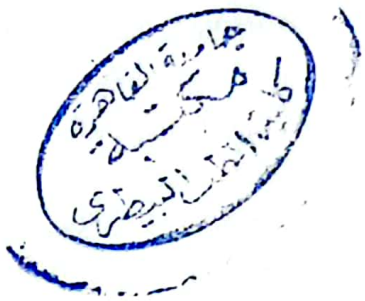
Vet.Med.J.,Giza. Vol.54, No.3. (2006): 649-669

EFFICACY AND PATHOGENICITY OF THREE LIVE INFECTIOUS BURSAL DISEASE VACCINES (INTERMEDIATE PLUS STRAINS) IN COMMERCIAL NATIVE CHICKENS BREED IN EGYPT.

ABDEL-ALIM, G. A, and KAWKAB, A. AHMED*
Faculty of Veterinary Medicine, Cairo University
Department of Poultry Diseases
* Department of Pathology

Received: 12. 4. 2006.
Accepted: 27. 4. 2006.



SUMMARY

In this study, three of live intermediate plus IBDV vaccines (A, B, & C) which are commonly used in Egypt were selected and their pathogenicity and efficacy were investigated. Groups of native breed chicks were vaccinated at 14 days of age with each vaccine by eye drop route and in drinking water then challenged with virulent field IBDV 14 days post vaccination (PV). The efficacy and pathogenicity of each vaccine were evaluated based on clinical signs, mortalities, gross lesion, Bursa/ body weight ratio (BF/BW), and histopathological lesions of the bursa. It was found that these vaccines are efficient as they conferred 100% protection in all vaccinated and challenged groups compared with 20% mortality in unvaccinated challenged group. However, they did not prevent bursal atrophy or histological lesions. The bursal atrophy was observed at 7days PV in groups vaccinated with vaccine (A) while it was observed at 10 days PV in groups vaccinated with vaccines (B) and (C). Vaccine (A) was proved to be more invasive compared with vaccines (B) and (C) as evidenced by higher bursal lesion scores and lower relative BF/BW ratios at certain intervals PV. When vaccine B and C were given in drinking water, moderate to severe bursal changes were observed. Meanwhile, when these vaccines (B & C) were given by eye drop route, mild to moderate changes in the bursa were observed indicating that vaccination by eye drop route would be better than in drinking water. It would be concluded that, all studied vaccines are efficacious and they vary in invasiveness and pathogenicity. Vaccines (B) and (C) are less pathogenic than vaccines (A) and the vaccination by eye drop route will result in less severe bursal le-

sions and better immune response than in drinking water.

INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious viral disease of young chickens which is characterized by destruction of the lymphoid cells in the Bursa of Fabricius; other lymphoid organs are also affected but to a lesser degree (Cheville, 1967). IBD virus (IBDV) is a member of the Birnaviridae family that contains two segments of double stranded RNA. Of the two serotypes, only serotype 1, which display a wide variation is pathogenic potential, is virulent (Lukert and Saif 2003). In fully susceptible chicken flocks (between 3 and 6 weeks of age), the disease is responsible for severe losses due to impaired growth and death, and from excessive condemnation of carcasses because of skeletal muscular hemorrhages (Lukert and Saif 2003). Susceptible chickens less than three weeks of age do not exhibit clinical signs (Kinenge et al., 1988) but have a subclinical infection characterized by microscopic lesions in the Bursa of Fabricius (Winterfield et al., 1983) and immunosuppression (Saif, 1991).

Because of the stability of IBDV in environment, control through sanitation and isolation is not practical for commercial poultry production (Benton et.al., 1967; Kibenge et al., 1988). The principal method of control is therefore by vaccination of the dam in order to obtain chickens which have passive immunity for the first 4 to 5 weeks of life. Egg yolk antibodies protect progeny against early subclinical infection. Because the level of passive immunity is variable and unpredictable , a common commercial practice to vaccinate all chicks against IBD with a live vaccine during the first 3 weeks of life (Winterfield et al., 1980). There are two kinds of live vaccines: those that have intermediate virulence and attenuated mild strains (Lukert and Saif 2003). Although both kinds of live vaccines are neutralized by maternal antibodies, the intermediate vaccine are superior to mild vaccines in giving immunity to commercial chickens with maternal antibodies because intermediate vaccines are less affected by maternal antibodies. However, intermediate vaccines vary in virulence; some often can induce severe bursal atrophy and immunosuppression in young chickens (Winterfield and Thacker 1978 Giambrone and Clay, 1986; Mazzariegos et al 1990). In addition, Muskett et al., (1979) reported on the increased virulence of an IBD live vaccine after it was passaged six times in chickens.

In Egypt, since it was first discovered by Elsergany et al., (1974), IBD causes severe economic losses in poultry industry and the occurrence severe outbreaks due to very virulent IBDV were reported (El-Batrawy,1990; Hassan et al., 2002 Abdel-Alim et al., 2003 and Eterradossi et al 2004). In spite of extensive and multiple administration of mild, intermediate and "hot: vaccine

theses severe outbreaks of IBD are still reported in both native and foreign commercial breeds (Saif-Edin et al., 1996; and El-Ebiary et al., 2001; Hassan et al., 2002). Live infectious bursal disease vaccines (intermediate plus) strains are now commonly used in Egypt in an attempt to control such acute IBD outbreaks. However, there is a lack of information regarding the safety and pathogenicity of such vaccines. Therefore, three of the commonly used live intermediate plus IBDV vaccines were selected and their pathogenicity and efficacy were investigated.

MATERIAL AND METHODS

Chickens

A total of 400, one day-old native breed chicks were used in this study. All chicks were obtained from a local commercial company and reared in clean, thoroughly disinfected, separated rooms and were provided with feed and water adlibtum.

Vaccines and viruses:

1- Infectious bursal disease (IBDV) vaccines

Three live commercial intermediate plus infectious bursal disease vaccines designated as A, B and C were used as follow:

- **Vaccine A:** freeze-dried live vaccine CEVA IBD L (serial N0. 1604N2S1A, CEVA-Phlaxia, Hungary).
- **Vaccine B:** freeze-dried live vaccine Bursine Plus (serial No. 1053183A, Forte. Dodge Animal Health, Fort Dodge, Iowa 50501 USA).
- **Vaccine C:** freeze-dried live vaccine Noblis Gumboro 228E (serial No 038916E, Intervet International, B.V. Boxmeer-Holland)

2- Newcastle disease virus (NDV) vaccine

Both live (B1 type, B1 strain) ND vaccine (serial No. 213701, Schering-Plough Animal Health, Millsoro, Delaware, USA), and live (B1 type, Lasota strain) ND vaccine (serial No. 1085208 A, Forte Dodg Animal Health, Iowa 50501, USA) were used for vaccination of chickens at 7th and 21st day of age, respectively via eye drop route.

3- Infectious bursal disease challenging virus

A bursal homogenate containing virulent Egyptian strain of IBDV that has been characterized earlier by RT-PCR-RFLP (Abdel-Alim et al., 2003) was used in this study. Virus titeration in chicken embryo was made by serial 10 fold dilutions of the bursal homogenate and inoculated onto chorioallantoic memberane route as described by Hitchner (1970). The titer was expressed as the 50% embryo infective dose (EID_{50}) per ml and was calculated by the Reed and Muench (1938). Each bird in challenged group was inoculated with $10^{3.5}EID_{50}$/ bird of bursal homogenate containing field virus by oculo-nasal route.

