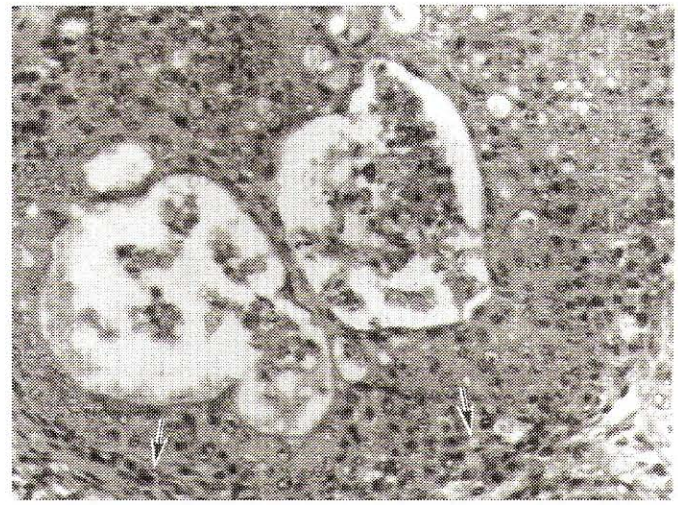

FIGURE 6. MICROGRAPHS OF THE BURSA OF FABRICIUS FROM 5 WK OLD CHICKEN, 7 DAYS AFTER IBD STANDARD VIRUS CHALLENGE. LYMPHOID FOLLICLES WITH SEVERE ACUTE NECROSIS AND LYMPHOID DEPLETION IN THE MEDULLARY AREA, AND REMANENTS LYMPHOCYTES IN THE CORTICAL AREA (ARROW). HE X 200.

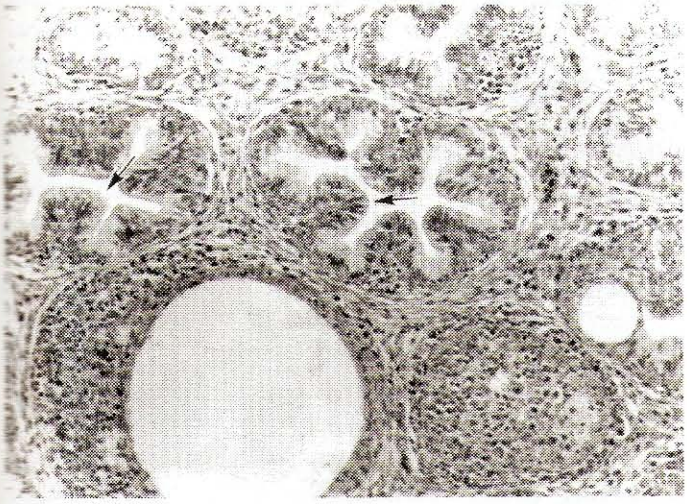

FIGURE 7. MICROGRAPHS OF THE BURSA OF FABRICIUS FROM 8 WK OLD CHICKEN, 24 DAYS AFTER IBD STANDARD VIRUS CHALLENGE. MOST LYMPHOID FOLLICLES REPLACED BY ADENOMATOUS STRUCTURES (ARROW) AND CYSTIC WITH SEVERE ACUTE NECROSIS AND LYMPHOID DEPLETION IN THE MEDULLARY AREA, AND REMANENT LYMPHOCYTES IN THE CORTICAL AREA. HE X 200.

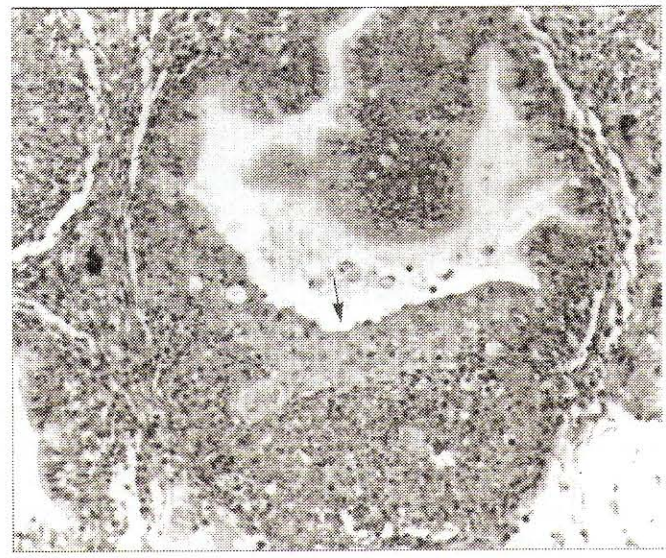

FIGURE 8. MICROGRAPHS OF THE BURSA OF FABRICIUS FROM 8 WK OLD CHICKEN, 24 DAYS AFTER IBD STANDARD VIRUS CHALLENGE. LYMPHOID FOLLICLE WITH SEVERE DEPLETION AND REPLACED BY ADENOMATOUS INTRAFOLLICULAR STRUCTURES IN THE MEDULLARY AREA (ARROW). HE X 200.

