# Infectious Bursal Disease infection and immunization in quail.

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**A B S T R A C T**

This work deals with vaccination and infection by infectious bursal disease IBD infection in quail. Monitoring of IBD antibodies in vaccinated quails using Serum neutralization test (SNT) revealed that all birds exhibited detectable specific antibodies started by the first week post vaccination with a mean titer of 8 to reach their peak (64) by the fourth week remaining with this level till the 6th month later, and through histopathological Challenges against the virulent IBD virus showed that all vaccinated quails were able to withstand the virulent virus showing neither clinical nor pathological changes in their bursa revealing 100% protection while the unvaccinated birds were unable to withstand the challenge virus showing characteristic pathological changes in the bursa which performed on the 3rd ,6th and 15th day from infection . So, it could be concluded that vaccination of quail is recommended to protect quail industry aiding in production of low price animal protein and to prevent suspected virus transmission to chickens.

**Keywords**: IBD, SNT, quail, Vaccination.

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## 1. INTRODUCTION

The quail is a small bird that inhabits woodland and forest areas around the world. There are thought to be more than 15 different species of quail, with each species of quail being found in different parts of the world and all have slightly different appearances depending on how they have adapted to their environment. In some parts of the world, quails are kept as poultry birds both for the small amount of meat that they contain and for the quail's brightly colored eggs. Infectious bursal disease virus (IBDV) is the causative agent of infectious bursal disease, (IBD) that affects young chickens about 3-6 weeks of age. It is a highly contagious and acute viral disease that is characterized by destruction of lymphoid cells in the bursa of fabricius. IBDV is classified as member of birnaviridaie (Brown, 1986). IBDV is a small, non-enveloped virus belongs to which characterized by bi-segmented dsRNA genome (Kibenge et al., 1988). The bursa of fabricius was evaluated in healthy and experimentally infected quails with IBDV. Infected bursae showed an initial increase in size which later decreased for a while before attaining a second peak histologically, the normal bursae showed the general plan of gastrointestinal tract structures with the lamina propria containing non-capsulated lymphoid follicles, which varied in arrangement and number. The infected bursae revealed inter follicular edema, lymphocytolysis, hemorrhages, fibroplasia and keratinization of the bursal substances. The destruction of immature B lymphocytes in the bursa creates an immunosuppression, which will be more severe in younger birds (Faragher, 1972 ). In addition to the impact on production and role in the development of secondary infections, this will affect the immune response of the chicken to subsequent vaccinations which are essential in all types of intensive animal production (Giambrone et al., 1976 ).

The present study was designed to spot the light on the possibility of quail to be infected with IBD virus and accordingly the possibility of immunization of such birds against such infection saving those bird populations aiding in increasing of national income.

## 2. MATERIAL AND METHODS

### *2.1. Quail:*

Thirty quails of about 2 weeks' old were obtained from a commercial market and they

were found to be free from IBD antibodies as screened by serum neutralization test (SNT). They divided into three groups (10birds/group) as follow: Group-1 was experimentally infected with IBD virulent virus. Group-2 was vaccinated with live attenuated IBD vaccine then challenged on the 21st day post vaccination with the virulent IBD virus. Group-3 was kept without vaccination and kept as control and divided on the time of vaccinated birds challenge into 2 subgroups (5birds/subgroup) where subgroup-1 was experimentally infected with the virulent virus while subgroup-2 was kept free.

*2.2. IBD vaccine:*

Four hundred blood samples were Live vaccine of IBDV (D78) was kindly supplied from (VSVRI) Veterinary Serum and Vaccine Research Institute (VSVRI) Abassia, Cairo. It was administrated for quail in drinking water for vaccinated group.

*2.2.1. Viruses:*

Virulent virus strain 1 and Vero cell culture adapted infectious bursal disease IBD virus were kindly supplied by veterinary serum and vaccine research institute (VSVRI) and used in SNT for challenge and evaluation of the quail immune response of vaccinated quail.

*2.3. Vero cell line:*

African green monkey kidney (Vero) cell monolayer cell cultures were supplied by VSVRI and used for estimation of IBD antibodies in sera of vaccinated quail using SNT.