Haemagglutination inhibition (HI) test:

Serum samples collected at first, 7th, 15th, 21th, 28th and 35th days of age from different groups were subjected to HI tests for determination of

haemagglutinating antibodies titer against NDV as described by David et al., (1998). Following completion of the test, the GMT was recorded for each group. These data were used to indicate the level of humoral immunosuppression caused by each treatment.

Quantitative agar gel percipitation test (QAGPT):

Quantitative AGP tests were performed with two fold dilutions of the sera with PBS, and the antibody titers were read 3 days later and antibody titers were calculated as described by Cullen and Wyeth (1975). The test was used to determine the weaning of maternal antibodies and antibodies to IBDV in vaccinated chicken sera using known positive precipitating antigen in form of bursal homogenates containing IBDV (obtained from FAHRP department, Wooster, the Ohio state university, the USA).

Bioassay:

In order to investigate the efficacy of each vaccine, all vaccinated groups and group 8 were inoculated with $10^{3.5}EID_{50}$/bird of velogenic field IBDV by oculo-nasal route. Group 7 were kept as non-vaccinated unchallenged control group. Clinical symptoms, gross lesions and mortality were monitored daily for up to 10 days post challenge (PC). Bursa samples were collected at 3 and 7 days PC for histopathology and determination of mean bursa weight/ body weight ratios and bursal lesion scores.

Histopathological examination:

Bursae from the vaccinated and control groups collected at 3, 7, 10, 14 and 21 days PV and at 3, and 7 days PC were fixed in 10% neutral buffered formalin, routinely processed and stained with Hematoxylin and Eosin (H&E) as described by Bancroft et al., (1990) and evaluated according to methods described by Rosales et al., (1989) as follow: 1= no lesions, normal; 2 = focal, mild cell necrosis or depletion; 3 = multifocal, 1/3 to 1/2 of the following show atrophy; and 4 = diffuse, atrophy of all follicle.

EXPERIMENTAL DESIGN:

A total of 400, one day-old commercial native breed chicks that have maternal antibodies were divided into 7 groups at 14 days of age (just before IBDV vaccination), each group was 50 chicks except group 7 was 100 chicks and then subdivided into group 7 and group 8 (50 chicks/ group) at 28 days of age (just before challenge). Groups 1- 6 were vaccinated with one of the studied IBDV vaccine at 14 days of age via eye drop route and in drinking water. At two weeks PV, all vaccinated groups (1-6) and group 8 were inoculated with $10^{3.5}EID_{50}$/bird of velogenic field IBDV by oculo-nasal route, while group 7 was kept as non vaccinated unchallenged control group. Blood samples were collected at 0, 7, 14, 21 and 28, and 35 days of age to demonstrate the presence of antibodies to IBDV and ND using QAGPT and HI, respectively. The experimental design and different treatments are summarized in the following table:

Groups	Type of vaccine	Route of vaccination at 14 days of age	Challenge with velogenic IBDV at 28 days of age
1	A	Drinking water	+
2	A	Eye drop	+
3	B	Drinking water	+
4	B	Eye drop	+
5	C	Drinking water	+
6	C	Eye drop	+
7	none	–	-
8	none	–	+

Observation of clinical signs, mortalities, gross lesions, histopathological examination of the bursa of Fabricius at different intervals post vaccination (PV) and post challenge (PC), relative bursa weight /body weight ratio, sero-conversion against IBDV by QAGPT and immunosuppression against ND vaccines were used as criteria for evaluation of the efficacy and pathogenicity associated with each vaccine.

Statistical analysis

Bursa weight / body weight (BF/BW) ratio was calculated for each bird by the following formula: (bursa weight / body weight) X 1000. The relative BF/ BW ratios of the vaccinated and challenged groups at each interval were compared with the negative control group for statistical analysis of significance by analysis of variance followed by Fisher least significant difference test as described by Snedecor and Cochran (1967).

RESULTS

Clinical signs and gross lesions

a- Before challenge:

No clinical signs or mortality were observed in all vaccinated and unvaccinated groups at any intervals post vaccination (PV). Bursal atrophy, as measured by the BF/BW ratio was observed at 7 days PV in groups 1 and 2 and at 10 days in groups 3, 4, 5, and 6 and remained throughout the experimental period as compared with control group. Neither signs nor mortality were observed in unvaccinated control group at any interval PV.

b- After challenge:

On the second day PC, typical IBD signs in the form of depression, ruffled feathers, watery diarrhea, prostration and finally death were observed in unvaccinated challenged

groups and ten birds were died with percentage of 20% mortality (10/50) through out the observation period. Gross lesions of dehydration, hemorrhages on the beast and thigh muscles, nephrosis and pathological changes in the bursa of Fabricius were also noticed in this group. Neither signs nor mortality were observed in all vaccinated and challenged groups except bursal atrophy as measured by BF/BW compared with unvaccinated unchallenged control group was observed at 3 and 7 days post challenge (PC).

Results of relative BF/BW ratios

Results of relative bursa weight/ body weight index are shown in Table (1).

a- Before challenge:

No significant differences were recorded between all vaccinated groups compared with unvaccinated groups at 3 days PV. Meanwhile, significant difference in BF/BW ratio was observed in both groups 1 and 2 vaccinated with vaccine (A) in drinking water and by eye drop route, respectively compared with unvaccinated control group at 7 days PV. At 10, 14 and 21 days PV, significant differences were observed between all vaccinated groups compared with unvaccinated control group. On the other hand, significant difference was also observed between group 2 that vaccinated with vaccine (A) by eye drop route and group 6 that vaccinated with vaccine (C) by eye drop route at 10 days PV(1.11+0.23 vs 2.49+ 0.53). Moreover, significant difference was observed between group 2 and group 4 that vaccinated with vaccine (B) by eye drop route at 21 days PV (1.21+ 0.13 vs 2.66+ 0.93). Table (1).

b- After challenge:

At 3 days PC, significant differences were observed between all vaccinated and challenged groups compared with unvaccinated unchallenged control group. At the same time, a significant difference was observed between unvaccinated challenged and unvaccinated unchallenged control group. At 7 days PC, significant differences were recorded between groups 1, 2, 3, 5, 6 and 8 compared with unvaccinated unchallenged control group. However, no significant difference was noticed between group 4 that vaccinated with vaccine (B) by eye drop and unvaccinated unchallenged control group at 7 days PC.

Results of bursal lesion scores

Results of the bursal lesion scores are presente in Table (2).

a- before challenge:

At 3 days PV, the lesion scores in groups (1, 3, 4, 5, 6, and 7) were (3, 2, 1.8, 1, 1.6, 1 ar 1), respectively indicating that a higher lesi score was observed in groups 1 and 2 that we vaccinated with vaccine (A) compared wi other groups and unvaccinated unchalleng groups. A bursal lesion scores between (2-

Table (1) Relative bursa weight/Body weight ratios of commercial native chickens breed vaccinated with live IBDV vaccine (intermediate plus strains) at 14 days of age and challenged with velogenic field IBDV at 28 days of age.