hexagonal arrangement surrounded by a single membrane, FIGS. 9,10,11.

At 24 days after challenge, the most common lesions observed were degeneration and necrosis of lymphoid cells, an increase in plasma cells and macrophages, proliferation of epithelial cells, and fibroplasias. By TEM, neither evidence of viral particles nor lymphocyte regeneration were seen.

Virus Isolation and Persistence

Virus isolation was performed in chicken embryos using homogenized bursal tissues from lines, cross-mating chickens and SPF chickens. The presence and persistence of the IBD standard virus was demonstrated. The homogenized bursal suspension on the chorioallantoid membrane (CAM) of embryonating eggs was positive to IBDV replication and ranged

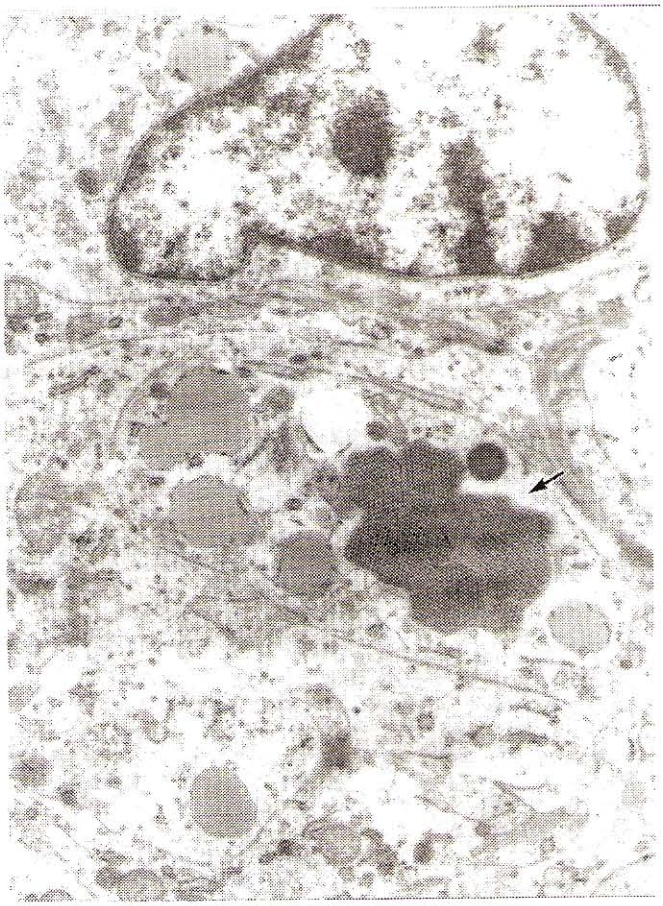

FIGURE 9. TRANSMISSION ELECTRON MICROGRAPH (TEM) OF THE BURSA OF FABRICIUS FROM 5 WK OLD CHICKEN, 7 DAYS AFTER IBDV CHALLENGE. IBD VIRAL INCLUSION IN A CRYSTALLINE FORM [ARROW] ORGANIZED IN A HEXAGONAL PATTERN IN THE CYTOPLASM OF A LYMPPHOID CELL. X 12,500.

from 40% to 93% for the total inoculated eggs, 3 days after challenge and 50% to 80% were positive, 7 days after challenge. Typical IBD lesions included edema and plaques on the CAM, and stunting, cutaneous hemorrhage, enlargement of liver and sometimes the spleen, pale liver, and poor feathers development were seen, FIG.12. The IBDV was isolated in low percentage, from 6% to 20% of the total SPF embryos inoculated with homogenized bursal samples from infected lines, cross-mating chickens and SPF chickens, 24 days after challenge. The IBD standard virus not only was possible to isolate and its persistence inside of infected experimental chickens at 3, 7, and 24 days after challenge were detected, but also IBDV was demonstrated by TEM when positive homogenized bursae samples were prepared by negative stain technique, FIG. 13.