*2.4. Sampling:*

Blood samples were obtained from the experimental quails through the wing vein puncture under complete aseptic conditions and allowed to form clots at 4°c over night. The serum was separated and centrifuged at 2000rpm for 15 minutes then kept in sterile screw capped vials at -20°C till subjected for serological and chemical examination. Such samples were obtained on week intervals up to 4 weeks then every 4 weeks up to 16 weeks post vaccination.

*2.5. Histopathology:*

Bursa from experimentally infected; challenged vaccinated and control quail groups were on the 3rd day of infection and 6$^{th}$ day of infection and 9th day of infection and after 15 days from infection and subjected for gross examination and histopathological examination.

*2.6. Serum Neutralization Test (SNT):*

SNT was carried out using the micro titer technique according to Bass et al. (1982) and the antibody titer was expressed as the reciprocal of the final serum dilution which neutralized and inhibited completely the CPE of 100 $TCID_{50}$ of the used virus according to Singh et al. (1967).

## 3. RESULTS

*3.1. Monitoring of IBD antibodies in vaccinated quail:*

Monitoring of IBD antibodies in vaccinated quails, SNT revealed that all birds exhibited detectable specific antibodies started by the first week post vaccination with a mean titer of 8 to reach their peak (64) by the fourth week remaining with this level till the 6th month later as tabulated in table (1).

*3.2. Evaluation of the induced IBD immunity in quails:*

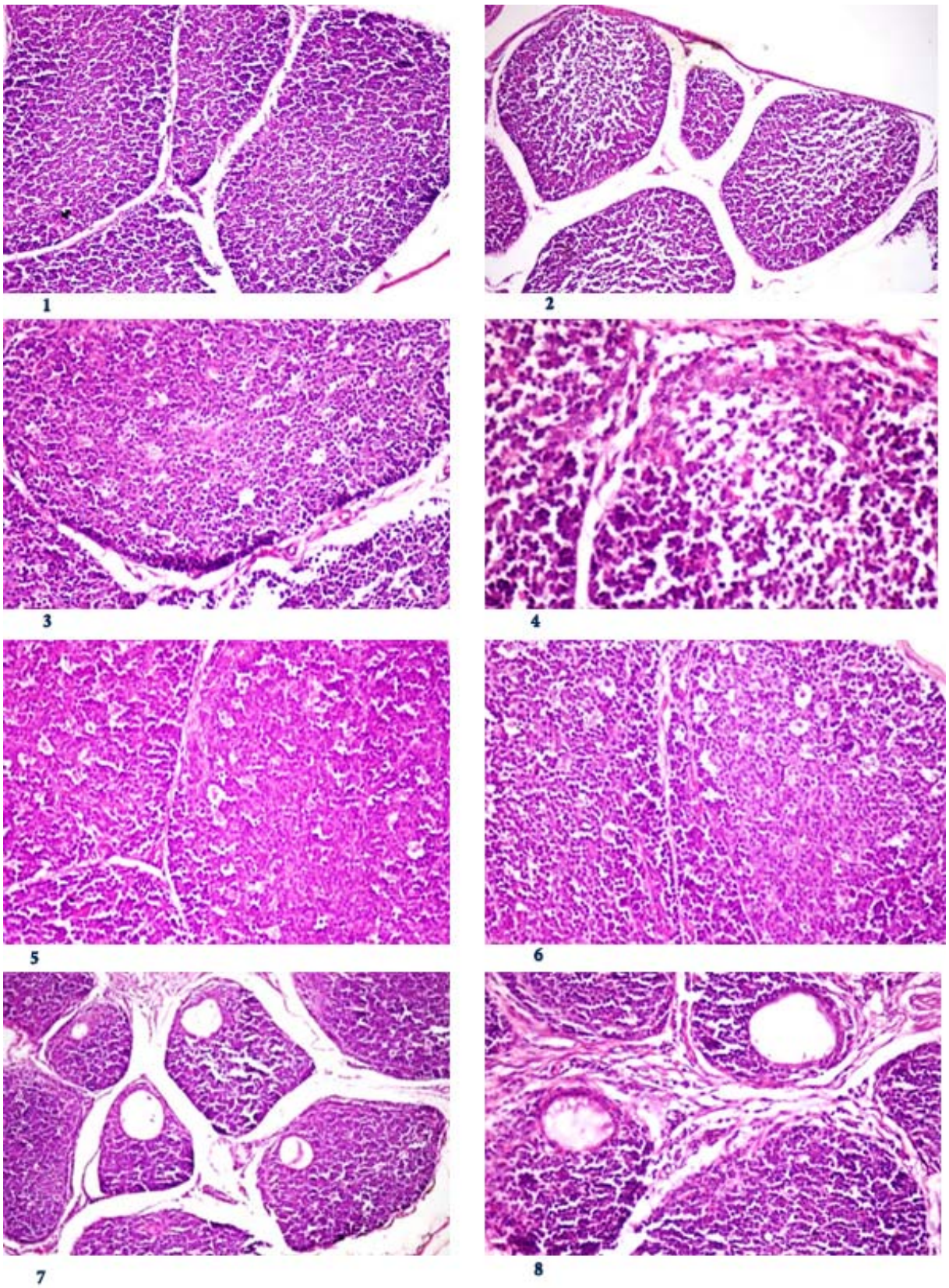
Twenty-one days' post vaccination; ten quails were randomly selected and challenged against the virulent IBD virus in addition to 5 unvaccinated quails.