Groups	Relative BF /BW ratio at days PV[A]					BF/BW ratio at days PC[A]	
	3days	7days	10days	14days	21days	3days	7days
1	3.83±0.93[a]	1.46±0.74[b]	1.58±0.54[b]	1.21±0.22[b]	1.67±0.12[b]	1.12±0.31[bc]	1.46±0.86[b]
2	3.21±0.55[a]	1.80±0.52[b]	1.11±0.23[ab]	1.18±0.26[b]	1.21±0.13[ac]	1.26±0.42[b]	1.53±0.86[b]
3	3.21±0.53[a]	2.41±0.73[a]	1.62±0.76[b]	1.48±0.67[b]	1.80± 0.38[b]	1.91±0.70[b]	1.29±0.74[b]
4	3.50±0.62[a]	3.52±1.50[a]	1.30±0.28[b]	1.15±0.36[b]	2.66±0.93[b]	1.42±0.17[b]	2.58±1.17[a]
5	3.80±0.98[a]	2.24±0.67[a]	1.17±0.25[b]	1.25±0.0[b]	1.44±0.45[b]	1.24±0.24[b]	1.11±0.19[b]
6	3.53±0.91[a]	2.72±1.86[a]	2.49±0.53[b]	1.86±0.45[b]	2.34±0.56[b]	1.43±0.39[b]	1.76±0.75[b]
7	3.75±0.50[a]	4.90±0.14[a]	4.13±0.56[a]	4.34±0.11[a]	4.86±0.30	3.36±0.55[a]	4.21±0.43[a]
8	----------					1.49±0.43[b]	1.24±0.25[b]

Each bird received live IBDV vaccine at 14 days of age and challenged with velogenic IBDV at 28 days of age.

A values represent the mean of five chickens per group.

Value within a column followed by the same superscript letter are not significantly different from others ($p < 0.05$).

PV = post vaccination PC = post challenge

Table (2) Bursa lesion scores of commercial native chickens breed vaccinated with live IBDV vaccine (intermediate plus strains) at 14 days of age and challenged with velogenic field IBDV at 28 days of age.

Groups	Days PV					Days PC	
	3days	7days	10 days	14 days	21 days	3 days	7 days
1	3.0	3.0	3.0	2.0	2.0	3.0	2.0
2	2.0	4.0	3.0	3.0	3.0	1.0	2.0
3	1.8	2.0	3.0	3.0	1.3	2.6	2.0
4	1.0	2.0	3.0	2.0	1.5	2.0	1.4
5	1.6	2.0	2.0	2.0	1.5	2.0	2.0
6	1.0	2.5	2.0	2.0	1.3	2.0	1.5
7	1.0	1.0	1.0	1.0	1.0	1.0	1.0
8	-	-	-	-	-	4.0	4.0

Chickens were vaccinated with IBDV vaccine at 14 days of age either by eye drop route and in drinking water

Chickens were challenged with $10^{3.5}$ EID_{50}/bird of velogenic IBDV at 28 days of age by oculo-nasal route

PV = post vaccination PC = post challenge

Table (3) Antibody responses in commercial native breed chickens vaccinated with live intermediate plus IBDV vaccines and NDV vaccines.

Groups	HI Geometric means against NDV (Age)						Mean of antibody titre against IBDV by OAPT / (Age)				
	0 D	7 D	14 D	21 D	28 Ds	35 D	0 D	7-D	14-D	21-D	28-D
1	7.6	6.6	3.2	2.6	3.2	4.0	1.3+ 0.80	1.0	0	1.45±31[a]	1.95± 0.30[a]
2	7.6	6.6	3.8	2.8	3.2	5.0	1.3+ 0.80	1.0	0	1.50±53[a]	2.00± 0.45[a]
3	7.6	6.6	3.6	3.0	3.6	4.2	1.3+ 0.80	1.0	0	1.57± 34[a]	2.15± 0.14[a]
4	7.6	6.6	4.0	3.0	3.4	5.6	1.3+ 0.80	1.0	0	1.62± 76[a]	2.30± 0.25[a]
5	7.6	6.6	3.0	2.8	3.2	5.8	1.3+ 0.80	1.0	0	1.61±33[a]	2.20± 0.39[a]
6	7.6	6.6	3.2	4.0	3.8	6.0	1.3+ 0.80	1.0	0	1.70±42[a]	2.61± 0.52[a]
7	7.6	6.6	3.8	4.3	5.7	6.4	1.3+ 0.80	1.0	0	0	0

All groups were vaccinated with Hitchiner B1 at 7 days of age and with La-sota vaccine strain of NDV at 21 days of age via eye drop route.

Groups 1- 6 were vaccinated with IBDV vaccine (intermediate plus strains) at 14 days of age by either by eye drop route and in drinking water.

Group 7= Blank unvaccinated with IBDV vaccine.

was observed in all vaccinated groups at 10 and 14 days PV. Low bursal lesion score was observed at 21 days PV in groups 3 and 4 that are vaccinated with vaccine (B), and in groups 5 and 6 that are vaccinated with vaccine (C). The highest bursal lesion score of 4 was noticed at 7 days PV in group 2 that was vaccinated with vaccine (A) by eye drop route (Table 2).

b- **After challenge:** A bursal lesion score between 2- 3 were noticed in all vaccinated and challenged groups at 3 and 7 PC. However, a bursal lesion score of 1.4 and 1.5 were observed in group 4 and group 6, respectively. A bursal lesion score of 4 was observed in unvaccinated unchallenged groups at 3 and 7 days PC.

Antibody responses to IBDV and NDV vaccinations

The antibody responses to IBDV and NDV vaccination were summarized in Table (3). The maternal immunity was not detected at 14 days of age as indicated by QAGPT. All vaccinated groups responded to IBDV vaccines and no significance difference in antibody titre was observed between vaccinated groups. Birds receiving any of the three studied IBDV vaccines were able to produce a high antibody titre against ND indicating that non of the IBD vaccines were immunosuppressive. A low HI GM tire was observed in group 1 and 3 (4.0 and 4.2 respectively) at 35 days of age (14 days after vaccination with Lasota strain) compared with other vaccinated groups.

Histopathological changes:

a- Before challenge:

Group (1): Lymphocytic necrosis in both cortex and medulla of the lymphoid follicles which was replaced by acidophilic fibrillar and nuclear debris (Fig.1), marked interfollicular edema associated with mononuclear leucocytic cells infiltrations were also noticed at 3 days PV. At 7 and 10 days PV, the interfollicular edema was more prominent associated with interfollicular leucocytic cells infiltration (Fig. 2). The lymphoid follicles showed lymphocytic necrosis, depletion and vacuolations. Moreover, at 14 and 21 days PV, the bursa showed similar histopathological alterations, the most conspicuous changes were interfollicular hemorrhage and leucocytic cells infiltrations.

Group (2): At 3 days PV, the bursa showed moderate lymphocytic necrosis, depletion and vacuolations (Fig. 3) accompanied with interfollicular edema and leucocytic cells infiltration. At 7 days PV, the examined bursae revealed atrophy of most lymphoid follicles associated with massive heterophilic cells infiltration in the stroma and also invading the

follicles (Fig. 4). At 10 and 14 days PV, some lymphoid follicles appeared repopulated with lymphocytes. Moreover, at 21 days PV, most lymphoid follicles appeared normal.

Group (3): At 3 days PV, the bursa showed lymphcytic necrosis and marked infiltration with heterophils (Fig. 5). At 7 and 10 days PV, the lymphoid follicles showed severe changes confined as complete necrosis and disintegration of cells leaving cyst like spaces of lymphoid depletion containing remnant of cellular debris (Fig. 6), heterophilic cells infiltration (Fig.7) and interfollicular edema and hemorrhage were also noticed. In addition to the previously mentioned changes, the bursal follicles also showed vacuolations and atrophy at 14 and 21 days PV (Fig.8).

Group (4): At 3 days PV, the bursas were histologically normal. While, the only histopathological change noticed at 7 days, was mild lymphocytic depletion in the medulla of some lymphoid follicles. Adversely, at 10 and 14 days PV, some lymphoid follicles appeared moderately vacuolated with lymphocytic necrosis and depletion as well as hemorrhages were also noticed (Fig. 9). Most lymphoid follicles repopulated with lymphocytes at 21 days PV.