Discussion and Conclusion

In experiment 1, results showed a relationship between degree of histopathological lesions in bursa of Fabricius and bursa: body weight ratios, TABLE I.

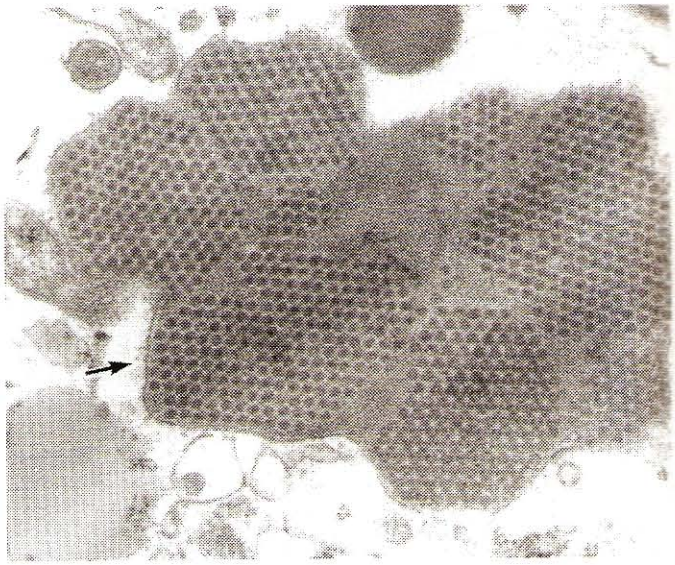

FIGURE 10. HIGHER MIAGNIFICATION FROM FIG. 9. IBD VIRAL INCLUSION SHOWING CRYSTALLINE FORM AND PARTIALLY SURROUNDED BY A SINGLE MEMBRANE [ARROW] AND ORGANIZED IN A HEXAGONAL PATTERN . X 40,000.

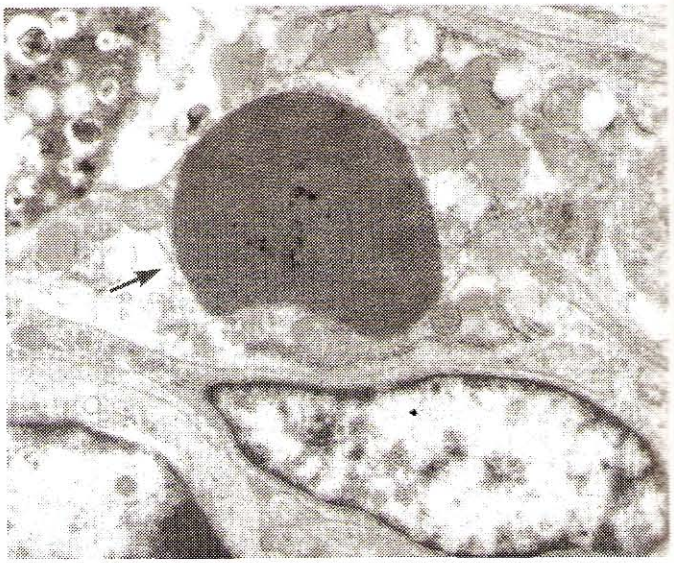

FIGURE 11. TRANSMISSION ELECTRON MICROGRAPH OF THE BURSA OF FABRICUS FROM 5 WK OLD CHICKEN, 7 DAYS AFTER CHALLENGE. IBD VIRAL INCLUSION IN THE CYTPLASM OF A MACROPHAGE SHOWING CRYSTALLINE ARRAY AND PARTIALLY SURROUNDED BY A SINGLE MEMBRANE (ARROW). X 30,000

Decreasing bursa: body weight ratios (B:BW) were observed 4 and 8 weeks after IBD vaccine administration which may imply a concurrent potential decrease in immune competence of Dekalb (lines and cross-mating) and SPF chickens, TABLE I. Similar results also have been reported by other investigators [15, 21, 22, 23, 26, 31, 44]. In this experiment, it

was demonstrated that moderate to marked bursal atrophy and bursal depletion occurred following D78 vaccine administration at 4 weeks of age. This demonstrate that an intermediate live vaccine virus, such as D78, may be indistinguishable from intermediate field IBD virus strains. A reduction in lymphocytes in the bursa of Fabricius implies potential dysfunction of the immune response and an increased disease susceptibility. Additionally, if the vaccine virus strain has the ability to remain viable outside the chicken and/or increase in virulence following serial chicken passages, it would establish a potential persistent infection in the poultry house.