All vaccinated quails were able to withstand the virulent virus showing no clinical and neither macroscopically post mortem findings nor microscopically histopathological changes in their bursa revealing 100% protection while the unvaccinated birds were unable to withstand the challenge virus showing macroscopically post mortem findings nor microscopically histopathological changes in the bursa as demonstrated in table (2).

*3.3. Histopathological examination:*

Bursa of control quails showed normal histological structure of the bursal follicles (Photo-1) while bursa of infected quail 3-days post infection showed mild to moderate lymphocytic depletion (Photo-2) of some lymphoid follicles with moderate degree of lymphocytic necrosis and nuclear pyknosis of some cells (Photo-3). The interfollicular connective tissue was moderately dispersed by edema.

The bursa of infected quail 15 days post infection showed severe lymphocytic depletion of the lymphoid follicles with extensive necrosis of the bursal follicular lymphoid cells (Photo-4).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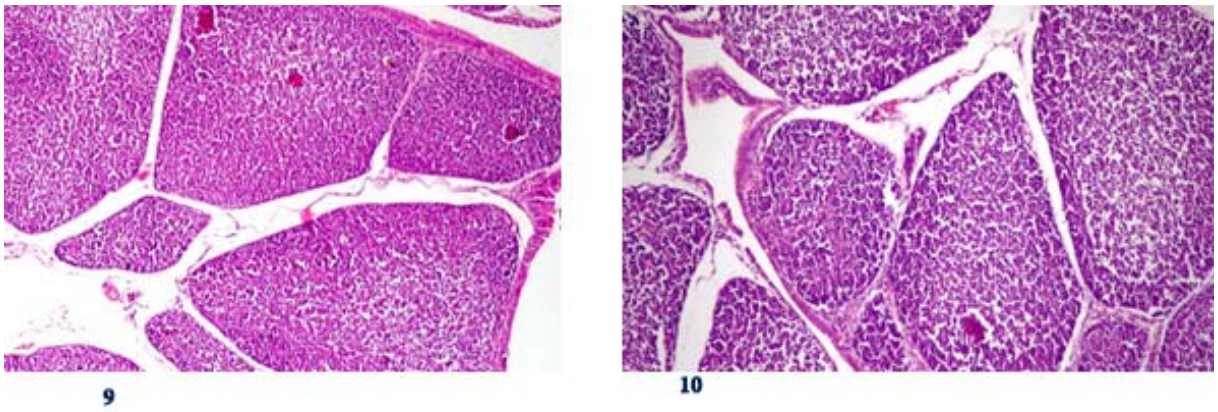