Group (5): At 3 days PV, the bursa showed almost normal lymphoid follicles (Fig. 10). Meanwhile, at 7 and 10 days PV, there were vacuolations of the lymphoid follicles, hyperplastic reticuloepithelial cells at the germinal centers. At 14 and 21 days PV, the most obvious changes were proliferation and hyperplasia of reticuloepithelial cells and increase the vacuolations of lymphoid follicles (Fig.11).

Group (6): At 3 days PV, the lymphoid follicles appeared apparent normal. While, at 7 days PV, the medullary portions of some lymphoid follicles appeared as a mass of cellular debris surrounded by vacuolated cortical remnants and scattered few lymphocytes that will regenerate. At 10 and 14 days PV, the bursa showed mild to moderate lymphocytic necrosis and depletion. At 21 days PV, the bursal lymphoid follicles were repopulated with lymphocytes (Fig. 12).

Group (7): No pathological changes were observed. (Fig. 13).

b- After challenge:

Group (1): At 3 days PC, the bursa showed proliferation of the bursal epithelial layer produced a glandular structure of columnar epithelial cells containing basophilic mucin, lymphocytic necrosis and depletion in some lymphoid follicles associated with intrafollicular cyst containing basophilic mucin as well as slight interfollicular hemorrhage (Fig.14). However, at 7 days PC, the bursa showed from mild to moderate lymphocytic depletion in the cortex and medulla of some lymphoid follicles as well as slight interfollicular edema (Fig. 15).

Group (2): At 3 and 7 days PC, the most conspicuous changes were proliferation of the bursal epithelial layer produced a glandular structure of columnar epithelial cells containing basophilic mucin, some lymphoid follicles showed mild to moderate vacuolations and atrophy (Fig. 16),. Some lymphoid follicles showed marked atrophy, other follicles showed lymphocytic necrosis in the medullary portion leaving eosinophilic fibrillar remnants.

Group (3): At 3 days PC, severe histopathological alterations were recorded which mentioned as, lymphocytic necrosis, marked intrafollicular and interfollicular heterophilic cells infiltrations, atrophied bursal follicles and marked interfollicular edema (Fig. 17). At 7 days PC, the bursa showed marked lymphocytic necrosis leaving acidophilic fibrillar debris.

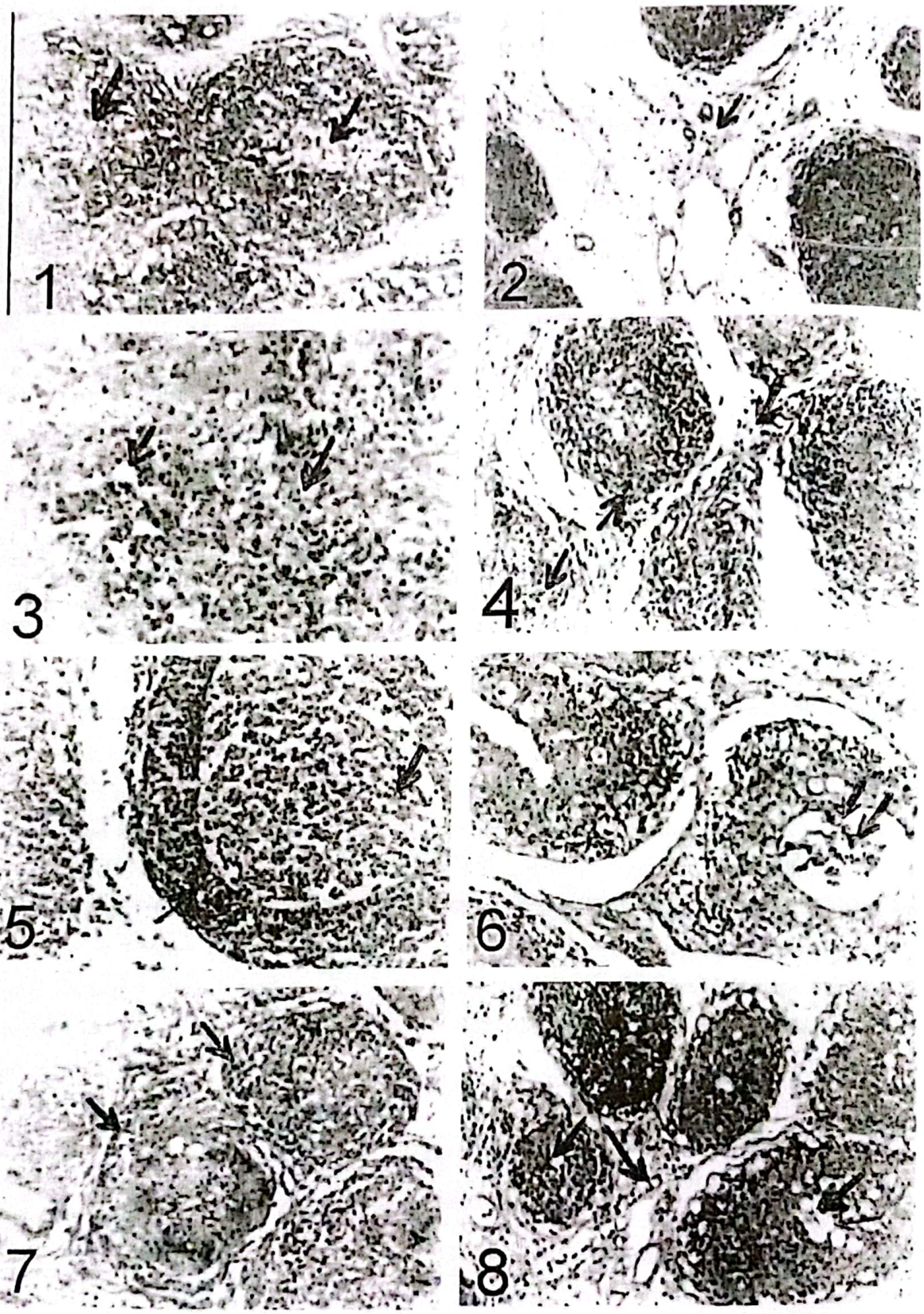
Group (4): At 3 days PC, the bursa showed proliferation of the bursal epithelial layer produced a glandular structure of columnar epithelial cells containing basophilic mucin, some lymphoid follicles showed atrophy associated with marked interfollicular edema. Meanwhile, at 7 days PC, most lymphoid follicles appeared histologically normal.

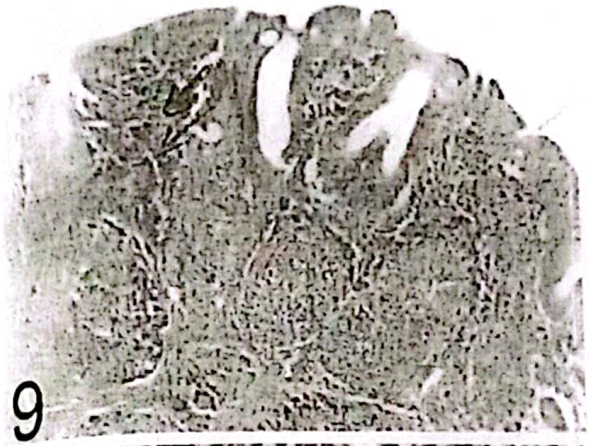
Group (5): At 3 and 7 days PC, there were lymphocytic necrosis leaving remnant of nuclear debris, lymphocytic depletion and vacuolations in lymphoid follicles (Fig.18) associated with atrophy of some follicles and moderate interfollicular edema with few leucocytic cells infiltration. Also, most lymphoid follicles showed from mild to moderate lymphocytic depletion in their medulla and few leucocytic cells infiltrating the interfollicular stroma (Fig.19).