Histopathological lesions in the bursae of Fabricius were demonstrated in this study 4 and 7 weeks after IBD vaccine administration. Likewise, B:BW ratios decreased in all lines and cross-mating chickens and SPF chickens. Confirmation that IBD vaccine virus caused lesions in the bursae of Fabricius was demonstrated by detection of ultrastructural lesions in lymphocytes after vaccine administration. Lesions included changes in cytoplasm organelles, nuclear chromatin margination, degeneration and necrotic lymphocytes, and macrophages with phagolysosoms. IBD viral particles were seen in the cytoplasm of lymphocytes and macrophages. Therefore, these findings suggest that the IBD virus strain used as vaccine was capable of replicating in bursa cells. The morphology and size of these virions were similar to those described in previous studies [11, 13, 31, 34, 37, 40]. These results could be explained by the presence of field IBD virus challenge. This must be considered, as in this study the experimental chickens and SPF chickens were not in isolation units, but battery type units. However, clinical signs of IBD or mortality rate were not observed in the lines and cross-mating chickens and SPF chickens after vaccination.

In experiment two, clinical signs and macroscopic observations after IBDV challenge were similar to those described in previous studies [5, 7, 18, 19, 22, 23, 24, 43]. Mortality observed after IBDV challenge was higher in the SPF chickens (29%) than in the lines and cross-mating chickens (4%). The course of the clinical IBD was between 4^{to} 5 days. The lines and cross-mating chickens showed few clinical signs while SPF chickens demonstrated marked clinical disease. Duration of clinical disease was short in both experimental chickens and SPF chickens. These results suggest that experimental chickens had better resistance to the IBDV challenge, probably because they were protected by residual maternal antibody titers or due to genetic resistance.

Microscopically, extensive bursa lesions were observed in experimental chicken and SPF chickens at 3 and 7 days after IBD standard virus challenge. Lesions persisted through the observation period of 24 days. The severity of bursa lesions were greater and more persistent in SPF chickens than in the experimental chickens. In this study, histopathological results were similar as described by Cheville [5, 6] and Faragher y col. [8].

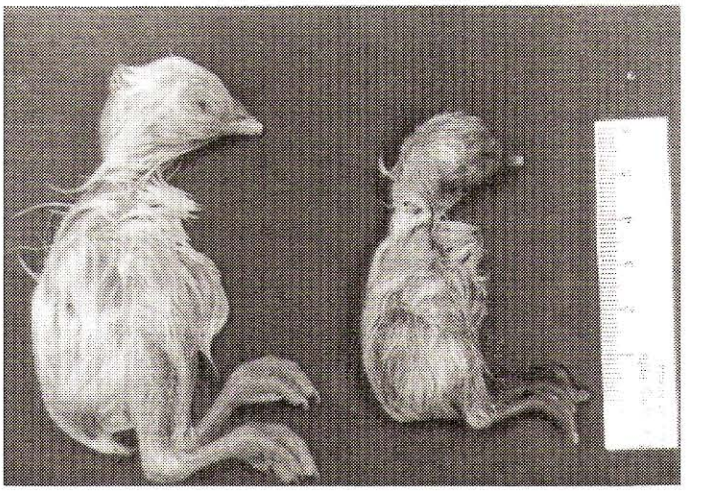

FIGURE 12. PICTURE OF SPF CHICKEN EMBRYOS 17 DAYS AFTER INCUBATION. EMBRYO ON LEFT WAS USED AS A CONTROL, EMBRYO ON RIGHT WAS INOCULATED WITH BURSA HOMOGENATE FROM EXPERIMENTAL CHICKENS INFECTED WITH IBD VIRUS AT 10 DAYS OF INCUBATION. INOCULATED EMBRYO WAS STUNTED AND WITH POOR FEATHERS DEVELOPMENT.