Photo (1): Bursa of control quail showing normal bursa follicles (H&E). Photo (2): Bursa of infected quail 3-days post infection showing moderate degree of lymphocytic depletion of the lymphoid follicles with marked edema of the interfollicular connective tissue. (H&E). Photo (3): Bursa of infected quail 3-days post infection showing necrotic changes of the bursal lymphoid cells with depletion. (H&E). Photo (4): Bursa of infected quail 15 days post infection showing severe lymphocytic depletion of the lymphoid follicles with appearance of necrotic lymphocytes, pyknotic nuclei (dashed arrow) and appearance of the underlying reticular mesh (H&E). Photo (5): Bursa of infected quail 15 days post infection showing marked necrosis of the follicular lymphocytes with appearance of apoptotic bodies (arrow) and tingible body macrophages (H&E). Photo (6): Bursa of infected quail 15 days' post infection showing large number of tingible body macrophages with various necrotic follicular lymphocytes. (H&E,). Photo (7): Bursa of infected quail 15 days post infection showing cyst formation within the lymphoid follicles and necrosis of the follicular cells as well as marked expansion of the interfollicular connective tissue by edema (H&E). Photo (8): Bursa of infected quail 15 days post infection showing expansion of the interfollicular connective tissue by edema and inflammatory cells with few heterophils (dashed arrow), notice cyst (C) formation in the follicles. (H&E). Photo (9): Bursa of vaccinated quail and virus challenged 3-days post challenge showing mild lymphocytic depletion (D) of some bursal follicles with mild interfollicular edema. (H&E,). Photo (10): Bursa of vaccinated quail and virus challenged 15 days post challenge showing near to normal appearance of the bursal follicles with very mild interfollicular edema. (H&E).

Table (1): Mean IBD serum neutralizing antibody titer in vaccinated quail

| Periods post Vaccination | Mean IBD serum neutralizing antibody titer* | |
|---|---|---|
| | Vaccinated quail | Unvaccinated quail |
| Pre-vaccination | 0 | |
| 1WPV** | 8 | |
| 2WPV | 16 | ↑ |
| 3WPV | 32 | Remained sero-negative |
| 4WPV | 64 | all over this |
| 2MPV*** | | period |
| 3MPV | ↑ | ↓ |
| 4MPV | 64 | |
| 5MPV | ↓ | |
| 6MPV | | |

*Mean IBD serum neutralizing antibody titer= the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100$TCID_{50}$ of IBD virus. **WPV= week post vaccination. ***MPV= month post vaccination

Table (2): Potency of IBD vaccine in quails

| Quail groups | Number of challenged quails | Number of survived quails | Protection percentage |
|---|---|---|---|
| Vaccinated | 10 | 10 | 100 |
| Unvaccinated | 5 | 1 | 20 |

The necrotic cells were either appeared with pyknotic nuclei or homogenous eosinophilic with appearance of the underlying reticular mesh. Many apoptotic bodies and large number of tingible body macrophages were observed within the depleted follicles (Photo- 5 and 6). Cyst formation was a conspicuous finding (Photo-7) within the destructed lymphoid follicles as well as necrosis of the follicular cells. Marked expansion of the interfollicular connective tissue by edema and inflammatory cells infiltration and few heterophils were noticed (Photo-8). However, examination of bursa of vaccinated and virus challenged quails 3-days post challenge showed marked protection achieved by the vaccine against the effect of the challenged virus, only mild lymphocytic depletion of some bursal follicles with mild interfollicular edema was observed (Photo-9) while 15-days post challenge, the bursa showed near to normal appearance of most of the bursal follicles with very mild interfollicular edema (Photo-10).

## 4. DISCUSSION

In an interesting investigation, quails vaccinated with IBD vaccine exhibited specific IBD antibodies where SNT revealed that such antibodies started by the first week post vaccination with a mean titer of 8 to reach their peak (64) by the fourth week remaining with this level till the 6th month later as tabulated in table (3).

Challenge of vaccinated quail, twenty one days post vaccination; showed that these quails were able to withstand the virulent virus showing no clinical and neither macroscopically post mortem findings nor microscopically histopathological changes in their bursa revealing 100% protection while the unvaccinated birds were unable to withstand the challenge virus showing macroscopically post mortem findings nor microscopically histopathological changes in the bursa as demonstrated in table (4) and experiment (2.3).