Group (6): No histopathological changes were observed in the collected bursae (Fig.20).

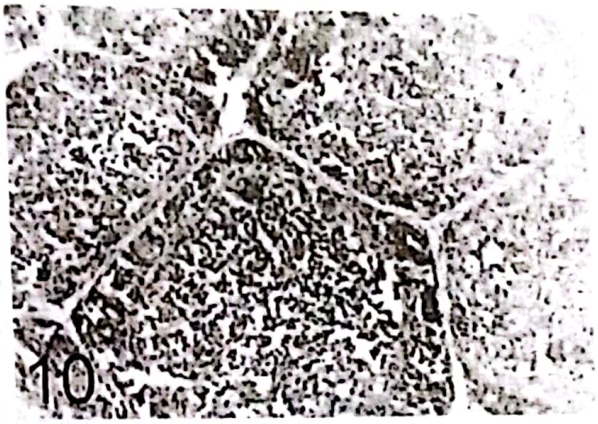
Group (7): No pathological changes were observed.

Group (8): Severe histopathological alterations were recorded at 3 days PC, the lymphoid follicles showed complete necrosis and disintegration of cells leaving cyst like spaces of lymphoid depletion containing homogenous eosinophilic material and remnant of cellular debris (Fig. 21), interfollicular edema and heterophilic cells infiltration were also noticed. In addition, at 7 days PC, the bursas showed marked lymphocytolysis, lymphoid atrophy, marked interfollicular edema and leucocytic cells infiltration (Fig. 22).