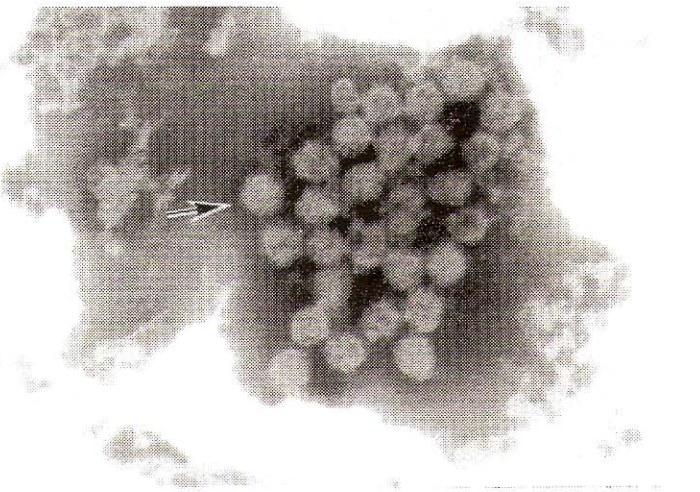

FIGURE 13. INFECTIOUS BURSAL DISEASE VIRUS (IBDV) NEGATIVELY STAINED WITH PTA. VIRUS PARTICLES SHOWING HEXAGONAL SHAPE (ARROW). X 105,000.

At 3 days post-infection, histopathological results were similar as described by Cheville [5] 48 hours after challenge. Lesions in the bursa were characterized by follicular lymphoid necrosis, depletion, and acute inflammation with edema, hemorrhages, and heterophils infiltration. The B:BW ratios increased in weight because of acute inflammation due to pathogenic effects of the IBDV in the bursa cells, TABLE II.

At 7 days post-infection, histopathological results for six lines and one cross-mating chickens, bursa weight returned to standard rate, while most of the other cross-mating and SPF chickens showed bursa atrophy. Lesions in the bursa were ba-

sically the same as described at 3 days but with more severity, TABLE II.

At 24 days post-infection, histopathological lesions increased with severe follicular lymphoid depletion and formation of central cysts lined by cuboidal epithelium, fibroplasia, mononuclear cell infiltration (macrophages, plasma cells) and B:BW ratios decreased for lines and cross-mating chickens and SPF chickens due to chronic inflammation with loss of bursal architecture and fibroplasia.

Ultrastructural lesions observed in the bursa of Fabricius after IBDV challenge were similar as described in prior reports [2, 5, 6, 31, 35]. Although some differences were found such as nuclear membrane disruption, bleb-like structures inside lymphocyte nuclei, and intra-nuclear virus-like particles. These findings have not been described before but there is no sufficient evidence to confirm that these nuclear changes were due to IBDV. Lesions consisted primarily of degeneration and necrosis of lymphoid cells which increased and persisted through 24 days after IBDV challenge. Viral inclusions inside phagolysosoms were seen in the cytoplasm of macrophages and viral inclusions with a hexagonal crystalloid arrangement surrounded by a single membrane were seen frequently in the cytoplasm of lymphocytes. These findings suggest that IBDV replicates in bursal lymphoid cells and macrophages. Käufer and Weiss [18,19] reported that IBDV replicated not only in lymphocytes and macrophages but also in heterohils. Cytolysis of lymphoid cells results in the development of the pathognomonic lesions and persistence immunosuppression due to destruction of immature B-cells in the bursa of Fabricius [2, 5, 6, 16, 41], FIG, 4.

The fact that circulating mature lymphocytes, which presumably left the bursa before IBDV infection, are not significantly infected, probably explains the high IBD antibody titers which are produces by surviving chickens after IBDV challenge [2]. However, a conclusive explanation about the stage of development which makes B lymphocytes the target cells to IBDV is still unknown. Therefore, there are a lot of questions about pathogenesis of IBDV which need to be answered.

Infectious bursal disease virus was isolated from bursa tissues from infected experimental and SPF chickens mainly at 3 and 7 days after challenge. Over 50% of the inoculated SPF eggs were positive of the total inoculated SPF embryos. IBDV was capable of producing typical lesions in inoculated SPF embryos. Affected embryos were stunted with edema, cutaneous hemorrhages, plaque on the CAM, enlargement of liver and sometimes spleen, and poor feather development. Negative staining of homogenated bursae positive to IBDV isolated from infected experimental chickens and SPF chickens showed viral particles 50-55 nm diameter and typical morphology, as described for IBDV [11, 13, 14, 29, 34, 40], FIGS. 12 y 13.

In conclusion, the gross, histopathologic, and ultrastructural changes in the bursa of Fabricius after IBD vaccine administration suggest lesions consistent with immunosuppression in experimental chickens and SPF chickens. In contrast, the gross, histpathologic, and ultrastructural changes in the bursa of Fabricius after IBDV challenge suggest that experimental chickens (lines and cross-mating) had better immune resistance to IBD virus than SPF chickens, which is possibly due to genetic factors.

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