Although there are nil data discuss IBD infection and vaccination in quail, some findings in chickens were found to be those obtained in quails where Jackwood et al. (1985) stated that the humeral immunity is the primary mechanism of the protective immune response; Darteil et al. (1995) said that the chickens can vaccinated with live IBD vaccine at 10-14 weeks of age; the same age of the present vaccinated quails; Zouelfakar et al. (1997) reported that chickens inoculated with live IBDV vaccine at 7 and 21 days of age were protected against mortality sings however the bursal: body weight ratios were significantly lower than those of control birds. In addition, EL-Ebiary et al. (1997) concluded that IBDV vaccination of chickens with various minimal requirements of the two commercially IBDV vaccines induced protection for 100% of examined chickens and Lukert and Saif (2003) reported that field exposure or vaccination with IBDV resulted in VN titers higher than 1:1000.

Challenge of vaccinated unvaccinated quail showed body temperature above normal then dropped markedly below 35o C before death. The clinical signs were evident by 24-48 hours post infection showing tremors, picking of the vent, ruffled feathers; by 48 hours there was a thick yellowish diarrhea. By 72 hours most birds had loss of appetite. Bursa of control quails showed normal histological structure of the bursal follicles (Photo-1) while bursa of infected quail 3-days post infection showed mild to moderate lymphocytic depletion (Photo-2) of some lymphoid follicles with moderate degree of lymphocytic necrosis and nuclear pyknosis of some cells (Photo-3).

The interfollicular connective tissue was moderately dispersed by edema. The bursa of infected quail 15 days' post infection showed severe lymphocytic depletion of the lymphoid follicles with extensive necrosis of the bursal follicular lymphoid cells (Photo-4). The necrotic cells were either appeared with pyknotic nuclei or homogenous eosinophilic with appearance of the underlying reticular mesh. Many apoptotic bodies and large number of tingible body macrophages were observed within the depleted follicles (Photo-5 and 6). Cyst formation was a conspicuous finding (Photo-7) within the destructed lymphoid follicles as well as necrosis of the follicular cells. Marked

expansion of the interfollicular connective tissue by edema and inflammatory cells infiltration and few heterophils were noticed (Photo-8). However, examination of bursa of vaccinated and virus challenged quails 3-days post challenge showed marked protection achieved by the vaccine against the effect of the challenged virus, only mild lymphocytic depletion of some bursal follicles with mild interfollicular edema was observed (Photo-9) while 15-days post challenge, the bursa showed near to normal appearance of most of the bursal follicles with very mild interfollicular edema (Photo-10).

Similar findings were obtained by Cho and Edgar (1970) who mentioned that in IBD infection, peak of depression was recorded during the 3rd and 4th day of infection. They showed that chickens died with IBD had atrophied bursae (84%); hemorrhages in the skeletal muscles (80%); excessive urates in kidneys (88%) and atrophied spleen (76%) while in experimentally infected chicks, the bursae began to be atrophied and surrounded by a yellowish gel-like film with maximum hypertrophy by 48 hours post infection and markedly atrophy by 120 hours. The liver and spleen were significantly hypertrophied by 72 hours. Okoye and Uzoukwu (1981) stated that the major gross lesions in birds died with IBD are hemorrhages in the thigh muscles and breast in addition to enlargement of the bursa of Fabricius which are sometimes hemorrhagic. Fadly and Nazerian (1983) infected chickens free from maternal immunity, experimentally with IBDV at 1, 5 and 11 weeks of age showed severity of microscopic lesions and frequency of detection of viral antigens in lymphoid organs of infected chickens at 5 weeks of age, were comparable to those of birds infected at 1 or 11 weeks of age. In addition, Lukert and Saif (1991) reported that the age of the greatest susceptibility to IBDV infection is between 3 and 6 weeks while chickens younger than 2 weeks do not exhibit clinical signs but have subclinical infections resulting in severe immune suppression.

Depending on the obtained results and although there is need to further studies, it could be concluded that quail showed be vaccinated against IBD to save their population and to prevent the possible virus transmission to chickens.

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