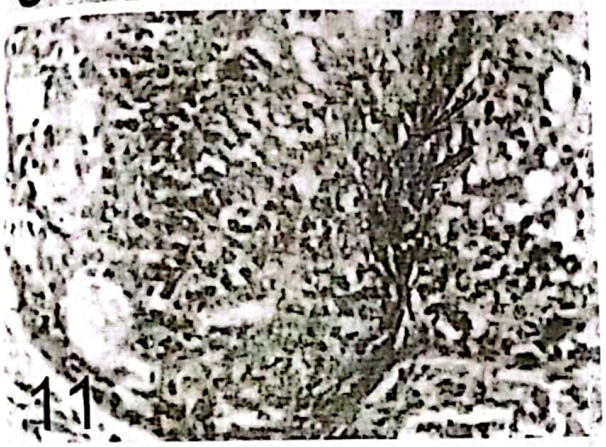

1
2
3
4
5
6
7
8



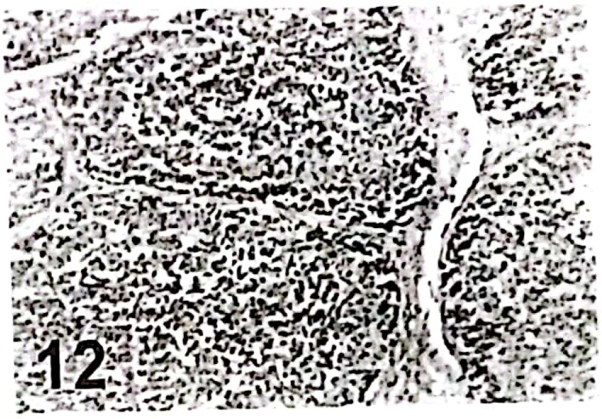
9



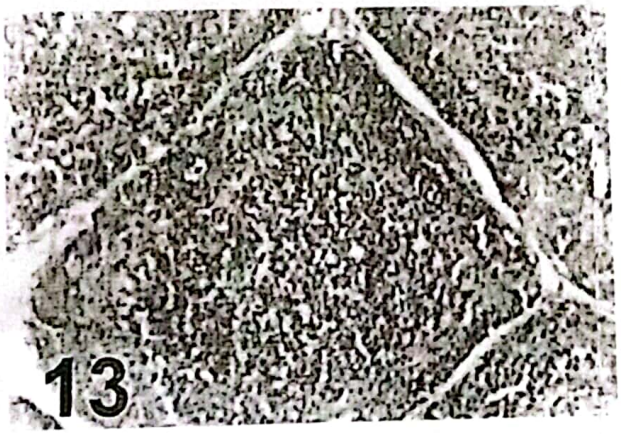
10



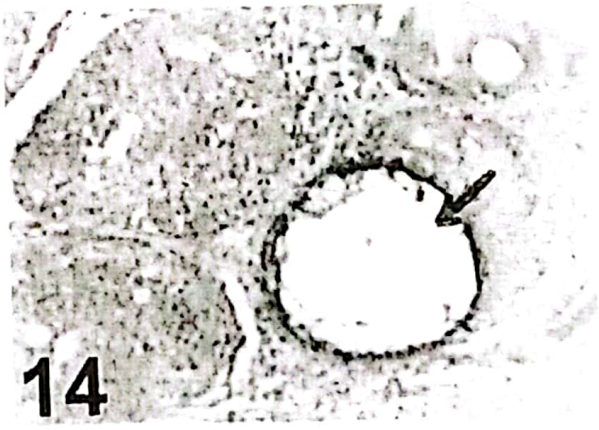
11



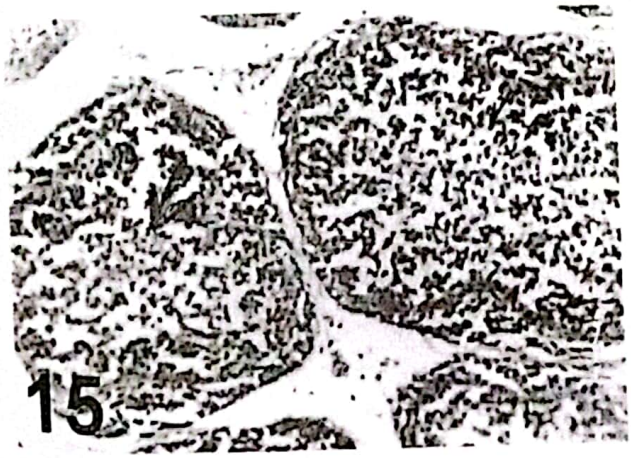
12



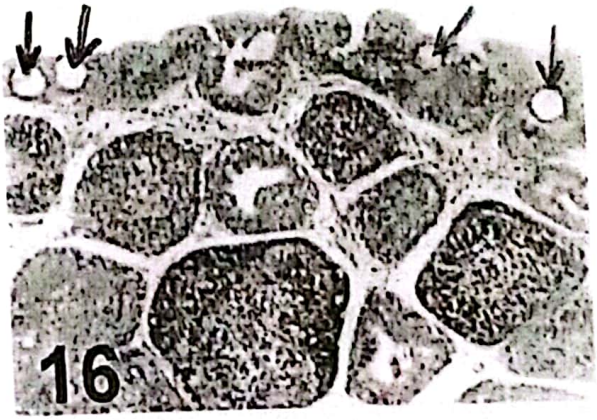
13



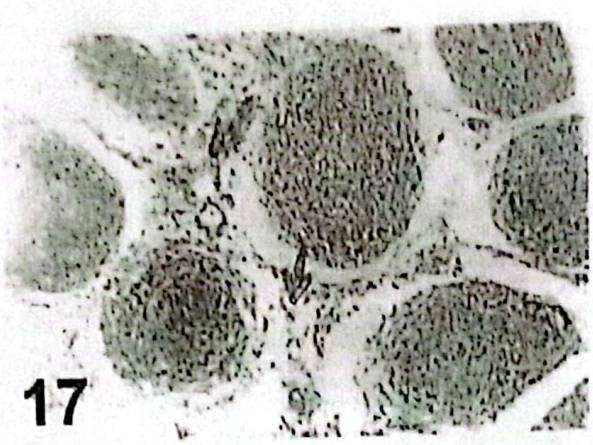
14



15

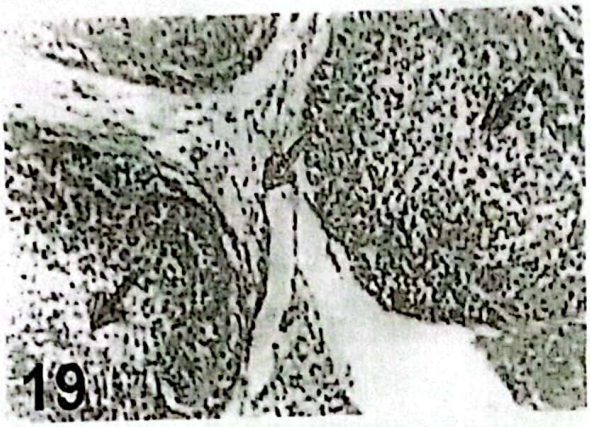


16

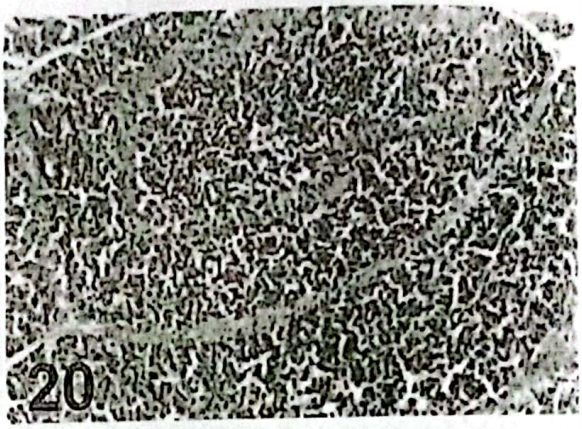


17

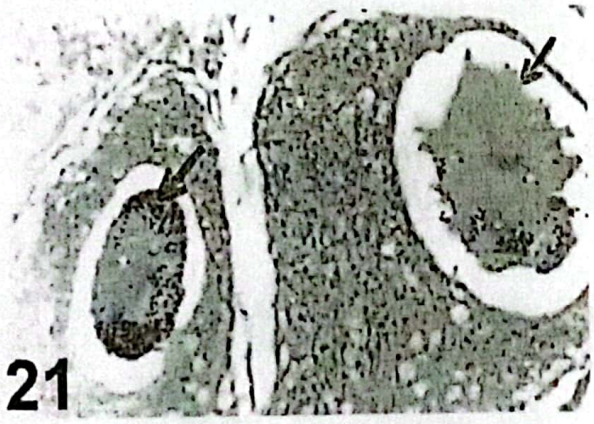
18



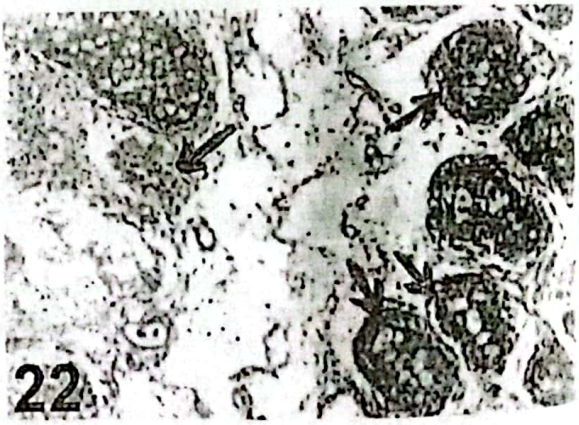
19



20



21



22

LEGANDS OF FIGURES:

Fig. (1): Bursa of fabricuis (BF) of chicken from group 1 (3 days PV) showing lymphocytic necrosis in both cortex and medulla of the lymphoid follicles which replaced by acidophilic fibrillar and nuclear debris. (H & E X 66).

Fig. (2): BF of chicken from group 1 (7 days PV) showing marked interfollicular edema associated with mononuclear leucocytic cells infiltrations. (H & E X 66).

Fig. (3): BF of chicken from group 2 (3 days PV) showing moderate lymphocytic necrosis, depletion and vacuolations. (H & E X 132).

Fig. (4): BF of chicken from group 2 (7 days PV) showing massive heterophilic cells infiltration in the stroma and also invading the follicles. (H & E X 66).

Fig. (5): BF of chicken from group 3 (3 days PV) showing lymphcytic necrosis associated with marked infiltration with heterophiles. (H & E X 66).

Fig. (6): BF of chicken from group 3 (7 days PV) showing complete necrosis and disintegration of cells leaving cyst like spaces of lymphoid depletion containing remnant of cellular debris. (H & E X 66).

Fig. (7): BF of chicken from group 3 (10 days PV) showing marked heterophilic cells infiltration. (H & E X 66).

Fig. (8): BF of chicken from group 3 (14 days PV) showing vacuolations and atrophy of bursal lymphoid follicles associated with interfollicular edema. (H & E X 66).

Fig. (9): BF of chicken from group 4 (14 days PV) showing hemorrhages. (H & E X 33).

Fig. (10): BF of chicken from group 5 (3 days PV) showing apparent normal lymphoid follicles. (H & E X 66).

Fig. (11): BF of chicken from group 5 (14 days PV) showing proliferation and hyperplasia of reticuloepithelial cells and vacuolations of the lymphoid follicles. (H & E X 132).

Fig. (12): BF of chicken from group 6 (21 days PV) showing repopulation of bursal lymphoid follicles with lymphocytes. (H & E X 66).

Fig. (13): BF of chicken from group 7 (control unvaccinated) showing the normal histology of lymphoid follicles. (H & E X 66).

Fig. (14): BF of chicken from group 1 (3 days PC) showing lymphocytic necrosis and depletion in some lymphoid follicles associated with intrafollicular cyst containing basophilic mucin as well as slight interfollicular hemorrhage. (H & E X 66).

Fig. (15): BF of chicken from group 1 (7 days PC) showing moderate lymphocytic de-

pletion in the cortex and medulla of lymphoid follicles as well as slight interfollicular edema. (H & E X 66).

Fig. (16): BF of chicken from group 2 (7 days PC) showing proliferation of the bursal epithelial layer produced a glandular structure of columnar epithelial cells containing basophilic mucin, some lymphoid follicles showing vacuolations and atrophy. (H & E X33).

Fig. (17): BF of chicken from group 3 (3 days PC) lymphocytic necrosis, marked intrafollicular and interfollicular heterophilic cells infiltrations as well as edema. Notice atrophied bursal follicles. (H & E X33).

Fig. (18): BF of chicken from group 5 (3 days PC) showing lymphocytic necrosis leaving remnant of nuclear debris, lymphocytic depletion and vacuolations in lymphoid follicles. (H & E X66).

Fig. (19): BF of chicken from group 5 (7 days PC) showing moderate lymphocytic lymphocytic depletion in their medulla and few leucocytic cells infiltrating the interfollicular stroma. (H & E X66).

Fig. (20): BF of chicken from group 6 (7 days PC) showing no histopathological alterations. (H & E X66).

Fig. (21): BF of chicken from group 8 (3 days PC) showing complete necrosis and disintegration of cells leaving cyst like spaces of lymphoid depletion containing homogenous eosinophilic material and remnant of cellular debris. (H & E X 66).

Fig. (22): BF of chicken from group 8 (7 days PC) showing marked lymphocytolysis, lymphoid atrophy, marked interfollicular edema and leucocytic cells infiltration (H & E X 33).

DISCUSSION

In the present study, no clinical signs or mortality were observed among all vaccinated unchallenged birds throughout the experimental period, while typical IBD signs and 20% mortality was recorded in unvaccinated challenged birds. Similar finding were reported by Eterradossi et al., (2004) who found that no mortalities or clinical signs were observed in SPF control group or in the group vaccinated with IBD L vaccine. Although these vaccines were efficacious and conferred 100% protection against mortality, none of them prevent the bursal damage or bursal atrophy. Similar finding were reported by other investigators (Hassan et al., 2002, Eterradossi et al., 2004 and Sultan et al., 2006) as they concluded that classical vaccines could protect against both mortality and clinical signs but did not prevent bursal lesions or bursal atrophy.

Bursal atrophy and significant difference in BF/BW were observed at 7 days PV in groups 1 & 2 that were vaccinated with vaccine (A), while it

was observed at 10 days PV in other vaccinated groups indicating that vaccine (A) induces post vaccination reactions earlier than those induced by vaccine (B) and (C). In addition, significant difference in the relative BF/BW ratio was observed between group 2 that received vaccine (A) by eye drop and group 6 that received vaccine (C) by the same route at 10 days PV (1.11+ 0.23 Vs 2.49 + 0.53). Moreover, significant difference in the relative BF/BW ratio was observed between group 2 and group 4 that received vaccine (B) by eye drop route at 21 days PV (1.21+ 0.13 vs 2.66+ 0.93) indicating that, the bursal atrophy was less severe in birds vaccinated with vaccine B and C by eye drop route than those vaccinated with vaccine A with the same route (Table 1). The bursal lesion scores were also high in groups 1 and 2 that received vaccine (A) either in drinking water or by eye drop route than in group 3 and 4 that received vaccine (B) and groups 5 and 6 that received vaccine (C) at 3 days PV. In addition, lower bursal lesion scores were observed at 21 days PV in groups vaccinated with vaccine (B) and (C) compared with groups received vaccine (A) which indicate that, the bursal damage induced by vaccine (B) and (C) are less severe than bursal damage induced by vaccine (A) Table (2). At 7 days PC, a lower bursal lesion score was observed in group 4 that received vaccine (B) by eye drop compared with groups 3 that received the same vaccine in drinking water (2 vs 1.4). The same observation was noticed where the bursal lesion score of group 6 that received vaccine (C) by eye drops at 7 days post challenge where lower than the bursal lesion score of group 5 that received the same vaccine (C) in drinking water (2 vs 1.5). These results indicated that vaccine (B) and (C) are less pathogenic and less invasive than vaccine A as indicated by relative body weight ratio and bursal lesion score, and the vaccination with eye drop route may have advantage over the drinking water route. Giambrone (1984) found that the coarse spray or eye drop route is more effective for IBD vaccination than drinking water. Lohren (1994) stated that, in many cases, especially when the farm had a history of virulent IBD, drinking water vaccination failed.

From histopathological examination, it was found that, bursae of each vaccinated and challenged groups showed 3 different histological characters based on the degree of reaction of the bursae against the virus into; mild, moderate, and severe reaction. In groups (1 and 2), moderate microscopic bursal lesions were observed at 3, 7, and 10 days PV, while at 14 and 21 days PV the vaccine produced mild lesions. After challenge, the bursa showed moderate reaction at 3 days (PC) then mild reaction at 7 days (PC).

In group (3), severe microscopical bursal lesions were observed in bursae of chickens all over the experimental period. After challenge the bursal reaction to the virulent IBDV was moderate. Meanwhile, when the same vaccine given

through the eye drop route (group 4), the bursa showed from mild to moderate changes at 7, 10 and 14 days PV, and repopulation of most lymphoid follicles with lymphocytes was observed at 7 days PC therefore, the bursa appeared histologically normal.

In group (5) moderate bursal reaction starting from the 7th day PV and continue the whole experimental period. On the other hand, when the same vaccine was given through the eye drop route in group (6), mild histopathological changes was observed and the bursa appeared histologically normal at 7 days PC.

Unvaccinated challenged group (8) showed severe histopathological lesions typical to IBDV infection and similar to those reported by Abdel-Alim and Saif (2001).

All vaccinated groups responded to IBDV vaccine as indicated by QAGPT and no significant difference was observed in the antibody titre (Table 3) which revealed that all used vaccines are immunogenic and elicited the immune response. Although the tested vaccines produced bursal atrophy and microscopic lesions, neither was immunosuppressive as measured by subsequent ND vaccination response. However, Birds vaccinated with vaccine (A) in drinking water revealed lower antibody response than those vaccinated by eye drop route. In addition, birds vaccinated with vaccine (B) in drinking water revealed lower antibody titre than those vaccinated by eye drop which indicated that the immune response in birds vaccinated with IBDV live intermediate plus vaccine by eye drop was better than those vaccinated in drinking water.

The following conclusion can be drawn: All studied three live intermediate plus vaccine of IBDV are immunogenic and efficacious as indicated by antibody response to IBDV and 100% protection against challenge with virulent IBDV; All vaccines have residual pathogenicity indicated by bursal atrophy and bursal damage in vaccinated birds; The bursal damage appeared earlier and was more severe in birds vaccinated with vaccine (A) than those vaccinated with vaccine (B) and (C); The eye drop route resulted in less severe bursal lesions and better immune response than drinking water route as evidenced mild pathological changes in the bursa of groups vaccinated by eye drop compared with those vaccinated with the same vaccine in drinking water.

REFERENCES

Abdel-Alim, G. A., Awad. M. H. H., andY. M. Saif. (2003): Characterization of Egyptian field strains of infectious bursal disease virus. Avian Dis., 47: 1452-1457.

Abdel-Alim, G. A., and Y. M. Saif. (2001): Detection and persistence of infectious bursal disease virus in specific-pathogen-free and commercial broiler chickens. Avian Dis., 45: 646 – 654.

Bancroft, J. D., Steven, A., and Tuner, D. V. (1990): Theory and practice of histopathological technique 3rd Edn. Churchill, Living Stone edingburgh, London, Melbourne and New York.

Benton, W.J., M. S. Cover, J.K. Rosenberger, and Lake. R. S. (1967): Physicochemical properties of the infectious bursal agent (IBA). Avian Dis., 11: 438- 445.

Cheville, N. F. (1967): Studies on the pathogenesis of Gumboro disease in the bursa of Fabricius, spleen, and thymus of the chicken. American J. Pathology, 51: 527-551.

Cullen, G. A. and S. Wyeth, P. J.(1975): Quantitation of antibodies to infectious bursal disease. Vet. Rec. 97, 315.

David, E. S., John, R. G., Mark, W. J., James, E. P., and Willie, M. R. (1998): Hemagglutination inhibition (HI). Alaboratory Manual for the Isolation and Identification of Avian Pathogens. 4th Edn., 255- 259.AAAP.

El-Batrawy, A. M. (1990): Studies on severe outbreaks of infectious bursal disease. Proceedings of the 2nd Sci., Con. Egyp. Vet. Poultry Ass. (pp. 239 – 252). Cairo, Egypt.

El-Ebiary, Elham, A., M. M. Abd El-Hakim., N. A. Sherif ., Susa. S. El-mahdy., and Nadia, M. Hassan.(2001). Comparative study on two commercial hot vaccinal strains of IBDV. Beni-Suef Vet. Med. J. Vol. XI. No. (2): 661 – 676.

El-Sergany, H. A., Ann, M. Saber, M. S. and Mohamed, M. A. (1974): A preliminary investigation on the occurrence of Gumboro disease. Egypt, J. of Vet. Sci., 11, 7 – 9.

Gerradossi, N., Gauthier, C., Reda, I., Comte, S., Rivallan, G., Tonquin, D., Biosseson, C, Lamande, J., Jestine, V., Morin, Y., Cazabau, C., and Borne, P. M. (2004): Extensive antigenic changes in an atypical isolate of very virulent infectious bursal disease virus with an antigenically classical live vaccine. Avian Pathology, 33 (4): 423 - 431.

Giambrone J. J. (1984). IBD spray vaccination. If water fails, try it. Broiler Ind., 40: 52 – 54.

Giambrone J. J. and Clay, R. P. (1986): Evaluation of immunogenicity, stability, pathogenicity and immunosuppressive potential of four commercial live infectious bursal disease vaccines. Poultry Science, 65: 1278 – 1290.

Hassan, M. K. Afify. M and Aly, M. M. (2002): Suseptibilty of vaccinated and unvaccinated Egyptian chickens to very virulent infectious bursal disease virus. Avian Pathology, 31: 149 – 156.

Hitchner, S.B. (1970): Infectivity of infectious bursal disease virus for embryonating eggs. Poultry Science, 49: 511-516.

Kibenge, F. S. B., Dhillon, A. S. and Russel, R. G. (1988): Biochemistry and immunology of infectious bursal disease virus. J of.General Virology, 69: 1757- 1775.

Lohren, H. (1994): Infectious bursal disease: Current situation and control by vaccination. In; proceedings of the international symposium on infectious bursal disease and chicken infectious anemia, Edited by Prof. E.Kaleta, Rauischolzhausen, Germany, 21-24 Jun 1994, pp 229- 234.

Lukert, P. D. and Y. M. Saif. (2003): Infectious bursal disease .In Y.M. Saif (Ed.) Diseases of poultry. 11th edn. (pp. 161 – 180). Ames, Iowa. Iowa State University.

Mazzariegos, L. A., Lukert, P. D. and Brown, J. (1990): Pathogenicity and immunosuppressive properties of infectious bursal disease "intermediate" strains. Avian

Dis., 34: 203 – 208.

Muskett. J. C., I. G. Hopkins, K. R. Edwars and D. H. Thornton. (1979): Comparison of two infectious bursal disease vaccine strains: efficacy and potential hazards in susceptible and maternally immune birds. Vet. Rec. 4: 332 – 334.

Reed, L. J., and H. A. Muench. (1938): Simple method for estimating fifty percent end point. Am. J. Hyg., 27: 493-496.

Rosales, A. G., P. Villegas., P. D. Lukert., O. J. Fletcher., M. A. Mohamed ., and J. Brown.(1989): Pathogenicity of recent isolates of infectious bursal disease virus in specific-pathogen-free chickens; protection conferred by an intermediate vaccine strain. Avian Dis., 33: 729 - 743.

Saif. Y.M. (1991): Immunosuppression induced by infectious bursal disease virus. Veterinary Immunology and Immunopathology, 30: 40- 50.

Saif-Edine, M., Aly, M. and Mousa, S. (1996): Status of infectious bursal disease in Egypt. Proceedings of the 4th Sci. Conf. Egypt, Vet. Poultry Asso. (pp. 56 – 81). Cairo, Egypt.

Snedecor, G. W., and W. F. Corchan (1967): Statistical methods. 6th Ed. Ames, Iowa. The Iowa State University.

Sultan, H. A., El-balal, S., Hussein, H. A., and Abdelrazek, A. (2006): Development of early protection induced by intermediate and intermediate plus infectiius bursal disease virus (IBDV) vaccines against very virulent IBDV (vvIBDV). Proceedings of the 7th Sci. Con. of the Egypt. Vet. Poultry Asso. (pp. 227 – 238). Cairo, Egypt.

Winterfield, R. W., A. M. Fadly., and A. Bickford (1983): Infectivity and distribution of infectious bursal disease virus in chickens, persistence of the virus and lesions. Avian Dis., 16: 622- 632.

Winterfield, R. W., Dhillon, A. S., Thacker, H. L. and Alby, L. J (1980): Immune response of white leghorn chicks from vaccination with different strains of infectious bursal disease virus. Avian Dis. 24, 179 – 188.

Winterfield, R. W. and L. H. Thacker.(1978): Immune response and pathogenicity of different strains of infectious bursal disease virus applied as a vaccine. Avian Dis., 22: 721 – 731.

كفاءة وضراوة ثلاث لقاحات حية (عترات فوق متوسطة) لمرض إلتهاب غدة فابريسى المعدى فى سلالة دجاج محلى فى مصر

د/ جمعة عبدالرحيم عبدالعليم* ، د/ كوكب عبدالعزيز أحمد **

* قسم أمراض الدواجن - كلية الطب البيطرى - جامعة القاهرة

** قسم الباثولوچى - كلية الطب البيطرى - جامعة القاهرة

فى هذه الدراسة تم قياس مدى كفاءة وشدة ضراوة ثلاث لقاحات حية (عترات فوق متوسطة) لمرض إلتهاب غدة فابريسى المعدى فى سلالة دجاج محلى فى مصر. تم تحصين مجاميع الطيور فى عمر ١٤ يوم بأحد هذه التحصينات عن طريق التقطير فى العين فى أحد المجاميع وفى مياه الشرب فى مجموعة أخرى وأجريت العدوى لمجاميع الطيور المحصنة والغير المحصنة بعد ١٤ يوم من التحصين (عمر ٢٨ يوم).

تم تسجيل النفوق والأعراض المرضية والصفة التشريحية وحساب الوزن النسبى لغدة فابريسى/وزن الجسم وكذلك متابعة التغيرات الباثولوچية فى غدة فابريسى على فترات بعد التحصين والعدوى كمقياس لمدى كفاءة وشدة ضراوة اللقاحات المستخدمة.

ولقد أظهرت هذه اللقاحات كفاءة عالية ١٠٠٪ حيث أنه لم تسجل أى نفوق فى القطعان المحصنة بالرغم من حدوث نفوق بنسبة ٢٠٪ فى القطعات الغير محصنة ومع أن هذه اللقاحات أثبتت كفاءة عالية فى منع حدوث النفوق إلا أنها لم تمنع حدوث ضمور وتغيرات باثولوچية فى غدة فابريسى ولقد لوحظ الضمور فى الغدة بعد ٧ أيام من التحصين فى المجاميع المحصنة باللقاح (أ) بينما تم ملاحظتها بعد ١٠ أيام من التحصين فى المجاميع المحصنة باللقاح (ب) واللقاح (ج).

ولقد أوضحت هذه الدراسة أن اللقاح (أ) أشد شراوة من اللقاح (ب) واللقاح (ج) كما ثبت من إرتفاع معدل التغيرات الباثولوچية وإنخفاض الوزن النسبى لغدة فابريسى/وزن الجسم فى بعض الفترات بعد التحصين فى المجاميع المحصنة باللقاح (أ) مقارنة بالمجاميع المحصنة باللقاح (ب) واللقاح (ج).

عندما أعطيت هذه اللقاحات (ب) و (ج) عن طريق ماء الشرب لوحظ حدوث تغيرات باثولوچية تتراوح من متوسطة - شديدة في الغدة بينما لوحظ تغيرات باثولوچية تتراوح من طفيفة - متوسطة عندما أعطيت نفس هذه اللقاحات عن طريق التقطير فى العين مما يدل على أن التحصين بالتقطير فى العين أعطى نتائج أفضل وتغيرات باثولوچية أقل من التحصين فى ماء الشرب .